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HYPERTENSION :

EXPERIMENTAL AND CLINICAL PHARMACOLOGICAL STUDIES

William Peregrine Pepperrell Leary

A thesis submitted to the Faculty of Medicine, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Doctor of Science (Medicine).

Durban, A.D. 1985.

ABSTRACT

The publications forming this submission cover two broad fields. A series of papers deal with experimental hypertension ; possible roles for angiotensin and prostanoid substances in the pathogenesis of hypertension were investigated. The results indicated that the capacity of kidney to inactivate angiotensin II could be quite profoundly altered by inducing hypertension using the one and two-clip Goldblatt methods or by altering the sodium chloride content of the diet. The possibility that a reduction in the endogenous formation of vasodilator prostaglandins might occur in experimental hypertension was investigated; there was a clear reduction in prostaglandin E_2 generation by kidneys of rats given increased dietary sodium and by the ischaemic kidneys of rats which had been subjected to uninephrectomy and clipping of the surviving renal artery. The lungs of genetically hypertensive rats inactivate both constrictor and vasodilator prostaglandins to a lesser extent than the lungs of normotensive controls.

Further papers describe clinical investigations of antihypertensive medicines in man or studies in healthy volunteers and patients on the ancillary effects of drugs commonly used in the treatment of hypertension. Several relevant reviews are also presented for consideration. These studies have provided some insights into aspects of drug efficacy and safety which are sometimes overlooked by the practising physician.

DECLARATION

I declare that this work is my own or has been aided, in some cases, by close associates with whom I have collaborated in laboratory or clinical work. Statements from Professor Reyes and Dr. Ledingham are presented. This work is submitted for the degree of Doctor of Science (Medicine) in the University of the Witwatersrand. Papers number 1 - 4 formed part of a thesis submitted for the degree of Doctor of Philosophy in the University of Oxford. No other portion of this work has been submitted for any other degree.



8th day of September 1985.

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TO WHOM IT MAY CONCERN

I was closely associated with Dr. W.P.P. Leary during his attachment to the Department of the Regius Professor of Medicine at Oxford. The idea that we might look at the catabolism of angiotensin II in isolated perfused organs was jointly generated. Thereafter all the laboratory work was undertaken by Dr. Leary, who was also responsible for analysis of the data and first draft of all the resulting publications. Whilst decisions about experiments were always jointly made, there was no doubt about who was to do them and who would make the first analysis of the results. In essence then, Dr. Leary was responsible for the major burden of work reflected in our joint publications.

J.G.G. Ledingham, MA, DM, FRCP
May Reader in Medicine
Honorary Consultant Physician
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This is to certify that I, PROFESSOR ARIEL JORGE REYES have been closely associated with Professor W.P.P. Leary in numerous clinical and laboratory studies during recent years. We have been equal partners in most of our research endeavours and, as a matter of course, have tended to rotate first authorship of publications arising from this work. Additional junior authors have been included, from time to time, out of courtesy and to encourage them in their future careers. All major work has been the result of the collaboration between Professor Leary and the undersigned.

Yours faithfully,

PROF. dr A.J. REYES

DEDICATION

This submission represents over 15 years work which could not have been completed without the assistance, patience and forebearance of many people including colleagues, patients, secretarial staff, loving friends and family. My thanks are due to them all and in particular, to my wife and to Mrs. Peta Gordon who compiled the papers which comprise this volume.

PREFACE

Several generations of medical scientists and clinicians have studied the complex problems presented by raised arterial blood pressure. Specialised units devoted to the investigation of hypertension commonly employ a multidisciplinary approach, using experimental animals, computer models, healthy volunteers, patients and analyses of hospital records and surveys in their efforts to determine the causes, prognosis and optimum management of this condition.

As the title implies, the publications which form this submission cover two broad fields. First a series of papers dealing with aspects of experimental hypertension is presented. These publications report studies in which possible roles for angiotensin and prostanoid substances in the pathogenesis of hypertension were investigated. In some cases laboratory work was actually carried out by the applicant, in others a major contribution to the initiation, direction and final presentation of studies was involved. Thereafter further papers are divided into 6 groups which either describe clinical investigations of antihypertensive medicines in man or studies in healthy volunteers and patients of the ancillary effects of drugs commonly used in the treatment of hypertension. Several relevant reviews are also presented for consideration. The applicant made a major contribution to each of these studies in terms of conceiving, initiating, directing and reporting them. The mathematical

theory applied to much of this work was conceived by Professor A.J. Reyes in its entirety.

SECTION A

A1
Pressor Response to Infusion of Angiotensin into the Portal Vein of the Rat: WP Leary, JGG Ledingham. Nature (1968) 220: 1-5.

A2
Removal of Angiotensin by Isolated Perfused Organs of the Rat: WP Leary, JGG Ledingham. Nature (1969) 222: 959-960.

A3
Inactivation of Angiotensin II Analogues by Isolated Perfused Rat Liver and Kidney: WP Leary, JGG Ledingham. Nature (1970) 227: 178-179.

A4
Renal and Hepatic Inactivation of Angiotensin in Rats: Influence of Sodium Balance and Renal Artery Compression: WP Leary, JGG Ledingham. Clinical Science (1970) 38: 573-582.

A5
Metabolism of Angiotensin I in the Pulmonary Circulation: JW Ryan, JM Stewart, WP Leary, JG Ledingham. Biochem J. (1970) 120: 221-223.

A6
In Situ Perfusion of Isolated Rat Lung: WP Leary, U Smith. Life Sciences (1970) 9 (1): 1321-1326.

A7
Catabolism of Angiotensin II: JG Ledingham, WP Leary. "Angiotensin". Ed. I. Page & M. Bumpus. Berlin: Springer Verlag (1974) 111-125.

A8
Impaired Prostaglandin Release from the Kidneys of Salt-Loaded and Hypertensive Rats: WP Leary, JG Ledingham, JR Vane. Prostaglandins (1974) 7: 425-432.

A9
Pulmonary Inactivation of Prostaglandin by Hypertensive Rats: WP Leary, AC Asmal, J Botha. Prostaglandins (1977) 13: 697-700.

A10
Enhanced Release of 'PGI_2-Like" Substance in Experimental Hypertension: JH Botha, WP Leary, AC Asmal. Prostaglandins and Medicine (1979) 3: 251-252.

A11
Enhanced Release of a 'Prostacyclin-Like' Substance from Aortic Strips of Spontaneously Hypertensive Rats: JH Botha, WP Leary, AC Asmal. Prostaglandins (1980) 19 (2): 285-289.

A12
Mechanical Reduction in Pressure and Pulse Pressure Decreases the Ability of Hypertensive Rat Aortas to Produce "PGI_2-Like" Activity: JH Botha, WP Leary. Prostaglandins and Medicine (1981) 6: 267-26.

A13
Platelets of Spontaneously Hypertensive Rats are not Abnormally Sensitive to PGI_2: JH Botha, WP Leary. Prostaglandins and Medicine (1981) 7: 499-500.

Thirteen original papers reporting basic laboratory studies are presented. The experiments described were designed to assess whether imbalances between naturally occurring vasodilator and vasoconstrictor substances might be associated with raised arterial pressures in certain animal models for hypertension. This approach is justified by the fact that the basic disorders which account for the occurrence of essential hypertension have yet to be identified, in spite of what is known about the regulation of blood pressure under physiologically normal circumstances and the extensive data which has accumulated in the course of critical studies in hypertensive man and animals over many years.

Since it is known that total peripheral arterial resistance is a fundamental determinant of arterial blood

pressure at any time, it follows that factors which influence resistance might, in turn, be involved in the homeostasis of arterial blood pressure. Naturally occurring vasoconstrictors and vasodilators might, theoretically at least, be counted amongst the factors which modulate arteriolar tonus. The early studies, carried out at Oxford University, concentrated upon the powerful vasoconstrictor angiotensin II which, at that time, was thought to be formed in plasma, notably during passage through the pulmonary circulation, by the action of a converting enzyme upon relatively inert angiotensin I. Subsequent investigations have indicated that angiotensin II formation also occurs locally in some tissues, certainly within the kidney. The Oxford studies examined the hypothesis that reduced enzymatic inactivation of the vasoconstrictor angiotensin II, which is destroyed during circulation through major organs, might contribute to the elevation of arterial blood pressure noted in various animal models for hypertension. This postulate was investigated by isolating certain organs in the rat and perfusing them with solutions containing angiotensin II. The results which emerged were interesting and indicated that the capacity of liver and kidney to inactivate angiotensin II could be quite profoundly altered by inducing hypertension using the one and two-clip Goldblatt methods or by altering the sodium chloride content of the diet. Other papers in this section dealing with angiotensin II describe techniques developed and used and provide an overview of the possible relevance of angiotensin catabolism to hypertension.

Subsequently the possibility that a reduction in the endogenous formation of **vasodilator** prostaglandins might occur in experimental hypertension was investigated. It was found that when comparisons with appropriate controls were made there was a clear reduction in prostaglandinE_2 generation by kidneys of rats given increased dietary sodium and by the ischaemic kidneys of rats which had been subjected to uninephrectomy and clipping of the surviving renal artery. Later work resulted in the finding that the lungs of genetically hypertensive rats inactivate both **constrictor** and **vasodilator** prostaglandins to a lesser extent than the lungs of normotensive controls.

The discovery of PGI_2 (prostacyclin) stimulated further investigations of a similar nature. It was found that aortic strips taken from genetically hypertensive rats as well as those from rats with experimentally induced hypertension generated more of this vasodilator than their controls, possibly representing an adaptive response to hypertension.

The subsequent finding that platelets taken from genetically hypertensive and normotensive rats are equally sensitive to prostacyclin indicated that the increased generation of prostacyclin might reduce platelet aggregation in hypertensive rats, thereby possibly retarding the genesis of degenerative vascular changes.

The relevance of all these findings to human hypertension remains uncertain. If nothing else they may illustrate the great complexity of the interactions involved

in blood pressure control; it appears unlikely that any valid, simple, explanation for the pathogenesis of essential hypertension will soon emerge.

SECTION B

B1
Treatment of Uncomplicated Essential Hypertension with Xipamide: WP Leary, AC Asmal, PC Williams, M Herron. Curr Ther Res (1978) 24 (8): 884-888.

B2
Evaluation of the Efficacy and Safety of Guanabenz versus Clonidine: WP Leary, AC Asmal, PC Williams. S Afr Med J (1979) 55: 83-85.

B3
Treatment of Hypertension with Verapamil: WP Leary, AC Asmal. Curr Ther Res (1979) 25 (5):747-752.

B4
Aldactone and Acebutolol in Treatment of Hypertension: WP Leary, AC Asmal, PC Williams, B. Marwick. J Int Med Res (1979) 7: 29-32.

B5
Antihypertensive Effects of Sotalol and Atenolol Given Once Daily: WP Leary, AC Asmal, P Brayshaw, P Williams. S Afr Med J (1980) 57, 692-695.

B6
Antihypertensive and Metabolic Effects of a Combination of Hydrochlorothiazide and Amiloride: WP Leary, AJ Reyes. S Afr Med J (1981) 60: 381-384.

B7
Effects of Low Doses of Xipamide Given as Monotherapy in Essential Hypertension: WP Leary, AJ Reyes. Curr Ther Res (1983) 34 (5): 888-899.

B8
Effects of a Hydrochlorothiazide and Amiloride Combination on Plasma Magnesium in Patients with Essential Hypertension: WP Leary, K. van der Byl. Mag Bull (1984) 4: 127-132.

B9
Once-Daily Administration of Captopril and Hypotensive Effect: AJ Reyes, WP Leary, TN Acosta-Barrios. J Cardio Pharma (1985) 7: S16-S19.

B10
Captopril Once Daily As Monotherapy in Patients with Hyperuricaemia and Essential Hypertension: WP Leary, AJ Reyes, TN Acosta-Barrios, B Maharaj. Lancet (1985) 1.

This section includes 10 publications describing trials in which hypertensive patients were treated with various medicines.

Under ideal circumstances and before a rational verdict upon the efficacy or safety of a new drug can be given, it is necessary to compare new and established medicines in substantial numbers of patients and in double-blind fashion. This obviously requires preparation of drug formulations and randomisation of patients in such a way that both doctor and patient are unable to distinguish which of the trial formulations is being administered at any time; a code held by another party can be broken in the event of an emergency. This approach removes much of the bias from clinical trials and allows relatively objective comparisons to be made between drugs.

During the early phases of drug testing in man and when a new or innovative approach is made to the use of an established drug it is common practice to maintain an open protocol, largely for ethical reasons. This approach ensures that the medical investigator is aware of the medication being used in each patient throughout the study and that immediate steps can be taken to intervene in the event of any untoward or adverse reaction. The results of such studies

are taken into account in the subsequent planning and design of scientifically objective, double-blind, studies used to test the validity of inferences based upon the preliminary open work.

The first study, although double-blind in the sense that neither the observer nor the patients were aware of the change from placebo to active therapy, was a simple preliminary investigation carried out to determine some characteristics of xipamide, a new antihypertensive diuretic. As reflected by the reference list, this was an early publication on the subject. The second paper describes a fairly typical clinical trial in which guanabenz, a recently developed substance, was compared to clonidine, an established drug of similar character.

Subsequent papers report upon the effects of various other compounds upon raised arterial pressure in man; several are of some interest: Paper B7, "Effects of low doses of xipamide given as monotherapy in essential hypertension", was one of the first studies to confirm earlier claims that antihypertensive diuretics markedly reduce blood pressure when administered at doses well below conventional diuretic dosages. This fact is of potential clinical importance since diuretics are commonly prescribed in antihypertensive regimens and the use of lower doses may well reduce the incidence of medium or long term side-effects of these drugs. Paper B8 records that the addition of amiloride to hydrochlorothiazide does not prevent the fall in plasma

magnesium level induced by hydrochlorothiazide during prolonged treatment, a finding at variance with earlier reports. The following study (B9) explored the theory that once daily treatment with the angiotensin converting enzyme inhibitor captopril would be effective in essential hypertension, particularly if sodium intake was restricted. A further novel finding with respect to captopril is presented as a letter (B10). Studies in healthy volunteers showed that single doses of captopril induced an increase in urinary uric acid excretion (paper C13). This stimulated a prospective study in hypertensive patients with gout which proved that, in the short term at least, captopril may be used to reduce both blood pressure and serum urate levels. Given the frequency with which hypertension and gout coexist, the potential significance of this finding is obvious, although it needs to be confirmed by prolonged studies.

SECTION C

C1
Comparative Effects of Xipamide, Furosemide and Hydrochlorothiazide in Healthy Adults: WP Leary, AC Asmal. Curr Ther Res (1978) 24 (6): 662-672.

C2
Xipamide: Diuretic Effects of Low Dosage in Healthy Adults: WP Leary, AC Asmal. Curr Ther Res (1978) 24 (6): 656-661.

C3
Urine Volumes and Flows after Oral Administration of Xipamide, Furosemide and Hydrochlorothiazide to Healthy Adults: AJ Reyes, WP Leary, AC Asmal. Curr Ther Res (1980) 28 (2): 230-234.

C4
A Mathematical Model for the Clinical Pharmacology of Diuretics: AJ Reyes, WP Leary. Curr Ther Res (1981) 30 (2): 227-235.

C5
A Formal Method for the Therapeutic Classification of Antihypertensive Diuretics: AJ Reyes, WP Leary. Curr Ther Res (1981) 30 (6): 1073-1088.

C6
Mathematical Evaluation of the Effects of Tizolemide, Furosemide and Placebo in Healthy Adults: WP Leary, AJ Reyes. S Afr Med J (1982) 61, 398-401.

C7
Urinary Magnesium and Zinc Excretions after Monodosing Healthy Volunteers with Chlorothalidone: AJ Reyes, WP Leary. Curr Ther Res (1982) 32 (1): 128-137.

C8
The Magnesiuric Effect of a Single Dose of Furosemide in Healthy Adults: AJ Reyes, WP Leary. Curr Ther Res (1982) 32 (3): 406-416.

C9
The Magnesiuric Effect of a Single Dose of Hydrochlorothiazide in Healthy Adults: WP Leary, AJ Reyes. Curr Ther Res (1982) 32 (3): 425-430.

C10
Urinary Magnesium and Zinc Excretions after Two Different Single Doses of Amiloride in Healthy Adults: WP Leary, AJ Reyes, K van der Byl. Curr Ther Res (1983) 34 (1): 205-216.

C11
Effects of Hydrochlorothiazide Plus Sotalol on Acute Urinary Electrolyte Excretion in Normal Subjects: WP Leary, AJ Reyes, K van der Byl. S Afr Med J (1984) 66, 680-681.

C12
Effects of Several Diuretic Formulations on Urinary Magnesium Output in Healthy Adults: WP Leary, AJ Reyes. Diuretics Jules B. Puschett (Elsevier Science Publishing Co., Inc.) (1984): 494-496.

C13
Effects of Captopril, Hydrochlorothiazide, and their Combination on Timed Urinary Excretions of Water and Solutes: WP Leary, AJ Reyes, K van der Byl, TN Acosta-Barrios. J Cardio Pharma (1985) 7 : S56-S62.

C14
The Effects of Single Doses of Muzolimine Upon Urinary Solute and Fluid Excretion: WP Leary, AJ Reyes, K van der Byl. Z Kardiol (1985) 74 (2): 135-140.

Fourteen publications are submitted, each describing certain pharmacological effects of diuretics. Diuretics are used by many clinicians throughout the world as medications of first choice in the treatment of hypertension. They are relatively cheap and, in negro patients at least, appear to be as effective as more expensive alternatives. Those who prefer to initiate treatment with a beta-receptor-antagonist or calcium channel blocker generally add an antihypertensive diuretic to the treatment regimen as a second step in managing patients who do not respond to monotherapy with the first-line drug used. Since diuretics occupy such an important place in the treatment of hypertension it is essential that all their characteristics be described in detail and that the possible adverse effects of both short-term and prolonged therapy should be identified.

Paper C1 reports upon a simple study comparing the responses of healthy volunteers to standard diuretic doses of furosemide (40mg), an established loop diuretic, and xipamide (20,40,60mg), a compound just introduced into medical practice at that time. A contemporary study by the same authors (C2) proved quite clearly that far lower doses of xipamide (5,10mg) could produce a satisfactory diuretic effect; it is unfortunate that this finding was largely

ignored at the time, particularly in the light of the subsequent finding that low doses of xipamide effectively reduce raised blood pressure (B7). The drug is still commonly prescribed in doses of 20-40mg daily.

Papers C3-C5 introduce the well-established concept of mathematical modelling to analysis of the diuretic and antihypertensive effects of drugs. The particular value of this method of presenting data is that it provides a relatively simple and accurate means of comparing the results of experiments involving different drugs, patients and investigators, provided that the protocols followed have certain similarities.

Papers C6-C11 deal with the urinary excretions of electrolytes in response to a variety of commonly prescribed diuretics. All the formulations studied are widely prescribed as monotherapy or in combination with other drugs for the treatment of hypertension. The effects that these compounds have upon plasma and tissue levels of ions such as sodium, potassium, magnesium, calcium and zinc may have an important bearing upon both serious and trivial adverse responses to prolonged therapy with diuretics.

The analytical technique employed is novel and has provided new insights into the excretion of certain electrolytes, notably magnesium. Since magnesium depletion appears to increase arterial tone and therefore elevate blood pressure it is important to determine the effects which antihypertensive regimens may have upon magnesium balance. Despite the fact that this work has illustrated apparent

differences between drugs, it has the weakness that results generated by acute studies in healthy volunteers cannot readily be accurately extrapolated to clinical situations in which the same medicines are administered chronically to patients with essential hypertension.

SECTION D

D1
Cardiovascular Effects of Salbutamol: A Comparison with Isoprenaline: AJ Coleman, WP Leary. S Afr Med J (1972) 46: 1177-1179.

D2
The Immediate Cardiovascular Effects of Pancuronium, Alcuronium and Tubocurarine in Man: AJ Coleman, JW Downing, WP Leary, DG Moyes, M Styles. Anaesthesia (1972) 27 (4): 415-422.

D3
The Immediate Cardiovascular Effects of Althesin (Glaxo CT 1341), a Steroid Induction Agent, and Thiopentone in Man: AJ Coleman, JW Downing, WP Leary, DG Moyes, M Styles. Anaesthesia (1972) 27 (4): 373-378.

D4
Cardiovascular Effects of Acebutolol (M & B 17803A), in Exercising Man; A Comparative Study with Practolol and Propranolol: AJ Coleman, WP Leary. Curr Ther Res (1972) 14 (10): 673-678.

D5
Some Hemodynamic Effects of Sodium Nitroprusside: M Styles, AJ Coleman, WP Leary. Anaesthesiology (1973) 38 (2): 173-176.

D6
Isoproterenol Blockade in Man: Comparison Between Penbutalol and Acebutolol: AJ Coleman, WP Leary, AC Asmal. Curr Ther Res (1974) 16 (1): 64-79.

D7
The Cardiovascular Effects of Etilefrine: AJ Coleman, WP Leary, AC Asmal. Europ J Clin Pharmacol (1975) 8: 41-45.

D8
Cardiovascular Effects of Intravenous Indoramine Hydrochloride in Man: AJ Coleman, WP Leary, AC Asmal. J Int Med Res (1979) 7: 511-518.

The drugs described in these papers all have some effect upon the cardiovascular system with resultant changes in arterial blood pressure. The medicines investigated are primarily used in the management of general anaesthesia, bronchial asthma, angina pectoris and also in hypertension per se. Clinically significant changes in blood pressure, cardiac output, pulse rate or peripheral resistance occur when these medicines are administered to patients with healthy cardiovascular systems and it is important that medical practitioners be aware of the possible occurrence of such changes both in normotensive and hypertensive patients. The same technique involving dye-dilution as a direct means of measuring cardiac output was used in all the studies included in this section of the submission. Each contributed new knowledge and represented an early investigation of the drug's cardiovascular effects **in man.**

SECTION E

E1
The Effects of Adrenergic B-blockade with Oxprenolol on Peripheral Metabolism: AC Asmal, AJ Coleman, WP Leary. Postgrad Med J (1975) 51: 173-177.

E2
Immediate Metabolic Effects of Adrenergic Beta-Blockade: WP Leary, AC Asmal, J Carboni, S. Wattrus. Pharmatherapeutica (1978) 2 (2): 70-75.

The ancillary or secondary effects of medicines may have a profound influence upon their overall efficacy and safety. Thus for example, the fact that beta-receptor antagonists influence a number of metabolic variables is of some importance when hypertensive patients with metabolic disorders such as diabetes mellitus are treated. The two papers presented report some peripheral metabolic effects associated with beta blockade by oxprenolol (E1) acebutolol, propranolol or practolol (E2). The effects of beta stimulation by isoprenaline were also assessed.

SECTION F

F1
Diuretics: WP Leary, AJ Reyes. S Afr Med J (1981) 59: 9-13.

F2
Diuretics, Magnesium, Potassium and Sodium: WP Leary, AJ Reyes. S Afr Med J (1982) 61: 279-280.

F3
Diuretics and Zinc: AJ Reyes, WP Leary, CJ Lockett, L Alcocer. S Afr Med J (1982) 62: 373-375.

F4
Magnesium Deficiency Provoked by Diuretics: AJ Reyes, WP Leary. S Afr Med J (1983) 63: 410-412.

F5
Prophylaxis and Treatment of Magnesium Depletion: WP Leary, AJ Reyes. S Afr Med J (1983) 64: 281-282.

F6
Magnesium and Sudden Death: WP Leary, AJ Reyes. S Afr Med J (1983) 64: 697-698.

F7
Magnesium and Deaths Ascribed to Ischaemic Heart Disease in South Africa: WP Leary, AJ Reyes, CJ Lockett, DD Arbuckle, K van der Byl. S Afr Med J (1983) 64: 775-776.

F8
Urinary Zinc Excretion, Diuretics, Zinc Deficiency and Some Side-Effects of Diuretics: AJ Reyes, JV Olhaberry, WP Leary, CJ Lockett, K van der Byl. S Afr Med J (1983) 64: 936-941.

F9
Drug Interactions with Diuretics: WP Leary, AJ Reyes. S Afr Med J (1984) 65: 455-461.

F10
The Antihypertensive Effect of Diuretics: AJ Reyes, WP Leary. Cont Med Educ (1984) 2: 85-93.

F11
Diuretic-Induced Magnesium Losses: WP Leary, AJ Reyes. Drugs (1984) 28 (1): 182-187.

F12
Cardiovascular Toxicity of Diuretics Related to Magnesium Depletion: AJ Reyes, WP Leary. Human Toxicol (1984) 3: 351-372.

Papers included in this section review aspects of the actions and uses of diuretics, medicines which are commonly prescribed in the management of hypertension.

The initial review provides a simple classification of these drugs and briefly discusses their pharmacokinetics, adverse reactions, interactions with other drugs and clinical uses. As in the subsequent reviews, reference is made to the authors' own work. The second brief review (F2) concentrates upon the effects diuretics have upon urinary sodium, potassium and magnesium excretions. F3 provides an insight into urinary zinc losses which may complicate the treatment of hypertension with certain diuretics.

Papers F4-F7 provide an overview of the theoretical relationship between diuretics, magnesium deficiency and ischaemic heart disease and could, logically, have formed a single paper; limited journal space resulted in division of

this work into 4 short articles. Paper F8 is complimentary to F3 but provides a much more detailed account of the relationship between zinc and diuretics. A significant proportion of the data presented was collected and analysed by the authors.

The two reviews which follow (F9, F10) deal in some detail with the antihypertensive effects of diuretics and with potential adverse or beneficial interactions which could take place between diuretics and other drugs, many of which might be coprescribed to hypertensive patients. The last two papers in this section (F11, F12) are similar in content. Both deal with the possible importance of urinary magnesium losses a consequence of prolonged diuretic administration to hypertensive patients. Much of the authors' original work is included in these reviews, which summarise many of the ideas presented in the shorter papers in this section. The suggestion is made that the failure of treatment with diuretics to reduce the incidence of sudden cardiac deaths in patients with hypertension might be explained by potassium and magnesium imbalances caused by prolonged treatment with these compounds.

SECTION G

G1
Tratamiento De La Hipertension: La Farmacologia De Los Agentes Vasoactivos: WP Leary. Medicina (1977) 37 (1): 102-109.

G2
Effectos Colaterales Adversos En El Tratamiento De La Hipertension Humana: WP Leary. Medicina (1979) 39 (1): 100-105.

The last 2 review articles in this submission are presented as light relief and to give some indication of the global interest which exists in the treatment of hypertension.

SECTION A : LABORATORY STUDIES, EXPERIMENTAL HYPERTENSION

The publications in this section deal with aspects of the renin-angiotensin system and of some prostanoid substances as they relate to experimental hypertension in animals.

The role played by the renin-angiotensin system in the cause, pathogenesis, prognosis and treatment of raised arterial pressure has been controversial for many years and continues to be the subject of intense investigation. Many studies, including those presented here, suggest that derangements of this system undoubtedly are of some importance, at least in experimental models of hypertension.

The finding that certain endogenous prostanoid substances, including prostacyclin, are vasodilators and may therefore be involved in the homeostasis of arterial pressure gave rise to the obvious hypothesis that a reduction in the synthesis of prostanoids might contribute to the development of hypertension. The findings in several of the studies presented here have been confirmed subsequently in man.

PAPER A1

Pressor Response to Infusion of Angiotensin into the Portal Vein of the Rat

This work was based on previously published investigations by quoted authors. I personally carried out all laboratory experiments. The paper was prepared in cooperation with Dr. J.G. Ledingham.

(*Reprinted from Nature, Vol.* 220, *No.* 5163, *pp.* 180–181, *October* 12, 1968)

Pressor Response to Infusion of Angiotensin into the Portal Vein of the Rat

THE physiological importance of enzymes that degrade angiotensin in plasma and in tissue extracts has been questioned by Johnson and Ryan[1]. They have shown that aqueous extracts of rabbit liver hydrolyse all the peptide bonds of Asp[1]-Ile[5]-angiotensin II amide and Asp[1]-Val[5]-angiotensin amide when incubated at *p*H 7·4 and 37° C. There is also evidence that the liver plays an active part in the destruction of angiotensin *in vivo*[2–5]. Bumpus *et al.*[2] have shown that the liver of nephrectomized rats infused with tritiated angiotensin accumulates more labelled peptide fragments than do other organs. Chamberlain *et al.*[3] found a smaller rise in the blood pressure of four dogs and two humans when Asp[1]-Val[5]-angiotensin amide was infused into the portal venous system than when it was infused into the femoral vein. It is difficult to interpret their results because full details of the techniques used are lacking, and it is not clear how the doses were matched against each other. Hodge *et al.*[4] have measured concentrations of angiotensin in circulating blood both before and after passage through various tissues in anaesthetized dogs. They find a marked decrease in the concentration of angiotensin after passage through the liver, as shown by the change in response of the isolated gut preparations used to assay angiotensin in their experiments.

In 1964, Methot *et al.*[5] reported that angiotensin caused a much smaller rise in the arterial pressure of Wistar rats when infused into the hepatic portal system than when infused into the femoral artery or the external jugular vein. We have repeated this work using smaller doses of angiotensin and have introduced some additional modifications to their technique.

Twenty Sprague–Dawley rats (eleven female and nine male) on mixed diets were used; weights ranged from 160 to 230 g (mean 200 g). Each rat was anaesthetized with intraperitoneal pentobarbitone (veterinary Nembutal, Abbott), 60 mg/kg, and given a subcutaneous injection of 2·5 mg/kg of pentolinium tartrate in polyvinyl-pyrrolidone (Ansolysen Retard, May and Baker) and atropine sulphate (1·5 mg/kg).

After a small flap of skin had been removed from the anterior aspect of the throat, a glass cannula was introduced into the trachea and the left external jugular vein and right common carotid artery were mobilized. A midline abdominal incision was made and the gut was drawn

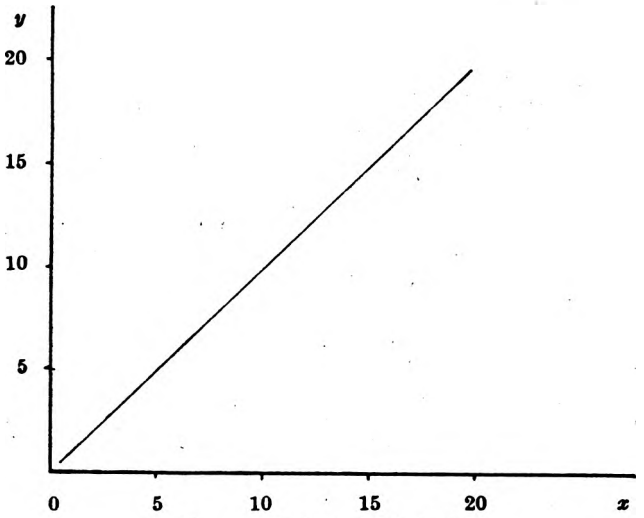

Fig. 1. Explanation in text.

to one side and covered with a gauze swab saturated with warm saline. The portal vein was opened and a polythene cannula was introduced and tied into place. The abdominal wound was either closed with surgical clips (three experiments) or covered with damp swabs. If any difficulty was experienced in setting up the preparation, the particular experiment was abandoned and a fresh one was prepared.

The external jugular vein and carotid artery were cannulated and the arterial cannula was connected to a Condon type manometer, recording the arterial pressure changes on a kymograph. Heparin (2,000 U/kg) was injected into the jugular vein before each experiment was continued.

Asp^1-Ile^5-angiotensin II was prepared in concentrations of 0·1 μg/ml. and 1·0 μg/ml., in sterile 0·9 per cent sodium chloride, *p*H 5·7. Injections of angiotensin were washed in with 0·2 ml. of 0·9 per cent sodium chloride, over a period of 10–20 s.

In eleven rats the pressor response to every infusion of angiotensin into the hepatic portal system was compared with infusions of two different strengths into the external jugular vein ("bracket assay", see Figs. 1 and 2). In nine rats various doses of angiotensin were infused and the pressor responses were measured, but a precise quantitative comparison was not made. In every experiment the smaller doses of angiotensin were given first and no doses in excess of 40 ng were given until the responses to the smaller doses had been assessed.

At the end of each experiment a suspension of carbon particles ('Statex *E*-12') was injected through the portal vein cannula. The liver was then examined and the perfused lobes were weighed.

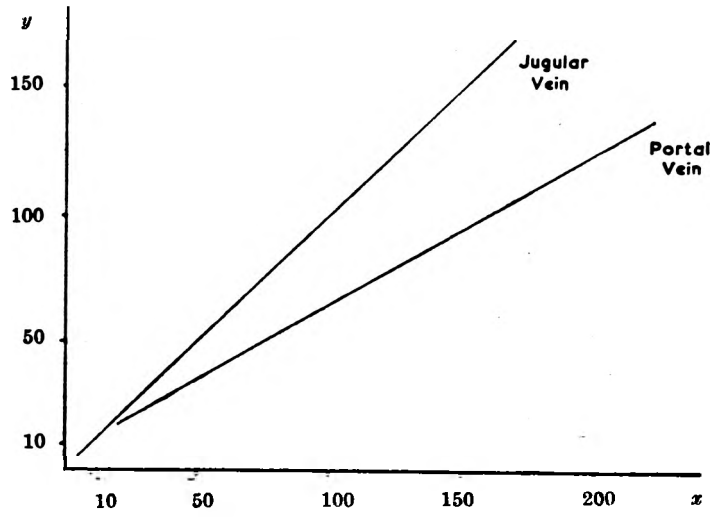

Fig. 2. Explanation in text.

Figs. 1 and 2 were prepared from the data collected in the first eleven experiments. The y axis represents the dose of angiotensin (ng) required to produce a given pressor response when infused into the external jugular vein; the values on the x axis are those of doses infused into the portal vein to produce an equivalent response. Fig. 1 shows that there is no difference between the response to infusions into the portal vein and the external jugular vein in the range of doses used (5–15 ng). Fig. 2 demonstrates that when doses of 20 ng or more are infused a marked difference appears ($P = 0{\cdot}001$).

Fig. 3 illustrates the pressor response obtained when doses of 5–80 ng were infused into the portal and jugular veins. The doses (y) are expressed logarithmically and the pressor response (x) in mm of mercury. There is a significant difference between the two dose response curves obtained ($P = 0{\cdot}01$).

Fig. 4 provides an example of pressor responses recorded on a kymograph after infusions of different strengths.

Methot *et al.*[5] suggest that the liver, by virtue of angiotensinase activity, plays a specific and important part in the metabolic destruction of circulating angiotensin. The strain of rat, the anaesthetic, the type of angiotensin and the *p*H of the infused solution were different in our work. In spite of these differences, we were able to confirm that when large doses of angiotensin were used the pressor response in the portal vein was relatively less than in the jugular vein: here there is no conflict. When smaller doses were used, the pressor responses were identical. This observation does not support the view that the liver usually plays any specific part in the de-

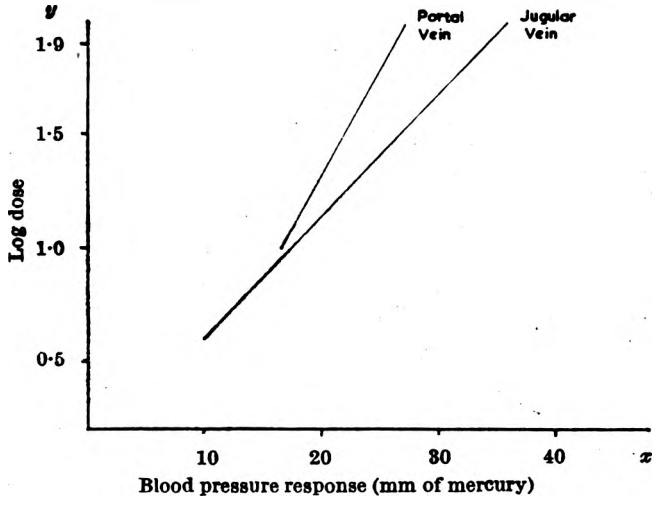

Fig. 3. Explanation in text.

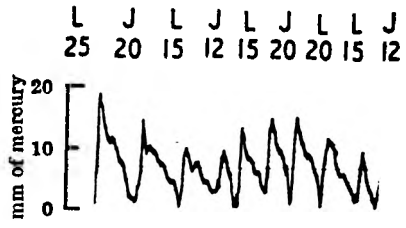

Fig. 4. Blood pressure response to infusions of angiotensin II into the hepatic portal vein (L) and external jugular vein (J). The figures 12 to 20 indicate the dose of angiotensin infused (mμg). It can be seen that 15 mμg angiotensin (L) has a pressor effect of between 12 and 20 ng (J).

struction of circulating angiotensin. Larger doses were observed to cause marked blanching of the liver in our preparations. Damage to hepatic cells with release of intracellular peptidases could explain the loss of pressor activity observed and this possibility is now being explored.

We thank Sir George Pickering for facilities and for encouragement, and Dr A. J. Honour for helpful advice. Financial assistance was provided by the Nuffield Foundation and the CSIR, Pretoria. Supplies of angiotensin were from Drs Riniker and Gross of Ciba.

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PAPER A2

Removal of Angiotensin by Isolated Perfused Organs of the Rat

The techniques used were developed in consultation with Professor H. Krebs and Dr. H.F. Woods. Every experiment was carried out by me and the publication was prepared with considerable contributions by Dr. J.G. Ledingham, who was also available for consultation throughout the period of experimentation.

(*Reprinted from Nature, Vol. 222, No. 5197, pp. 959–960, June 7, 1969*)

Removal of Angiotensin by Isolated Perfused Organs of the Rat

Tissue peptidases are the enzymes chiefly responsible for the removal of angiotensin II in rat liver and kidney

by

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KNOWLEDGE of metabolic processes in individual organs has grown considerably since the introduction of the techniques of isolated perfusion. We have applied these techniques to the study of angiotensin removal in the rat.

We used female Sprague–Dawley animals, weighing 180 to 250 g, fed on a standard small animal diet (Spillers Ltd, Banbury, England), and given unrestricted access to water. Rats were anaesthetized by intraperitoneal injection of pentobarbitone (Veterinary Nembutal: Abbott), 60 mg/kg. Perfusion of the liver and kidney was performed as described by Hems *et al.*[1] and Nishiitsutsuje-Uwo *et al.*[2] with some modifications.

A technique for the perfusion of rat lung was developed (Woods and W. P. L., unpublished work) in which the chest of an anaesthetized rat was opened and small bags of crushed ice applied to the lungs and heart. The inferior vena cava was tied and a nylon catheter introduced into the cavity of the right ventricle through the superior vena cava and secured by a ligature around the right atrio-ventricular groove. A catheter was placed in the left ventricle by way of the ascending aorta which was then tied off. Medium was circulated through the right heart catheter and the 5 to 10 ml. of blood washed out in this way was discarded before perfusion was started using the same medium and apparatus that is used in liver perfusion. All glass surfaces were coated with silicone. No more than 10 min elapsed from thoracotomy to lung perfusion with angiotensin.

All three organs were perfused *in situ* and Asn[1] Val[5] Angiotensin II was added to the medium to provide an

accurately measurable concentration of 133 ng per ml. Organs were perfused at rates ranging from 2 to 4 ml./g of tissue per min. In the case of liver perfusions the effect of adding washed aged human red cells, rat red cells and concentrations of angiotensin of up to 1,100 ng per ml. was assessed. The time course of angiotensin II destruction by medium alone and by perfused liver, kidney or lung was determined by bioassay of serial samples of perfusate using techniques previously described[3,4]. Destruction was expressed in ng of angiotensin per g wet weight of tissue and corrected to a standard flow rate. Perfused organs did not produce detectable pressor or depressor substances, nor did circulating medium destroy angiotensin in the absence of liver or kidney. The addition of washed aged human red cells or rat red cells did not alter the angiotensinase activity of the medium, nor of the perfused organs.

Three other methods of assessing angiotensin destruction in vascular beds have been described[5–7]. The techniques favoured by Biron and his colleagues have drawbacks which the authors themselves have emphasized[8]. The method of Regoli and Vane[6], involving superfusion of a series of assay organs, is perhaps the most physiological technique available, but it is complex.

The techniques of Hems *et al.*[1] and Nishiitsutsuje-Uwo *et al.*[2] can be quickly mastered and the apparatus required is simple. A perfusion can be set up and an experiment completed in less than 1 h. Results are remarkably reproducible so that small numbers of experiments produce definitive answers. Pharmacologically active substances can be added or subtracted at will.

Rates of destruction of angiotensin II are shown in Table 1. The inability of the lung to extract angiotensin II has previously been noted[8,9]. Survival of the peptide recirculating through the lung for up to 10 min adds weight to the view that this tissue bed plays no important part in angiotensin II disappearance *in vivo*.

The perfused liver and kidney both extracted angiotensin II rapidly, depending on the amount of peptide added. At a concentration of 133 ng per ml. the kidney removed angiotensin II at a faster rate than the liver ($P < 0{\cdot}001$). From 0·5 to 1 per cent of the total angio-

Table 1. DISAPPEARANCE OF ASN[1] VAL[5] ANGIOTENSIN II ± STANDARD ERROR OF THE MEAN IN THE FIRST 6 MIN OF PERFUSION

Organ	Angiotensin concentration (ng/ml.)	Inhibitors added	Angiotensin loss (ng/g/min)	No. of experiments
Lung	133	—	No loss detected	3
Liver	67	—	238·9 ± 11·6	14
	67	Edetic acid 3×10^{-3} M	174·8 ± 12·9	4
	133	—	498·1 ± 5·1	5
	133	Dimercaprol 4×10^{-4} M	No loss detected	2
	1,100	—	4,475·5 ± 130·37	2
Kidney	133	—	841·4 ± 36·5	8
	133	Edetic acid 3×10^{-3} M	389·6 ± 80·7	4

tensin II lost could be detected as vasoactive substance in the urine collected during kidney perfusions. It was not possible to determine the concentration of inactive peptide fragments. The mechanism of the disappearance of angiotensin II in the liver and kidney is not known. β Aspartyl-angiotensin II has been found resistant to the action of plasma aminopeptidases, but is lost in tissues at the same rate as its α analogue[8]. Such loss could be caused by tissue enzymes distinct from those identified in plasma or, as suggested by Biron, to storage at receptor sites. The destruction of β aspartyl-angiotensin II reported by Biron *et al.*[8] had been previously investigated with conflicting results[10]. Edetic acid and dimercaprol are potent inhibitors of angiotensin destroying enzymes[11]. Addition of these compounds to the perfusing medium resulted in significant reduction in the loss of angiotensin II suggesting a dominant if not exclusive role for tissue peptidases.

The techniques that we have used provide a simple and reproducible means of examining organ destruction of angiotensin in a variety of situations. Preliminary work[12] has indicated that the rate of angiotensin disappearance can be altered profoundly by changes in sodium balance or blood pressure in albino rats.

We thank Sir G. Pickering and Sir H. Krebs for making facilities available and Drs H. F. Woods, A. J. Honour and D. Hems for technical instruction and advice. W. P. L. is a Nuffield Dominions Fellow and is also grateful to the CSIR, Pretoria, for their support.

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PAPER A3

Inactivation of Angiotensin II Analogues by Isolated Perfused Rat Liver and Kidney

The techniques used were developed in consultation with Professor H. Krebs and Dr. H.F. Woods. Every experiment was carried out by me and the publication was prepared with many contributions by Dr. J.G. Ledingham, who was also available for consultation throughout the period of experimentation.

(Reprinted from Nature, Vol. 227, No. 5254, pp. 178–179, July 11, 1970)

Inactivation of Angiotensin II Analogues by Isolated Perfused Rat Liver and Kidney

CHANGES in the activity of enzymes which destroy angiotensin may be concerned in the development of hypertension, but many relevant studies have involved tissue homogenates[1–7] which may not reflect accurately the capacity of intact organs to remove circulating angiotensin. Intracellular enzymes, that normally have limited or no access to perfusing blood, are released when tissue extracts are prepared. Others[8–11], however, recognizing this problem, investigated angiotensin inactivation by perfused organs *in vivo*, and demonstrated the dominant role of organ vascular beds in the inactivation of angiotensin in rats and dogs and the relative unimportance of circulating angiotensinases. A tentative explanation is that tissue enzymes are involved rather than storage of angiotensin at receptor sites (results of W. P. L., J. G. L. and J. W. Ryan, unpublished).

Alterations in sodium balance or the application of a silver clip to one renal artery are followed by changes in the capacity of isolated perfused rat kidneys to inactivate [Asn^1, Val^5]-angiotensin II, but angiotensin inactivation by the isolated perfused rat liver is not so affected[12,13]. We report here similar findings when rat kidney and liver were perfused with medium containing other analogues of angiotensin II.

Isolated rat kidney and liver were perfused as described[13,14]. One group of female Sprague–Dawley rats (Carworth, Alconbury) weighing 180 to 280 g were given a standard small animal diet (Spillers Ltd, Banbury) and unrestricted access to tap water. The estimated daily intake of sodium was 1 mequiv and of potassium 4 mequiv per rat. Another group of similar animals were given the standard diet but tap water was replaced by 1 per cent NaCl–0·2 per cent KCl in water. The daily intake for each rat was approximately 11 mequiv Na^+ and 5 mequiv K^+. For kidney perfusion, [Asn^1, Val^5]-angiotensin II, β-[Asp^1, Val^5]-angiotensin II, α-[Asp^1, Val^5]-angiotensin II, and α-[Asp^1, Ile^5]-angiotensin II were used at a substrate concentration of 133 ng/ml. medium. For liver perfusion, the [Asn^1, Val^5]-angiotensin II and β-[Asp^1, Val^5]-angiotensin II analogues were used.

Experimental conditions were maintained for at least 2 weeks before perfusion and organs from the two experimental groups were perfused with different angiotensin analogues in random order to minimize the influence of chance factors or improvements in technical skill. The rate of angiotensin destruction was determined as described[13,14] and expressed as ng peptide destroyed per g wet weight of perfused organ after 6 min perfusion. The

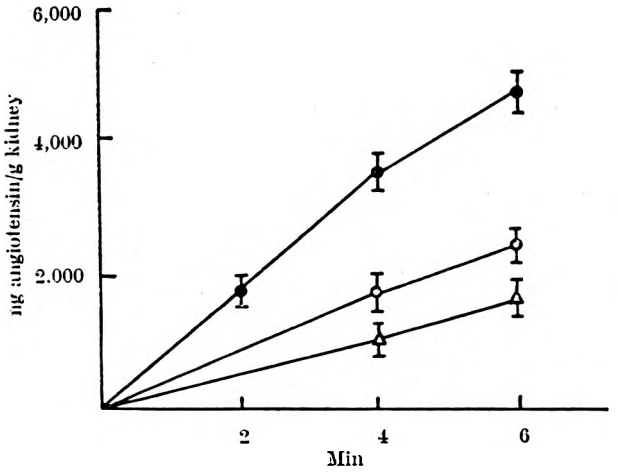

Fig. 1. Kidney perfusion. Inactivation of angiotensin II analogues by rats given the standard diet and tap water. Mean peptide removed at 2, 4 and 6 min (±s.e.) expressed as ng angiotensin/g kidney, corrected for flow rate. ●—●, Perfusions with [Asn¹, Val⁵]-angiotensin II; ○—○ and △—△, those with [Asp¹, Val⁵]-angiotensin II and [Asp¹, Ile⁵]-angiotensin II, respectively.

initial concentration of angiotensin presented to the perfused organs was 133 ng/ml.

Differences in the rates of disappearance of the various analogues during kidney perfusion are documented in Fig. 1 and Table 1. α-[Asp¹, Val⁵]-, α-[Asp¹, Ile⁵]- and [Asn¹, Val⁵]-angiotensin II were all rapidly inactivated, but β-[Asp¹, Val⁵]-angiotensin II was not detectably destroyed after 6 min perfusion. The perfused isolated kidneys of rats drinking saline removed significantly less [Asn¹, Val⁵]-, [Asp¹, Val⁵]- or [Asp¹, Ile⁵]-angiotensin II from the perfusate than kidneys from control animals in group 2 (Table 2, Figs. 2 and 3).

β-[Asp¹, Val⁵]-angiotensin II and [Asn¹, Val⁵]-angiotensin II were inactivated by the isolated liver at similar rates (Table 3). In single experiments it was shown that [Asp¹, Ile⁵]- and [Asp¹, Val⁵]-angiotensin II were also destroyed. An increase in dietary intake of Na^+ did not

Table 1. INACTIVATION OF ANGIOTENSIN II ANALOGUES BY ISOLATED PERFUSED KIDNEYS OF RATS DRINKING TAP WATER

Analogue	Disappearance of peptide after 6 min perfusion, corrected for flow rate: Peptide loss (ng/g kidney) (mean ± s.e.)	No. of experiments
β-[Asp¹, Val⁵]-angiotensin II	No loss detected	5
[Asn¹, Val⁵]-angiotensin II	4,797·7 ± 246·6	10
α-[Asp¹, Val⁵]-angiotensin II	2,447·9 ± 192·3	9
α-[Asp¹, Ile⁵]-angiotensin II	1,665·0 ± 208·4	3

Table 2. INACTIVATION OF ANGIOTENSIN II ANALOGUES BY ISOLATED PERFUSED KIDNEYS OF RATS DRINKING 1 PER CENT SALINE

Analogue	Disappearance of peptide after 6 min perfusion corrected for flow rate: Peptide loss (ng/g kidney) (mean ± s.e.)	No. of experiments
[Asn¹, Val⁵]-angiotensin II	2,694·3 ± 196·9	8
α-[Asp¹, Val⁵]-angiotensin II	917·7 ± 87·5	4
α-[Asp¹, Ile⁵]-angiotensin II	780·8 ± 68·1	3

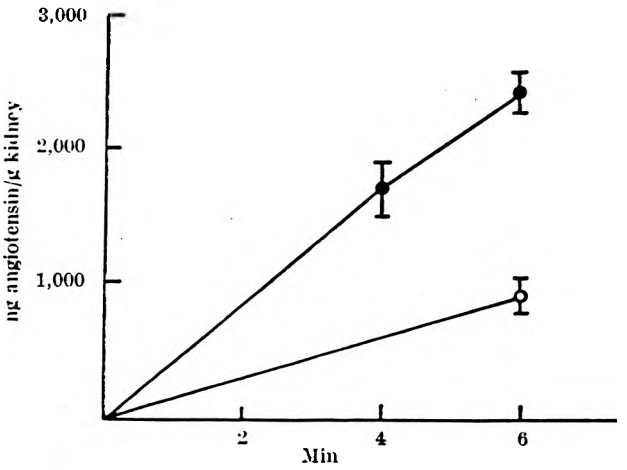

Fig. 2. Kidney perfusion. Effect of increased dietary sodium on inactivation of [Asp¹, Val⁵]-angiotensin II. Mean peptide loss at 4 and 6 min (±s.e.) expressed as ng angiotensin/g kidney, corrected for flow rate. ●—● and ○—○, animals drinking tap water and 1 per cent saline respectively.

alter the rate of removal of angiotensin II amide by the isolated perfused liver.

The perfused rat liver inactivates a number of angiotensin analogues at similar rates, suggesting a variety of active enzymes, whereas the kidney destroys α-aspartic acid analogues of angiotensin II at less than half the rate of [Asn¹, Val⁵]-angiotensin II and does not inactivate β-[Asp¹, Val⁵]-angiotensin II. The latter findings resemble those reported in studies of human plasma by Khairallah and Page[15], who identified two distinct aminopeptidases and an endopeptidase. One aminopeptidase (angiotensinase A_1) destroyed only [Asn¹, Val⁵]-angiotensin II and was highly active at *p*H 7·4. The other aminopeptidase (angiotensinase A_2) was relatively inactive at this *p*H and specific for [Asp¹]-angiotensin analogues. Neither of these peptidases destroyed β-angiotensin and both could be inhibited by EDTA. The endopeptidase (angiotensinase B) was similar to that previously described[16]. At *p*H 7·4 it was 10 per cent as active as angiotensinase A_1 and it was not inhibited by EDTA. This peptidase destroyed [Asn¹, Val⁵]-angiotensin II twice as rapidly as other angiotensin analogues. Destruction of angiotensin by peptidases in the rat kidney such as leucine-aminopeptidase, which inactivates [Asn¹, Val⁵]-angiotensin II more rapidly than [Asp¹]-angiotensin II analogues[16], or enzymes similar to those described by Khairallah and Page[15] in human plasma, could explain the results of the kidney perfusion experiments.

The finding that the rate of destruction of angiotensin

Table 3. INACTIVATION OF ANGIOTENSIN II ANALOGUES BY ISOLATED PERFUSED LIVERS OF RATS DRINKING TAP WATER

Analogue	Disappearance of peptide after 6 min of perfusion: Peptide loss (ng/g liver) (mean ± s.e.)	No. of experiments
[Asn¹, Val⁵]-angiotensin II	376·5 ± 78·4	4
β-[Asp¹, Val⁵]-angiotensin II	289·7 ± 32·9	3

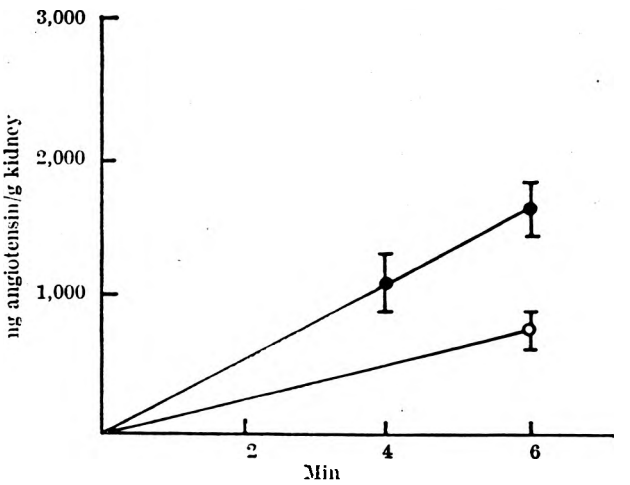

Fig. 3. Kidney perfusion. Effect of increased dietary sodium on inactivation of [Asp¹, Ile⁵]-angiotensin II. Mean peptide loss at 4 and 6 (±s.e.) expressed as ng angiotensin/g kidney, corrected for flow rate. ●—● and ○—○, rats drinking tap water and 1 per cent saline respectively.

by the isolated rat kidney is altered by changes in sodium balance has implications in the understanding of the renin–angiotensin–aldosterone system[13]. The effect of sodium loading is as clearly seen with naturally occurring analogues as with the synthetic compound, which further implicates renal angiotensinase in the regulation of tissue levels of angiotensin in the kidney.

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PAPER A4

Renal and Hepatic Inactivation of Angiotensin in Rats: Influence of Sodium Balance and Renal Artery Compression

The techniques used were developed in consultation with Professor H. Krebs and Dr. H.F. Woods. Every experiment was carried out by me and the publication was prepared in consultation with Dr. J.G. Ledingham.

Clinical Science (1970) **38**, 573–582.

RENAL AND HEPATIC INACTIVATION OF ANGIOTENSIN IN RATS: INFLUENCE OF SODIUM BALANCE AND RENAL ARTERY COMPRESSION

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(*Received* 26 *September* 1969)

SUMMARY

1. Rates of inactivation of asn^{1}val^5 angiotensin II by isolated perfused rat liver and kidney have been investigated in a variety of experimental conditions.

2. The liver inactivates angiotensin at a rate independent of sodium balance.

3. Sodium loading reduces and sodium depletion enhances the capacity of the kidney to remove angiotensin.

4. In the presence of a silver clip on one renal artery with contralateral kidney intact, the clipped organ destroys angiotensin at a normal rate, and the capacity of the contralateral intact kidney to inactivate the peptide is reduced.

5. The kidney with its renal artery constricted by a silver clip, when the contralateral kidney has been removed, has a reduced ability to destroy angiotensin.

Many attempts have been made to associate increases in blood pressure in experimental or naturally occurring hypertension with changes observed in the renin–angiotensin–aldosterone system. Repeated measurements have been made of kidney renin content, circulating renin and angiotensin and of aldosterone metabolism, but to date the evidence that any, or all, of these factors are critically involved remains conflicting (Page & McCubbin, 1968; Pickering, 1968; Lee, 1969). The possibility that changes in the activities of enzymes destroying angiotensin might be concerned in the development of high blood pressure has been considered previously in studies relating to the disappearance of the peptide in plasma or in tissue homogenates (Dexter, 1942; Bing, 1962; Hickler, Lauler & Thorne, 1963; Lagrue & Meyer, 1963; Biron, Landesman & Hunt, 1964; Birbari & Hickler, 1965; Itskovitz & Miller, 1966; Itskovitz, Dudrick & Dyrda, 1967). The results of these studies are of doubtful significance. The capacity of organs to destroy angiotensin is unlikely to be reflected accurately by work using tissue homogenates (Johnson & Ryan, 1968).

Vane and his colleagues have recently demonstrated the importance of organ vascular beds

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in the inactivation of angiotensin *in vivo* and the relative unimportance of circulating angiotensinases (Vane, 1969). Vane's observations have been supported by Biron, Meyer & Pannisset (1968) and by Leary & Ledingham (1969). It is critical therefore to determine whether or not manoeuvres known to alter electrolyte balance or blood pressure can simultaneously alter the capacity of individual organs to inactivate angiotensin. This point has been investigated using the techniques of isolated organ perfusion in the rat.

METHODS

Female Sprague-Dawley rats in a weight range of 180–250 g were supplied by Carworth Europe, Alconbury, England.

Perfusion techniques

Perfusion of the liver was performed as described by Hems, Ross, Berry & Krebs (1966) and of the kidney by the method of Nishiitsutsuji-Uwo, Ross & Krebs (1967) with certain modifications. Both the organs were perfused *in situ* for periods of not less than 6 min and were isolated by ties placed around appropriate vessels. Rates of perfusion were measured in each experiment and varied between 3·5 and 9 ml min^{-1} g $kidney^{-1}$ or 13 and 20 ml/g liver. No attempt was made to regulate perfusion rates outside these limits. Livers were perfused at a constant hydrostatic pressure of 23 cm water, and kidneys by pulsatile flow at pressures adjusted to 120–200/60–100 mmHg. Details of anaesthetic, perfusion medium, apparatus used and angiotensin bioassay have been previously described (Leary & Ledingham, 1969; Hems *et al.*, 1966; Nishiitsutsuji-Uwo *et al.*, 1967). By these techniques adequate oxygen to preserve biochemical function can be provided without added red cells (Ross, 1966). Red cells were therefore omitted from the perfusing medium in this series of experiments.

Addition of angiotensin

In all experiments asparagine^{1}valine^5 angiotensin II (asn^{1}val^{5}angiotensin II) was used. The peptide was added to the medium 3 min before an organ was included in the circuit, allowing time for equilibration to take place. The concentration of angiotensin initially presented to the organ was 133 ng/ml of perfusate. The time course of angiotensin inactivation by medium alone and by perfused kidney or liver was determined by bioassay of serial samples of perfusate taken at 2 min intervals. Destruction of angiotensin was expressed as ng/g wet weight of tissue per min, corrected to a standard flow rate per g wet weight of perfused tissue. Livers were weighed at the end of perfusion. In the case of the kidney the weight of the organ was taken to be that of the unperfused contralateral kidney or, in those experiments when one renal artery was constricted, the ischaemic kidney was weighed after the experiment and a correction made for the change in weight induced by perfusion.

Inactivation of angiotensin II by the isolated perfused liver and/or kidney was studied in groups of rats on the following regimes:

1. Standard small animal diet (Spillers Ltd, Banbury, England) with unrestricted access to tap water. The estimated daily intake of sodium was 1 mEq and of potassium 4 mEq per rat. (Liver perfusion in four and kidney perfusion in ten rats.)
2. Same standard diet with replacement of tap water by 1% sodium chloride solution. Daily

intake of sodium was approximately 11 mEq and of potassium 5 mEq per rat. (Liver perfusion in five and kidney perfusion in eight rats.)

3. Standard diet and 2% sodium chloride solution to drink. Fluid intake was higher in this group and estimated daily sodium intake was 30 mEq, and potassium 4 mEq per rat. (Kidney perfusion in five rats.)

4. Standard diet, unlimited tap water, one renal artery constricted by the application of a silver clip, leaving the contralateral kidney intact. (Liver perfused in three, ischaemic kidney in five and intact kidney in five rats.)

5. Standard diet, tap water or 1% sodium chloride solution to drink, uninephrectomy. (Kidney perfusion in five rats drinking water and five taking 1% saline.)

6. Standard diet, tap water or 1% sodium chloride solution, with hypertension induced by renal artery clip and contralateral nephrectomy. (Liver perfusion in three rats; kidney perfused in five rats taking water and in five taking 1% sodium chloride solution to drink.)

7. Sodium depletion induced by a single peritoneal dialysis with 5% dextrose in water, followed by tap water to drink and a diet modified from that of Struyvenberg, de Graeff & Lameijer (1965) providing approximately 0·2 mEq sodium and 1·3 mEq potassium per rat per day. (Liver perfusion in two and kidney perfusion in seven rats.)

8. Same fluid and diet as Group 7, with supplements of sodium chloride bringing the sodium intake to 5 mEq per rat per day. (Liver perfusion in three and kidney perfusion in seven rats.)

The Struyvenberg diet of Groups 7 and 8 differed from the Spillers diet of Groups 1–6 not only in sodium and potassium content but also in the amount of other ions present, notably calcium.

In each of these groups of rats, constant experimental conditions had been maintained for not less than 2 weeks before perfusion was undertaken. Organs from the eight experimental groups were perfused in random order over a period of 6 months so that any influence of chance factors or improvement in technical performance could be minimized.

The blood pressures of the animals in all groups except 7 and 8 were measured. Conscious animals were warmed to 39° for 10–20 min and systolic pressure was recorded using a pneumatic tail cuff and a distal pressure transducer attached to an oscilloscope (Beilin, Garcia & Blackwell, 1969).

RESULTS

Blood pressure. All five animals in Group 6 were hypertensive (systolic pressure over 150 mmHg) within 2 weeks of surgery. Hypertension also developed in six of the thirteen animals in Group 4, but the other rats in this group were found to have systolic pressures between 110 and 140 mmHg. The blood pressures of animals in the other experimental groups examined were less than 120 mmHg systolic.

Effects of changes in sodium intake

Liver perfusion. After 6 min perfusion, the livers of animals taking 1 mEq sodium per day inactivated from 230·4 to 558·0 ng angiotensin II, mean 376·5 ± 78·4 (SEM) ng. These results were not significantly altered by giving rats 1% sodium chloride solution to drink (range

264·0 to 528·5 ng; mean 359·3±30·1 ng). These results are shown in Fig. 1. The livers of sodium depleted animals inactivated 313·8 to 329·8 ng with a mean of 321·8±8·0 ng which was not significantly different from their sodium replete controls (range 267·2 to 350·3, mean 297·6±26·4 ng).

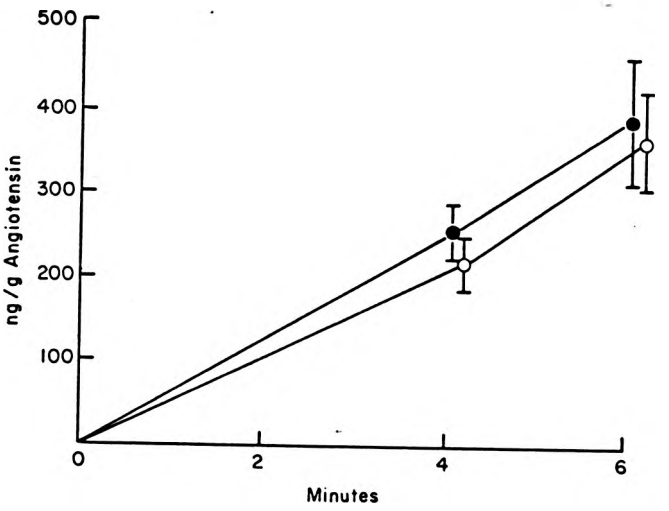

FIG. 1. Liver perfusions: effect of salt loading on inactivation of angiotensin. Inactivation of asn^{1}val^5 angiotensin II ng per g wet weight of tissue±SEM. Open circles represent animals of Group 2 given 1% sodium chloride solution ($n=5$) and closed circles rats of Group 1 taking a normal diet ($n=4$). There was no significant difference in angiotensin loss at 6 min between the two groups ($P>0·45$).

Kidney perfusion. In contrast to the findings in the liver, Figs. 2 and 3 show considerable changes in the rates of inactivation of angiotensin by the kidneys of salt loaded or salt depleted animals compared with controls. The amount of peptide destroyed at 6 min was reduced by almost 50% in those animals which had drunk 1% saline and even more in those given 2% saline (Fig. 2). Renal inactivation of angiotensin by salt depleted animals (Group 7) was significantly more than that by animals from Group 8, whose diet was identical apart from sodium intake (Fig. 3).

It is apparent from Figs. 2 and 3 that kidneys of animals taking 0·2 mEq sodium in the Struyvenberg diet (Group 7) inactivated approximately the same amount of angiotensin as did those of rats on Spiller's diet taking 11 mEq sodium per day (Group 2). The potassium intake was four times greater in animals taking the Spiller's diet than those of Groups 7 and 8 and major differences in the content of other ions including calcium were also present.

Kidney perfusion: effects of renal artery clip

Unilateral clip: contralateral kidney intact. There was no significant difference between the rates of inactivation of angiotensin by kidneys with clipped arteries and kidneys of intact animals. The kidneys contralateral to those with the arterial clip, on the other hand, destroyed

considerably less peptide (Fig. 4). There was no detectable relationship between the presence or absence of hypertension and change in the capacity of the artery-clipped or contralateral kidneys to inactivate angiotensin.

Unilateral clip with contralateral nephrectomy. The effects of uninephrectomy alone were

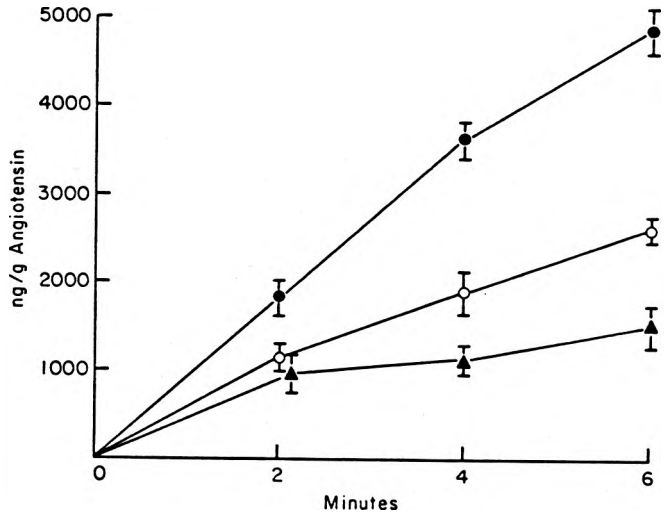

FIG. 2. Kidney perfusions: effect of salt loading on inactivation of angiotensin. Removal of asn^{1}val^5 angiotensin II ng per g wet weight of tissue ± SEM. Closed circles represent control animals of Group 1 taking a normal diet ($n=10$); open circles animals of Group 2 given 1% sodium chloride solution ($n=8$); closed triangles animals of Group 3 given 2% sodium chloride solution ($n=5$). The 6 min loss of angiotensin was significantly decreased in both the saline groups compared with controls ($P<0{\cdot}0025$).

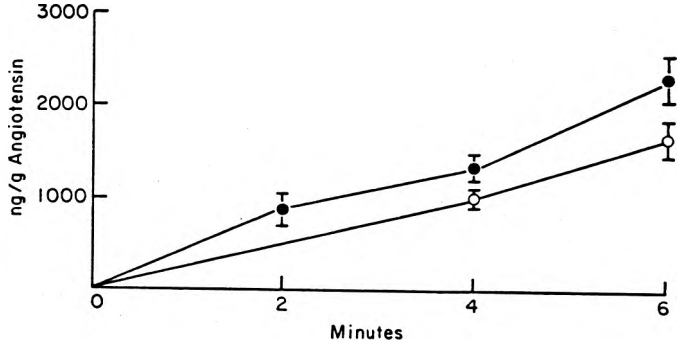

FIG. 3. Kidney perfusions: effect of salt depletion on inactivation of angiotensin. Removal of asn^{1}val^5 angiotensin II ng per g wet weight of tissue ± SEM. Open circles represent animals of Group 8 on a normal salt intake ($n=7$); closed circles sodium depleted animals of Group 7 ($n=7$). The difference between the two groups was significant after 6 min perfusion ($P<0{\cdot}05$).

studied. The remaining hypertrophied kidney destroyed angiotensin at the same rate as did organs taken from animals with both kidneys intact. The effects of salt loading were also similar in these two groups (Fig. 5). However, if the solitary kidney had had an arterial clip applied the situation was different and the clipped organ destroyed much less angiotensin than control

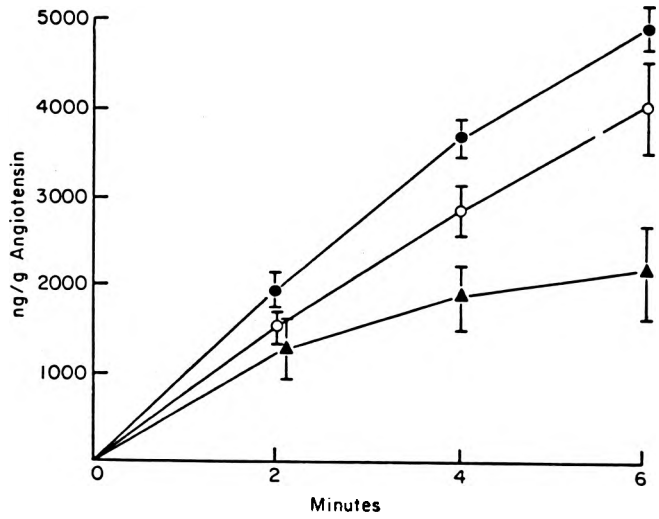

FIG. 4. Kidney perfusions: effects of unilateral arterial clip with contralateral kidney intact on inactivation of angiotensin. Removal of asn^{1}val^5 angiotensin II ng per g wet weight of tissue ± SEM. Closed circles represent control animals of Group 1 with both kidneys intact and taking a normal diet ($n=10$), open circles experiments in which a kidney with artery clipped was perfused ($n=5$) and closed triangles those experiments in which the contralateral unclipped kidney was perfused ($n=5$). With a clip on one renal artery, the contralateral organs destroyed less angiotensin at 6 min than normal control kidneys ($P<0{\cdot}0005$) or than the organs with clipped arteries ($P<0{\cdot}05$). There was no difference between kidneys with arterial clip and normal control organs ($P>0{\cdot}2$).

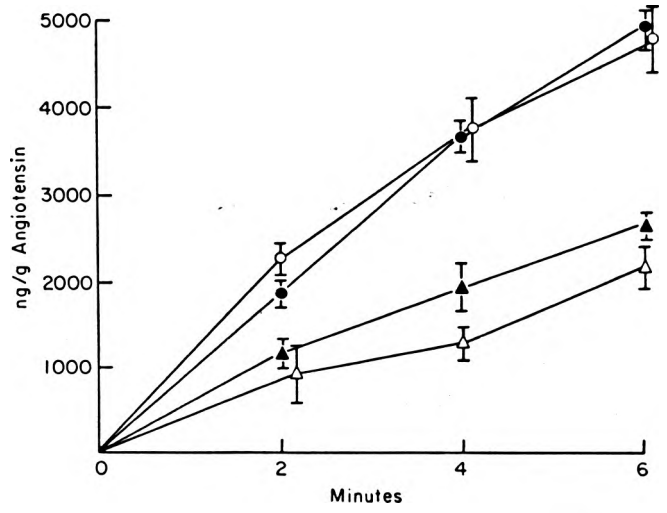

FIG. 5. Kidney perfusions: effects of uninephrectomy and salt loading on inactivation of angiotensin. Removal of asn^{1}val^5 angiotensin II ng per g wet weight of tissue ± SEM. Closed circles represent a control group on a normal diet with kidneys intact ($n=10$), open circles uninephrectomized animals on the same diet ($n=5$). Animals given 1% sodium chloride solution are represented by closed triangles (both kidneys intact, $n=8$) and open triangles (uninephrectomized animals, $n=3$). Loss of angiotensin at 6 min was significantly reduced by salt loading in both groups ($P<0{\cdot}0025$). Loss was not significantly different during perfusion of kidneys from the intact animals and from the uninephrectomy group, regardless of sodium intake.

kidneys or than kidneys similarly clipped with the contralateral kidney intact (Fig. 6). In the absence of the contralateral kidney the capacity of the clipped organ to destroy angiotensin could not be reduced further by increasing dietary salt intake.

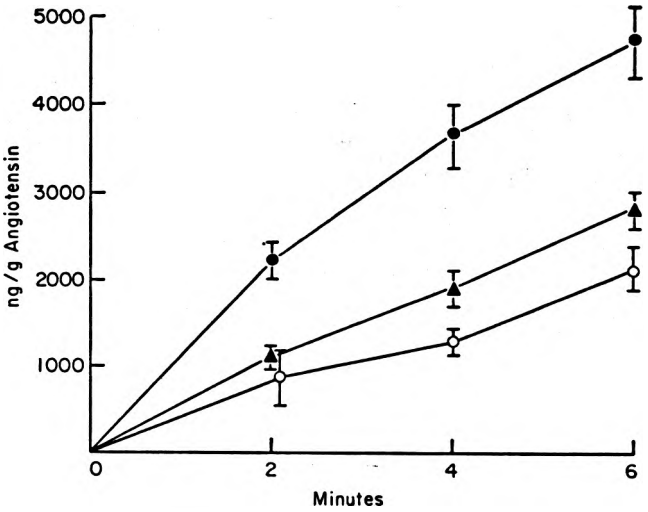

FIG. 6. Kidney perfusions: effects of unilateral nephrectomy and of arterial clip and salt loading on inactivation of angiotensin. Removal of asn^{1}val^5 angiotensin II ng per g wet weight of tissue± SEM. Closed circles represent animals with unilateral nephrectomy alone ($n=5$), closed triangles animals with uninephrectomy, a Goldblatt clip and systolic BP 150 mmHg ($n=5$). Open circles represent uninephrectomized animals given 1% sodium chloride solution ($n=3$). Loss of angiotensin was reduced at 6 min in the presence of a Goldblatt clip ($P<0{\cdot}0025$) or after salt loading ($P<0{\cdot}0025$).

Liver perfusion: effects of renal artery clip

The isolated rat liver inactivated angiotensin II amide at rates unaltered by renal artery constriction ($P>0{\cdot}05$). The perfused livers of rats with renal artery constriction and uninephrectomy removed 223·0–313·5 ng angiotensin in 6 min, mean 254·4 ng, SEM 29·6. The livers of rats with unilateral renal artery constriction and an intact contralateral kidney inactivated from 329·6–437·1 ng in 6 min (mean 380·9±31·1). In control animals the range was 230·4–558·0 ng (mean 376·57±8·4).

DISCUSSION

Perfusion of the isolated rat liver and kidney is a reproducible method of studying the rate of disappearance of angiotensin passing through these organs (Leary & Ledingham, 1969). The liver inactivates the peptide at rates which are not altered by sodium loading or depletion. In contrast the changes in capacity of the isolated kidney to inactivate angiotensin with altered sodium intake are great. Angiotensin destruction varied inversely with salt-intake in rats given the Spiller's diet (Groups 1–6) and in rats taking the Struyvenberg diet (Groups 7 and 8).

Kidneys from animals on the Struyvenberg diet containing 0·2 mEq sodium per day (Group

7) inactivated less angiotensin in 6 min than those of animals on the Spiller's diet of 1 mEq sodium per day (Group 1). In addition the kidneys of animals taking 5 mEq sodium in the Struyvenberg diet (Group 8) destroyed angiotensin at rates similar to animals on the Spiller's diet taking 30 mEq sodium daily (Group 3). These inconsistencies deserve comment. Major differences in constituents of the two diets other than sodium may be important. Eggleston & Krebs (1969) have shown that 3–5 fold differences in the activities of pyruvate-kinase and α-glycerophosphate dehydrogenase can be induced in the livers of rats of various strains by alterations in diet. Enzymes destroying angiotensin may also be responsive to changes in intake of many dietary constituents other than sodium, and direct comparisons of angiotensinase activity between rats of Groups 1–6 (Spiller's diet) and 7 and 8 (Struyvenberg diet) cannot properly be made.

In rats with one renal artery constricted and the other intact, considerably less peptide was removed by the intact kidney than by the ischaemic kidney, while ischaemic kidneys inactivated angiotensin at about the same rate as controls. In rats with one kidney removed and the remaining renal artery constricted by a silver clip the ischaemic kidney removed less peptide from perfusate than its controls and inactivation was not further reduced when these animals had been given saline to drink.

Bing (1962) showed that in the rat the capacity of kidney extracts to destroy asn^{1}val^{5}angiotensin II was reduced when DOCA-salt or renovascular hypertension had been established. Blaquier *et al.* (1961) using similar methods and investigating the same peptide, were unable to demonstrate any difference in angiotensinase activity between normal rat kidneys and those taken from animals rendered hypertensive by arterial clip. Our findings do not support those of Blaquier's group, but extend and largely confirm those of Bing.

The question arises as to whether the changes observed are of physiological or pathological significance or whether they are artefacts. Our studies were performed on organs taken from single strains of one species: high concentrations of angiotensin had to be used to achieve reliable bioassay results; the perfusing medium did not contain plasma or red cells, and the use of an anaesthetic could not be avoided. All these points must be taken into account in assessing the possible significance of the results. However, the difference induced in the behaviour of the kidney by the various experimental manoeuvres used were great, consistent and statistically highly significant. No such changes were observed in the liver experiments. The changes observed in renal inactivation of angiotensin are not limited to the synthetic peptide, asn^{1}val^{5}angiotensin. Recent experiments indicate that the amount of naturally occurring angiotensin II free acids inactivated during kidney perfusion is significantly reduced in rats drinking 1% sodium chloride solution when compared with those given water (Leary, 1969).

The possible mechanisms by which the isolated kidney may inactivate angiotensin at widely differing rates depending on experimental circumstances is not clear. Disappearance of the peptide during perfusion could result from breakdown of the molecule by enzymes (Bakhle, Reynard & Vane, 1969; Leary & Ledingham, 1969) or by attachment to receptor sites in the perfused organs (Biron *et al.*, 1968). The observation that inactivation of angiotensin by tissue vascular beds can be inhibited by edetic acid or dimercaprol (Leary & Ledingham, 1969; Bakhle *et al.*, 1969) favours an enzymatic process, but does not distinguish with certainty between these two possibilities.

Alteration of the rate of removal of angiotensin by the kidney could reflect change in the

affinity of enzymes or binding sites for the peptide, or could reflect changes in the distribution of blood flow between areas of the kidney with different inherent capacities to inactivate angiotensin. Horster & Thurau (1968) have demonstrated differences in filtration rate between superficial and juxtamedullary nephrons of rat kidneys. In rats taking a standard diet containing approximately 1 mEq sodium/day superficial nephrons have a lower individual glomerular filtration rate than do juxtamedullary nephrons. This difference in individual nephron GFR and distribution of renal bloodflow is reversed if sodium intake is increased to 5 mEq/day. If renal angiotensinase were located mainly in juxtamedullary nephrons, animals with a normal sodium intake would inactivate angiotensin at a faster rate than those of salt-loaded animals, in which perfusion of juxtamedullary nephrons would be reduced. This redistribution of renal bloodflow could possibly be mediated by the changes in kidney renin content that occur in response to renal artery clipping and changes in sodium balance (Gross *et al.*, 1964). This theory must remain unproven until the angiotensinase activity of superficial and juxtamedullary nephrons has been measured.

The changes in the capacity of the rat kidney to inactivate angiotensin are of potential importance in any assessment of the role of the renin–angiotensin–aldosterone system in states of altered electrolyte balance or blood pressure. Decreased removal of angiotensin in the kidney would induce increased local tissue concentrations of the peptide with a resultant rise in renal vascular resistance. The concentration of angiotensin in the renal venous blood and later in the arterial circulation would not necessarily be detectably altered. Reduced kidney blood flow with increased vascular resistance, particularly in the efferent arterioles, is a consistent finding in all states of high blood pressure (Goldring *et al.*, 1941; Friedman, Selzer & Rosenblum, 1941; Bradley *et al.*, 1950; Cargill & Hickam, 1949; Bolomey *et al.*, 1949). The possibility exists therefore that tissue concentrations of angiotensin in the kidney could be altered critically in certain circumstances, with resultant renal vasoconstriction caused by angiotensin, but not reflected by alterations in circulating concentrations of renin, angiotensin or aldosterone.

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PAPER A5

Metabolism of Angiotensin I in the Pulmonary Circulation

This publication was the product of biochemical expertise lent by Professors Ryan and Stewart and theoretical and laboratory skills provided by me. Dr. Ledingham edited the final draft of the paper.

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Short Communication

Metabolism of Angiotensin I in the Pulmonary Circulation

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There is a growing body of evidence suggesting that angiotensin I is converted into angiotensin II during passage through the pulmonary circulation. The major finding in support of this hypothesis is that the venous effluent of lungs perfused with angiotensin I has a greater capacity to contract the rat colon and raise systemic arterial blood pressure than can be explained by the concentration of angiotensin I entering the pulmonary artery (Ng & Vane, 1967, 1968*a*,*b*; Biron & Huggins, 1968). These results are obtained by using lungs perfused with blood or Krebs–Henseleit solution (Krebs & Henseleit, 1932), indicating that the postulated conversion cannot be entirely accounted for by enzymes in blood. Bakhle, Reynard & Vane (1969) have also shown that the enhanced biological effects are not due to release of endogenous catecholamines or prostaglandins E_2 or F_{2a}.

The change in biological activity of angiotensin I after passage through the lung could be due to conversion of the decapeptide into angiotensin II by tissue enzymes. However, this possibility is not proved by the bioassay of unfractionated perfusate. The biological assay preparations that have been used are highly selective in terms of known compounds, but previous studies have not ruled out the possibility that angiotensin I acts on the lung to stimulate the release of an unknown compound capable of contracting the rat colon and raising arterial blood pressure. The possibility also exists that metabolic products of angiotensin I potentiate the effects of the parent compound, perhaps as the *C*-terminal pentapeptide of bradykinin potentiates the effects of bradykinin on the guinea-pig ileum (Garbe, 1967).

Conversion of angiotensin I into angiotensin II in the pulmonary circulation could be of major physiological importance and therefore an attempt has been made in this study to obtain biochemical evidence of any such conversion.

Perfusion studies were performed by using female Spraque–Dawley rats, weighing 0.2–0.4kg, fed on a standard small-animal diet (Purina Laboratory Chow) and given unrestricted access to water. Three rats were given tap water and three were given tap water containing 2% (w/v) NaCl. The latter three rats received the salt water for 10–12 days before use. All rats were anaesthetized by an intraperitoneal injection of chloral hydrate (300 mg/kg body wt.). Lungs were perfused *in situ* as described by Leary & Ledingham (1969) by a method developed by F. Woods & W. P. Leary (unpublished work).

All glassware except the chromatography column was coated with silicone (Siliclad, Clay–Adams; Inc., New York, N.Y., U.S.A.). Krebs–Henseleit solution, aerated with O_2+CO_2 (95:5) and heated at 37°C, was pumped through the lungs at a rate of 5–6ml/min until the venous effluent was free of blood (2–3min). Then (10-L-[^{14}C]leucine)-angiotensin I (25μCi/μmol, lot no. 6901; Schwarz Bio-Research Inc., Orangeburg, N.Y., U.S.A.) was added to the Krebs–Henseleit solution to a concentration of 700μg (about 0.5nmol)/ml and the perfusion rate was maintained at 6ml/min. Venous effluent from the perfused lung was collected over the next 3min into an Erlenmeyer flask containing 20ml of cold 10% (w/v) trichloroacetic acid stirred constantly with a magnetic stirring device.

This perfusate was centrifuged at 1690*g* for 10 min at 4°C to remove a small (0.2ml) brown precipitate. The supernatant was extracted five times with 2 vol. of diethyl ether. The aqueous layer was evaporated to dryness in a rotary evaporator. Its residue was dissolved in 1.1ml of 1M-pyridine, and 1.0ml of the solution was chromatographed on a Bio-Gel P-2 column (98cm × 1.2cm). The column was eluted with 1M-pyridine, and 50–100 2ml fractions were collected. The column was calibrated previously to determine elution volumes of angiotensin I, angiotensin II, histidyl-leucine and leucine (Fig. 1*a*).

A 20μl sample of each fraction was applied to pieces (1cm × 1cm) of filter paper (Whatman no. 1) to detect radioactivity. The papers were dried and then placed in scintillation vials containing 18ml of an 0.4%(w/v) solution of Omnifluor (New England Nuclear Corp., Boston, Mass., U.S.A.) in toluene. The radioactivities of vials were counted in a Unilux II liquid-scintillation counter (Nuclear–Chicago).

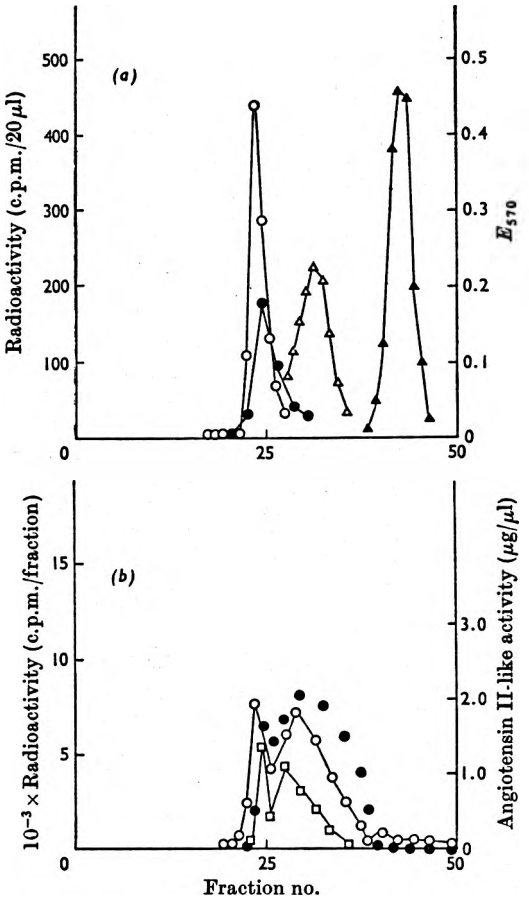

Fig. 1. (*a*) Molecular-sieve chromatography of angiotensin I and selected lower homologues. Angiotensin I and some of its homologues were chromatographed on a column (98 cm × 1.2 cm) of Bio-Gel P-2. Fractions (2 ml) were collected. Fractions containing (10-L-[^{14}C]leucine)-angiotensin I and (5-L-[^{14}C]isoleucine)-angiotensin II (45 μCi/μmol; Schwarz BioResearch Inc.) were located by scintillation counting. Histidyl-leucine and leucine were located with ninhydrin (E_{570}). ○, Angiotensin I; ●, angiotensin II; △, histidyl-leucine; ▲, leucine. (*b*) Molecular-sieve chromatography of the effluent of lungs perfused with (10-L-[^{14}C]leucine)-angiotensin I. The effluent was treated with trichloroacetic acid and extracted as described in the text. The extract was chromatographed on a column (98 cm × 1.2 cm) of Bio-Gel P-2 and fractions were collected as described above. Samples were tested for radioactivity and for ability to contract the rat colon and raise arterial blood pressure. ○, Radioactivity; □, blood pressure; ●, ability to contract rat colon.

In four experiments the fractions were recombined to correspond to peaks of radioactivity. In two experiments fractions were freeze-dried individually. Each residue was dissolved in 2 ml of water and tested for effects on the rat colon (Regoli & Vane, 1964) and on the arterial blood pressure of the rat (Peart *et al.* 1966).

Further analysis of radioactive peaks was carried out by paper electrophoresis at pH 1.9 (formic acid–acetic acid–water, 6:13:81, by vol.), pH 5 (0.1 M-acetic acid adjusted to pH 5 with pyridine) or pH 9 [1% (w/v) $(NH_4)_2CO_3$]. Whatman no. 1 paper was used for analytical runs and Whatman 3MM paper was used for preparative electrophoresis. With any of these systems electrophoresis was carried out at 22 V/cm, 10–20 mA, for 1 h.

In all experiments the activity of the solution entering the lungs was compared with that of the venous effluent in terms of ability to raise mean arterial blood pressure. In none of these experiments was there a clear increase or decrease of activity. In contrast, the ability of the venous effluent to contract the rat colon was 2–5 times greater than could be accounted for by angiotensin I. On average, the rat colon was 15 times as sensitive to angiotensin II as to angiotensin I.

In the two experiments in which column fractions were assayed individually, as much as half of the pressor activity and well over half of the colon-contracting activity was found to be eluted much later than expected for angiotensin I or angiotensin II (Fig. 1*b*). The identity of the substance(s) of the second peak is not known. Control experiments were performed to rule out the possibility that the second peak of biological activity is a substance leached from the tubing of the perfusion circuit. Chromatography of the perfusate obtained by pumping angiotensin I through tubing but not through lungs showed a single peak of activity corresponding in elution to angiotensin I. Under the conditions of our chromatography an impurity of the column matrix would not be expected to be eluted as a peak.

The substance of the first biologically active peak was identified as angiotensin II. Its elution volume corresponded to that of authentic angiotensin II, being slightly less than that of angiotensin I. Fractions corresponding to the first peak of activity were combined and freeze-dried. The residue was dissolved in 0.5 ml of pyridine–acetic acid buffer, pH 5. The entire sample was applied to a strip of Whatman 3MM paper and electrophoresis was carried out at pH 5, 22 V/cm, 10–20 mA, for 1 h. Arginine and picric acid were included in the run as reference compounds. Radioactivity was located by strip counting (Actigraph III; Nuclear–Chicago). Then the paper was cut at 1.0 cm intervals from the origin towards the cathode. Each piece of paper was eluted with 2.0 ml of Tyrode's solution, and the eluates were tested for effects on the rat colon and arterial blood pressure. Biological activity was found as a single peak (R_{Arg} 0.18–0.27) correspond-

ing to the migration of authentic angiotensin II (R_{Arg} 0.24). This area of the electrophoretogram was devoid of radioactivity.

The electrophoretogram contained two peaks of radioactivity, a small one (60c.p.m; background 6c.p.m.) corresponding in migration (R_{Arg} 0.47) to angiotensin I, and a larger peak (700c.p.m.), which migrated much faster (R_{Arg} 0.69). The second peak, judged from its early elution from the Bio-Gel P-2 column and its rapid migration, is probably a large *C*-terminal fragment of angiotensin I.

As shown in Fig. 1(*b*), over half of the radioactivity applied to the column was eluted in fractions expected for peptides of two to five amino acid residues. None of the radioactivity was accounted for as free leucine. This point rules out the possibility that angiotensin I is converted into angiotensin II by the stepwise removal of leucine and then histidine (Ng & Vane, 1967).

Subsequent analysis showed that virtually all of the radioactivity in fractions 28–40 was incorporated into histidyl-leucine. Fractions 28–32 and 33–40 were freeze-dried and analysed by electrophoresis at pH 5 and pH 9. Samples were run with or without the addition of carrier histidyl-leucine. Carrier was located after reaction with ninhydrin. At either pH value radioactivity of fractions 28–32 and 33–40 was not distinguishable from that of authentic histidyl-leucine (pH 5, R_{Arg} 0.71; pH 9, $R_{picric\ acid}$ 0.53).

Our results show that angiotensin I is converted into angiotensin II during a single circulation through rat lung washed free of blood. Conversion occurs by removal of the *C*-terminal dipeptide, histidyl-leucine, and not by the stepwise action of a carboxypeptidase. Much of the radioactivity associated with the large-molecular-size (mol.wt. about 900) material can be accounted for as a large *C*-terminal homologue of angiotensin I. This suggests that the converting enzyme of lung competes with an aminopeptidase. Angiotensin II is not the only biologically active substance found in the venous effluent of lungs perfused with angiotensin I. There is a second substance, separable by chromatography on Bio-Gel P-2, that contracts the rat colon and raises arterial blood pressure. The relatively late elution of the second substance suggests that it either is of small molecular size or is adsorbed by the Bio-Gel matrix. Whether the second substance is an endogenous compound in lung or a lower homologue of angiotensin I is not known. The *C*-terminal heptapeptide of angiotensin II is known to have biological activity. However, this peptide would not be expected to separate from angiotensin I or angiotensin II during chromatography on Bio-Gel P-2. It remains to be determined whether smaller homologues are capable of contracting the rat colon and raising arterial blood pressure.

This work was supported in part by grants from the Florida Heart Association, the Population Council (M69148) and the National Institutes of Health (HE12843). W. P. L. was supported in part by the Nuffield Dominions Foundation, Oxford, and the Medical Research Council, Pretoria, South Africa.

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PAPER A6

In Situ Perfusion of Isolated Rat Lung

This paper was my own with the exception of electron microscopy all of which was performed by Dr. Una Smith.

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IN SITU PERFUSION OF ISOLATED RAT LUNG

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Enzymes that convert angiotensin I and angiotensin II or inactivate both peptides have been found in extracts of animal and human tissues (1-4). The role of these enzymes in living animals is uncertain, since non-specific pep-- tidases capable of degrading angiotensin may be released by cells damaged in the preparation of tissue extracts. Hodge, Ng and Vane (5) and other workers (6-7) have recognised this problem and advocated the use of intact perfused organs in the study of angiotensin metabolism.

A simple method for perfusing the rat lung in situ, with minimal damage to parenchymal cells, is described in this paper. This technique was used to study the conversion of (10-L-leucine-^{14}C)- angiotensin I (25 u ^{Ci}/u mole, lot. No. 6901; Schwarz Bioresearch, Inc., Orangeburg, N.Y., U.S.A.) to angiotensin II by the rat lung (8).

Methods

Six female Wistar rats were anaesthetised with sodium pentobarbitone (Veterinary Nembutal, Abbott), 60mg/kg, intraperitoneal and fixed to a perspex dissection platform placed above a collecting vessel. The lung was prepared for perfusion by a modified technique based on that of Woods and Leary (9). The sternum was exposed and skin flaps reflected to expose the rib-cage. The chest was opened and the anterior chest wall removed en-bloc to the mid-axillary line bilaterally. Polyethylene bags of crushed ice were applied to the lungs and heart. The inferior vena-cava was tied below its junction with the right atrium. A nylon cannula (size PP270, Portland Plastics Ltd., Hythe) was intro-

duced into the cavity of the right ventricle through the right precaval vessel and secured by a ligature tied around the right atrioventricular groove. An identical cannula was placed in the left ventricle through the ascending aorta and tied in place at the level of the coronary vessels. The left border of the heart was displaced anterolaterally so that the left precaval and azygos veins could be mobilised and tied.

Perfusion medium, prepared as previously described (7), was passed into the right heart cannula at 6 ml/min by a pressure dependent finger pump (Process and Instruments, Brooklyn, U.S.A.). The first 10 ml of perfusate was discarded after passage through the lung to avoid contamination of medium in the collecting vessel with plasma or erythrocyte peptidases. Lungs were aerated by a mixture of O_2 and CO_2 (95:5) bubbled through the perfusion medium. In some experiments lungs were ventilated with a Starling pump which delivered air through a tracheostomy tube at 30 ml/min. Perfusion medium was kept at 37°C and pH 7.4 (9), and lungs were perfused with (10-L-leucine-^{14}C) angiotensin I at a concentration in the perfusate of 700 mg/ml for 10 min before fixation for electron microscopy. Venous effluent from the perfused lung was collected into an Erlenmeyer flask containing 20 ml of cold trichloroacetic acid 10 g% w/v, stirred constantly with a magnetic device. Analytical methods for peptide fragments and angiotensins I and II in the effluent perfusate are described elsewhere (8).

To determine what damage, if any, the lungs suffered from the perfusion, specimens were examined by electron microscopy after a full 10 min period of perfusion. Tissue was fixed by perfusion with 0.25 M glutaraldehyde in 0.5 M cacodylate buffer at pH 7.4 and containing 0.17 M sucrose. After 3 min perfusion at 37°C both lungs were removed and cut into small cubes approximately 0.2 mm of side and transferred to fresh fixative. To ensure rapid penetration of the fixative a light vacuum was applied via a water aspirator until the specimens sank to the bottom of the vial. After fixation overnight the tissue pieces were washed in several changes of 0.05 M cacodylate buffer containing 0.34 M

sucrose and placed in 1% osmium tetroxide in veronal acetate buffer (10) at pH 7.4 for 90 min at 4°C. Dehydration was carried out in an ethanol series and the tissue was imbedded in Araldite (A.R.L. Ciba, Duxford, England) via propylene oxide. Sections were cut on an L.K.B. Ultrotome III, mounted on copper grids, double stained using lead citrate and saturated ethanolic uranyl acetate and examined in a Philips EM 100 or EM 200 electron microscope.

Results

Clear evidence was obtained that the isolated rat lung preparation remained enzymatically competent and has been published elsewhere (8). Angiotensin I was converted to angiotensin II during a single passage through the lung washed free of blood. Analysis of peptide fragments indicated conversion by removal of the C-terminal dipeptide His-leu.

Large areas of lung were scanned in the electron microscope. Fig. 1 is a representative field showing that the general arrangement and relationships between the component cell types within the lung are preserved. The vessels are conspiciously free of blood cells and plasma. Fig. 2 illustrates that the pinocytotic vesicles of the endothelial cells retain their circular profiles and that the intercellular junctions between capillary cells retain their integrity. There is no evidence of stretching or excessive pressure in the vessels. Apart from the absence of blood Figs. 1 and 2 illustrate those features which are presumed to obtain in the living tissue and provide good evidence that the fine structure of the lung has been preserved during perfusion.

Discussion

It is widely recognised that adequate fixation of mammalian tissue for electron microscopy cannot be achieved by simple diffusion and requires perfusion techniques that satisfy certain biochemical, pharmacological and physiological criteria of preservation, lung tissues examined in the electron microscope may show inadequate clearance of blood cells and plasma and considerable mechanical damage as a result of over-dilatation of vessels (12). This damage may be manifested by broken cell membranes, stretched intercellular junctions,

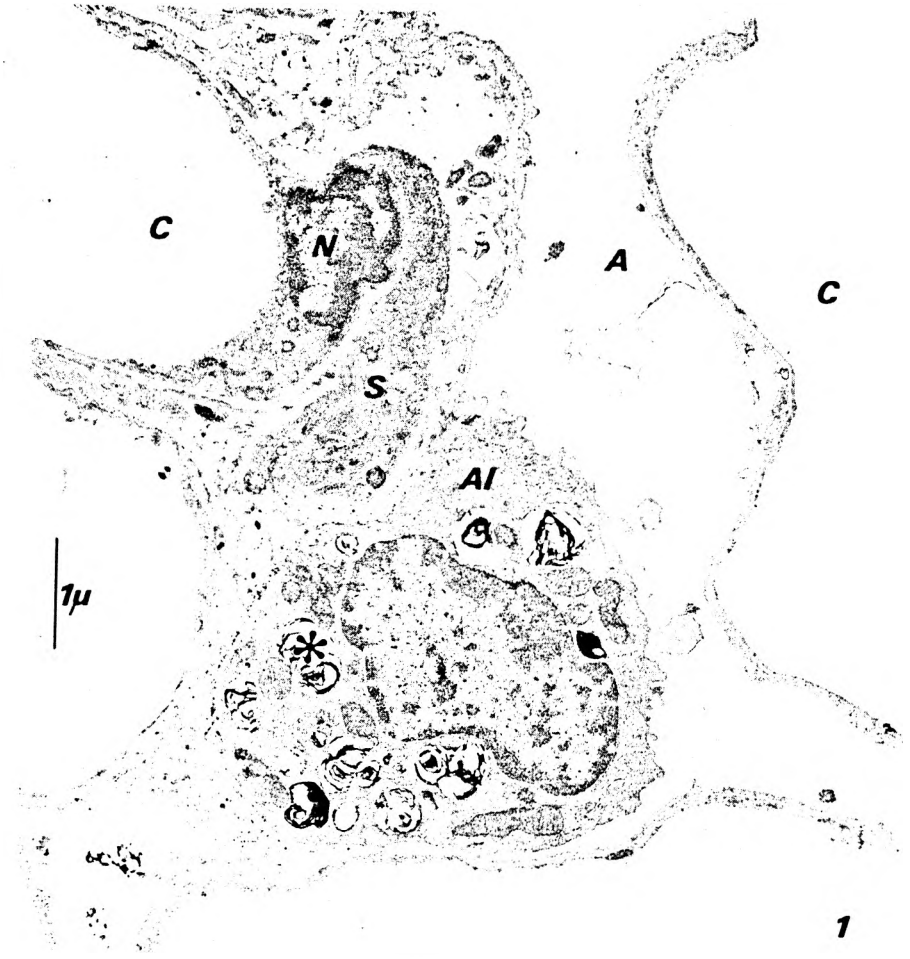

FIG 1.

Electron micrograph showing the relationship between capillaries (C) and alveolar space (A). An endothelial nucleus (N), septal cell (S) and giant alveolar cell (Al), containing lamellar bodies (*) and mitochondria (M) are also shown. Note absence of blood cells within lumina of blood vessels.

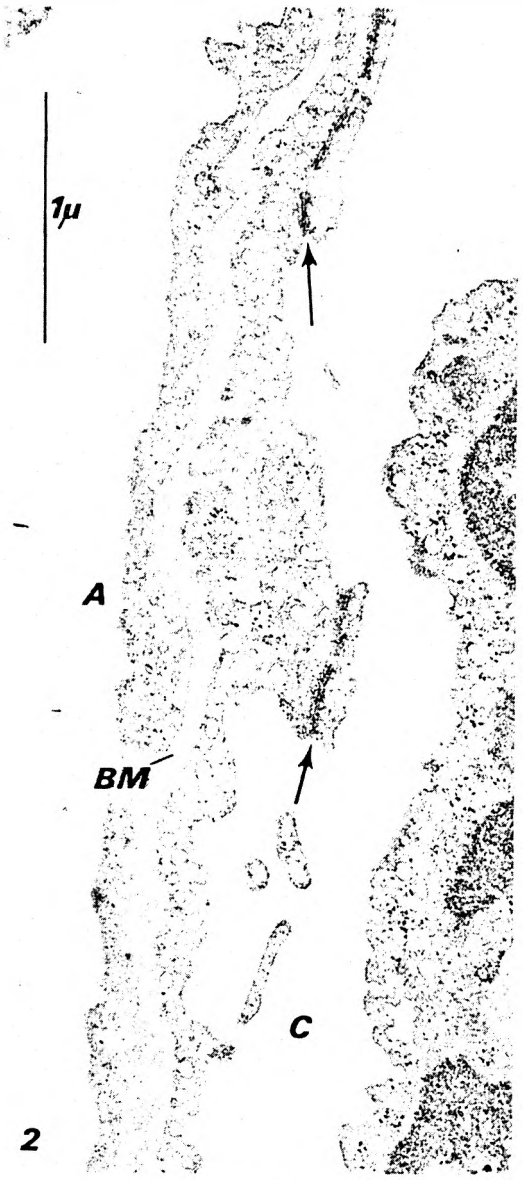

FIG 2.

Micrograph showing portions of the endothelium lining the capillary lumen (C), basement membrane (BM), and epithelium bordering on the alveolar space (A). Intercellular junctions between the endothelial cells are shown by arrows. Except in areas where the endothelium is extremely narrow, the circular profiles of pinocytotic vesicles feature prominently.

extruded cytoplasmic contents and swollen mitochondria. These changes were not found after perfusion of the lungs by the method described in this paper.

The technique described appears to provide a useful means of distinguishing physiological changes in peptide metabolism within the lung from changes due to cell disruption such as that which occurs in the preparation of tissue extracts. It may be used in conjunction with established techniques for perfusing the liver and kidney (5, 6, 7, 9).

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PAPER A7

Catabolism of Angiotensin II

This paper reviews the field in which papers 1-6 are placed. It was prepared in full collaboration with Dr. J.G. Ledingham.

Catabolism of Angiotensin II

J. G. LEDINGHAM and W. P. LEARY

Catabolism of Angiotensin II

It has long been evident that there are substances in blood and tissues which are capable of destroying angiotensin. The effects of temperature, pH changes and dialysis on angiotensin survival in renin assay systems indicated that enzymes were probably involved (BRAUN-MENENDEZ and FASCIOLO, 1939: PAGE and HELMER, 1940). The generic term "angiotensinase" is used to describe these enzymes, although their specificity is unproven.

The study of angiotensinases in tissue homogenates, lysed red cells, plasma and serum *in vitro* has provided good data about the biological pathways by which angiotensin may be destroyed. This approach probably has no value in the evaluation of the contribution of specific organs or tissues to the metabolism of angiotensin in health and disease. The presence of these enzymes in blood and in tissue extracts of kidney, liver, lung, uterus, intestinal mucosa, adrenal, pancreas, spleen, brain, skeletal and cardiac muscle (FASCIOLO et al., 1940; ITSKOVITZ and MILLER, 1967; MATSUNAGA et al., 1969) does not imply that they are in any way specific for angiotensin or necessarily play any part in the metabolism of Ang. II *in vivo*. For instance homogenates of lung contain angiotensinases in amounts comparable to those in other organs (ITSKOVITZ and MILLER, 1967; MATSUNAGA et al., 1969) but studies of intact lungs indicate that this peptide is not detectably metabolized in the pulmonary circulation in man or other animals (HODGE et al., 1967; LEARY and LEDINGHAM, 1969; BIRON et al., 1969; BIRON and CAMPEAU, 1971; OSBORN et al., 1969).

Nature of Angiotensinases

Yeast aminopeptidase, pepsin, trypsin, chymotrypsin, carboxypeptidases and papain have been shown to destroy angiotensin by CROXATTO and CROXATTO (1942), CROXATTO et al. (1943), and PLENTL and PAGE (1943). The availability of synthetic angiotensin in 1958 led to further studies into the nature and source of angiotensinases in plasma, serum, red cells and tissue slices or homogenates. Enzymes capable of hydrolyzing the N-terminal bond (aminopeptidases), the middle of the peptide (endopeptidases) and the C-terminal bond (carboxypeptidases) of Ang. II have been described (Table 1).

Aminopeptidases (Angiotensinase "A"). GLENNER et al. (1962) found an enzyme specific for N terminal α-L-glutamyl and α-L-aspartyl residues in rat kidney microsomes. KHAIRALLAH et al. (1963) later described a peptidase of very similar properties, in human plasma and lysed washed red cells. Both enzymes were calcium-dependent, pH optima were similar and neither would destroy β-Asp^1-Ang. II. These enzymes, which had much in common, were named Aminopeptidase A by GLENNER et al. and Angiotensinase A by KHAIRALLAH et al. (1963). NAGATSU

et al. [1965 (1 and 2) and 1970] then produced evidence that separate amino-peptidases were responsible for hydrolyzing the Asn^1 and Asp^1 analogues of Ang. II. KHAIRALLAH and PAGE (1967) suggested the enzyme active at pH 7.4, which hydrolyzed the Asn^1 peptide, be called Angiotensinase A_1 and the peptidase specific to [Asp^1]-Ang. II Angiotensinase A_2. MATSUNAGA et al. (1969) have found similar neutral

Table 1. *Identified angiotensinases*

Angiotensinase	Authors	pH Optimum	Inhibitors	Source	Site of Action
Aminopeptidase A	GLENNER et al., 1962	7.0	EDTA	Kidney Microsomes	Asp^1-Arg^2
Angiotensinase A_1	KHAIRALLAH and PAGE, 1967	7.4	Sodium pyrophosphate	Human plasma	Asn^1-Arg^2
Aminopeptidase	NAGATSU et al., 1965, 1970		EDTA	Human serum Human red cells Kidney extracts	
Angiotensinase A_2 Aminopeptidase	NAGATSU et al., 1965 KHAIRALLAH and PAGE, 1967; NAGATSU et al., 1970	7.4	EDTA	Human serum Red cell and kidney extracts	Asp^1-Arg^2
Microsomal Aminopeptidase	MATSUNAGA and MASSON, 1970	7.4	EDTA	Kidney Microsomes (SH group independent)	Asn^1-Arg^2
		7.4	EDTA and Thiol reagent	Liver microsomes (SH group dependent)	Asp^1-Arg^2 Asn^1-Arg^2
Angiotensinase B (Endopeptidase)	REGOLI et al., 1963; KHAIRALLAH and PAGE, 1967	7.4	? EDTA	Rat serum Human plasma Rat kidney	Tyr^4-Val^5
Lysosomal Endopeptidase	MATSUNAGA, 1971	5.5	SH group dependent	Kidney and liver lysosomes	Tyr^4-Val^5
Carboxypeptidase	JOHNSON and RYAN, 1968	7.4	N.E.M.	EDTA dialyzed Rabbit-liver extract	Pro^7-Phe^8
Angiotensinase C Carboxypeptidase	YANG et al., 1968	5.6 to 5.7	D.F.P.	Hog kidney Human urine	Pro^7-Phe^8
Lysosomal Carboxypeptidase	MATSUNAGA, 1971	5.5	D.F.P.	Kidney and liver lysosomes	Pro^7-Phe^8

amino-peptidases in the microsomal fragments of many organs, most concentrated in kidney and liver (Table 2). Liver cell sap aminopeptidase may be dependent on sulphhydryl (SH) groups in contrast to the renal microsomal enzyme.

Endopeptidases (Angiotensinase "B"). The effects of rat serum, human plasma and rat kidney homogenate on six analogues of Ang. II were observed by REGOLI et al. (1963). Analogues stable to aminopeptidases were destroyed by all three media, and chromatography of the peptide fragments showed individual amino acids and the tetrapeptide Asp^1-Arg^2-Val^3-Tyr^4. The C-terminal tetrapeptide was

not found intact, but the evidence was taken to suggest the presence of an endopeptidase, hydrolyzing the tyrosine--valine linkage. A similar plasma endopeptidase, active at pH 7.4 and inhibited by diisopropyl-fluorophosphate (D.F.P.) was described by KHAIRALLAH and PAGE (1967) and labelled Angiotensinase B. MATSUNAGA (1971) has recently shown chymotrypsin-like activity in the presence of dithiothreitol (D.T.T.) and inhibited by D.F.P. in the lysosomal fraction of rat kidney and liver. This SH group-dependent lysosomal enzyme may be identical to that described by REGOLI et al. (1963) but differs from the Angiotensinase B of KHAIRALLAH and PAGE in that it is not inhibited by D.F.P.

Carboxypeptidases (Angiotensinase "C"). Pancreatic Carboxypeptidase A was shown to release phenylalanine from Ang. II by LENTZ et al. (1956) and JOHNSON and RYAN (1968) have found evidence of carboxypeptidase activity in aqueous extracts of rabbit liver. Another enzyme with the capacity to hydrolyze the

Table 2. *Neutral aminopeptidases in extracts of various tissues* (Adapted from MATSUNAGA et al., 1969)

Tissue	Activity per 10 ng of tissue	Protein mg
Kidney	343	0.55
Liver	309	0.76
Spleen	175	0.70
Adrenal	—	0.35
Lung	137	0.30
Heart	28	0.24
Brain	35	0.16
Muscle	17	0.17

[a] Activity is expressed in nanograms of angiotensin inactivated by 0.46 ng of protein at 37 °C for 15 min.

terminal peptide bond when proline is the penultimate amino-acid has recently been described by YANG et al. (1968). This enzyme, isolated from porcine kidney cortex or human urine has been called Angiotensinase C. It can be distinguished from Carboxypeptidase A, Cathepsins and other known enzymes; it is inhibited by D.F.P., though not by chelating agents, and has a pH optimum of 5.6 to 5.7.

MATSUNAGA and his colleagues (SAITO et al., 1969; MATSUNAGA et al., 1969; MATSUNAGA, 1971) have shown interest in the angiotensinases found in the lysosomal fraction of various tissues, particularly kidney. The evidence now suggests that the main enzyme of this fraction is a carboxypeptidase, inhibited by D.F.P., active at acid pH, and independent of SH groups.

Angiotensinases in Plasma, Serum, and Blood

Peptidases in plasma, serum or whole blood have been the subject of intensive investigation. *In-vitro* methods have suggested the presence of both aminopeptidases and endopeptidases in plasma and serum [REGOLI et al., 1963; KHAIRALLAH and PAGE, 1967; PICKENS et al., 1965; NAGATSU et al., 1965 (1 and 2), 1970]. Studies of the metabolism of (^{35}S) Phenyl thiocarbamyl-Ang. II in human plasma confirm the presence of both types of enzymes, but suggest a dominant role for amino peptidases (DOYLE et al., 1967). Degradation of Ang. II in blood,

once thought all-important, is now known to be a slow process of minor significance compared with the rapid catabolism of the peptide in tissue vascular beds (HODGE et al., 1967; NG and VANE, 1968; BAKHLE et al., 1969).

Kidney Angiotensinases

Tissue Extracts. Renal angiotensinases have been measured in homogenates of dog, rat and rabbit kidney by FASCIOLO et al. (1940), REGOLI et al. (1963), LEE (1965), ITSKOVITZ and MILLER (1967) and JOHNSON (1967). YANG et al. (1968) reported the presence of a carboxypeptidase in extracts of hog kidney cortex while other workers have fractionated kidney homogenates and demonstrated that most angiotensinase is present in microsomal and lysosomal fractions (DENGLER and REICHEL, 1960; GLENNER et al., 1962; MATSUNAGA et al., 1968). The nature of these peptidases has been investigated by incubation of [Asn^1-Val^5]-Ang. II or its analogues with kidney homogenates under standard conditions with subsequent bioassay of residual pressor activity. In some experiments, peptide fragments have been identified by chromatography (REGOLI et al., 1963; SAITO et al., 1969; MATSUNAGA et al., 1969; MATSUNAGA, 1971).

Table 3.

Species	Fraction	Angiotensinase identified	Authors
Rat	Microsomes	Aminopeptidase	GLENNER et al. (1962)
Rat	Homogenate	Aminopeptidase and Endopeptidase	REGOLI et al. (1963)
Pig	Cortex	Carboxypeptidase	YANG et al. (1968)
Rat	Lysosomal	Endopeptidase	SAITO et al. (1969)
Rat	Lysosomal	Endopeptidase and Carboxypeptidase	MATSUNAGA (1971)
Rat	Microsomal	Aminopeptidase	MATSUNAGA and MASSON (1970)

Data collected from these studies is presented in Table 3.

Intact Organ Experiments. The probability that nonspecific intracellular enzymes with limited access to perfusing blood are released in the preparation of tissue homogenates has stimulated the development of other techniques for the measurement of angiotensinases in the kidney.

HODGE et al. (1967) used the superfusion technique of REGOLI and VANE (1966) to study angiotensin metabolism in anaesthetized dogs. Blood drawn from the renal vein superfused a series of assay organs before returning to the dog's circulation. Ang. II was infused into the renal artery or directly into blood perfusing the assay organs. Comparison of the assay responses to peptide infused by these two routes allowed the calculation of angiotensin loss during passage through the intact kidney. BIRON et al. (1968) studied renal angiotensinase activity in the anaesthetized rat and dog, using the animals own blood pressure response to assay the peptide. Angiotensin analogues were infused into the renal vein at a rate producing a stable elevation of 20 to 25 mmHg in arterial pressure. The same analogue was then infused into the renal artery at gradually increasing rates to produce the same diastolic B.P. response. Both HODGE et al. (1967) and BIRON et al. (1968) were able to show significant inactivation of angiotensin in the intact kidney, but did not prove that this was due to angiotensinases. In a similar study, OSBORN et al. (1969) found renal inactivation of [Asn^1-Val^5]-Ang. II and (^{35}S) Phenylthiocarbamyl-Ang. II in the sheep and dog. These findings were confirmed in the rat

by LEARY (1970) who measured pressor responses to [Asp^1-Ileu^5]-Ang. II infused into the renal artery, jugular vein and inferior vena — cava in the same animal.

The technique of isolated organ perfusion has been applied to the study of renal angiotensinases in several species by BAKHLE et al. (1969) and LEARY and LEDINGHAM (1969). BAKHLE et al. (1969) studied Ang. II metabolism in isolated kidneys perfused with Krebs' solution to distinguish angiotensinase activity in the renal vascular bed from activation of plasma or erythrocyte peptidases during passage through the organ. Isolated kidneys from cats and dogs were perfused through the directly cannulated renal artery. Renal veins were cut to allow perfusate to escape from the organ. In rats both kidneys were perfused by tying a cannula into the aorta below the origin of the renal arteries. The aorta was tied above the renal arteries and kidneys and aorta then excised. The excised preparation was suspended in a perspex chamber and perfused with Krebs' solution gassed with 95% O_2 and 5% CO_2 at 37 °C. Angiotensin inactivation was measured by comparing the responses of rat colon to [Asn^1-Val-]-Ang. II infused proximal or distal to the perfused kidney.

LEARY and LEDINGHAM (1969) perfused isolated rat kidney by the methods of NISHITSUTSUJE-UWO et al. (1967) with certain modifications [LEARY, 1970; LEARY and LEDINGHAM, 1970 (1)]. Rat kidney was isolated from the general circulation by ligation of appropriate vessels and perfused *in situ* for periods of not less than 6 min with an oxygenated artificial medium at pH 7.4 and 37 °C. The time course of Ang. II destruction by perfusate alone and by the isolated kidney was determined by a rat bioassay technique (PEART, 1955; LEE, 1965). BAKHLE et al. and LEARY and LEDINGHAM both reported inactivation of [Asn^1-Val^5]-Ang. II in the isolated perfused rat kidney and their results have been confirmed by BIRON and CAMPEAU (1971). BAKHLE et al. (1969) also described inactivation of the peptide by cat and dog kidneys and LEARY and LEDINGHAM (1969) found that angiotensin loss in their preparation was partially inhibited by edetic acid 3×10^{-3}M. Later it was demonstrated that α-[Asp^1-Val^5]-Ang. II and α-[Asp^1-Ileu^5]-Ang. II were inactivated by isolated rat kidney whereas the β-Asp^1-Val^5 analogue was not [LEARY and LEDINGHAM, 1970 (2)]. OSBORNE et al. (1970) have also perfused isolated rat kidneys by the method of NISHITSUTSUJI-UWO et al. (1967) using [Asp^1-H_3-Tyr^4-Val-]-Ang. II as substrate and the superfusion technique of REGOLI and VANE (1966) to assay biological activity of the peptide. Identification of radioactive compounds in the effluent perfusate was achieved by Sephadex filtration, immunoabsorption, paper chromatography and immunoassay. Disappearance of Ang. II during perfusion was found to be due to enzymatic hydrolysis rather than to specific or non-specific binding to receptor sites. The presence of 2-8 heptapeptide, 3-8 hexapeptide and 1-4 tetrapeptide in the perfusate suggests predominant aminopeptidase and endopeptidase activity in the isolated perfused kidney.

Indirect evidence for the importance of the kidney in angiotensin inactivation has been presented by several workers. BUMPUS et al. (1964) showed that in rats infused with tritiated angiotensin, the radioactivity was concentrated largely in kidney, uterus and the adrenals. BOURGOIGNIE et a. (1969) reported an increased sensitivity to infused angiotensin in nephrectomized rats. Using an immunoassay technique in the dog BAILIE et al. (1971) have found that Ang. II in the renal vein plasma is less than that in arterial plasma, indicating renal extraction of the peptide from plasma. Since no evidence was found to indicate significant plasma angiotensin entry into renal lymph, it was suggested that hydrolysis of the peptide occurred in the kidney.

Conclusions. Inactivation of Ang. II analogues by the kidney has been measured in a variety of circumstances. Methods of study include incubation of the peptide

with kidney homogenates or sub-cellular fragments and perfusion of intact organs in vivo and in vitro. Inactivation has been expressed in biological terms and, in some studies, peptide fragments have been identified by chromatography. This evidence, taken in conjunction with the behaviour of kidney peptidases in the presence of enzyme inhibitors, suggests that an aminopeptidase and one or more other angiotensinases exist in mammalian kidney.

Liver Angiotensinases

Tissue Extracts. In a study comparing angiotensinase activities from various tissues in the rat ITSKOVITZ and MILLER (1967) found extracts of intestinal mucosa, kidney cortex and liver to be the most potent sources. MATSUNAGA and his colleagues, in their investigation of microsomal and lysosomal angiotensinases in rat tissues, have found liver extracts second only to kidney in content of acid and neutral angiotensinases (MATSUNAGA et al., 1969). JOHNSON and RYAN (1968) investigated the degradation of [Asn^1-Val^5]-Ang. II and its Ileu^5 analogue by aqueous extracts of rabbit liver, buffered at pH 7.4. Chromatography after incubation showed hydrolysis of all bonds in each peptide, implying the presence of several enzymes. When aminopeptidases were inhibited by dialysis of the extract against ethylene-diamine-tetraacetic acid (EDTA), angiotensinase was reduced by 60%. The residual peptidase activity was shown to be due to a carboxy peptidase, similar to pancreatic Carboxypeptidase A.

Intact Organ Experiments. Following the claim that experimental renal hypertension in dogs can be reversed by anastamoses of the renal and portal veins, METHOT et al. (1964) in the rat and CHAMBERLAIN et al. (1964), in dogs and man, performed experiments to show whether or not intact liver was capable of destroying Ang. II. Both groups compared portal with systemic venous injections of [Asn^1-Val^5]-Ang. II, and both found considerable reduction in blood-pressure response when the peptide was given via the portal vein. WERNZE and FUJII (1966), using the same technique in rats confirmed hepatic inactivation of [Asn^1-Val^5]-Ang. II when injected into the portal vein, but found no difference between the pressor effects of the β-Asp^1-Val^5-peptide given by femoral or portal vein. LEARY and LEDINGHAM (1968) also compared pressor responses to injections of angiotensin given by portal and jugular veins of rats but used small doses (5 to 15 ng) of [Asp^1-Ileu^5]-Ang. II in contrast to the much larger doses (500 to 1000 ng) of Asn^1 analogue given by both METHOT et al. and WERNZE and FUJII. No difference was found in pressor response to portal and jugular vein injections between 5 ng and 15 ng; but as the doses of angiotensin were increased from 20 ng to 200 ng there was an increasing and significant deficit in the portal vein pressor response. The failure to detect loss of angiotensin in the hepatic circulation of the rat when small amounts of peptide were injected, may reflect the insensitivity of pressor assay used. BIRON et al. (1968) report 60 to 70% inactivation of [Asn^1-Val^5]-Ang. II and of its 86% pure β-Asn^1 analogue in the hepatoportal circulation of both rats and dogs.

The *in vivo* studies so far discussed necessitate the use of very large amounts of Ang. II. More physiological doses can be assessed by the superfusion techniques of REGOLI and VANE (1966). Losses of about 70% were found during infusions containing 1 to 5 ng/ml of [Asn^1-Val^5]-Ang. II into the hepatic circulation of dogs (HODGE et al., 1967).

The isolated liver perfusion technique of LEARY and LEDINGHAM [1969; 1970 (1)] allows the effects of peptidase inhibitors to be assessed in the inactivation

of angiotensin analogues by the intact rat liver. Livers perfused with an artificial Krebs' medium are very active in extracting α-[Asn^1-Val^5]-, α-[Asp^1-Val^5]-, α-[Asp^1-Ileu^5]- and β-[Asp^1-Val^5]-Ang. II (LEARY and LEDINGHAM 1970 (2)]. Inactivation of α-[Asn^1-Val^5]-Ang. II is partially inhibited by EDTA, 3×10^{-3}M, and dimercaprol 4×10^{-4}M in the medium completely prevented hepatic extraction of this analogue (LEARY and LEDINGHAM, 1969).

Conclusions. A variety of experimental techniques indicate conclusively that the liver is an important site of Ang. II catabolism. The precise nature of the angiotensinases concerned is not yet known, but the large number of analogues inactivated by the liver, and the effects of inhibitors suggest the presence of a number of different enzymes.

Lung Angiotensinases

Tissue Extracts. Ang. II is inactivated when incubated with extracts of rat and dog lung (ITSKOVITZ and MILLER, 1967; BAKHLE, 1968; MATSUNAGA et al., 1969).

Intact-Organ Experiments. Inactivation of Ang. II in the intact lung has been studied in man and animals by a variety of methods. No change in systemic-pressor response to the peptide was observed in rats by GOFFINET and MULROW (1965) in rats and dogs by BIRON et al. (1968) or in sheep and dogs by OSBORN et al. (1969) whether they infused the peptide proximal or distal to the pulmonary circulation. Subsequently BIRON et al. (1969) repeated these experiments in man and found that the pressor potency of Ang. II given into the pulmonary artery was equivalent to that obtained from aortic injection of the peptide.

HODGE et al. (1967) detected no apparent inactivation of Ang. II in the lungs of anaesthetized dogs investigated by the infusion — superfusion technique of REGOLI and VANE (1966).

BAKHLE et al. (1969), LEARY and LEDINGHAM (1969) and BIRON and CAMPEAU (1971) reported negligible losses of Ang. II biological activity during passage of the peptide through isolated lung of several species perfused with erythrocyte free solutions.

In the studies reported above, angiotensinase activity was measured in the lung by bioassay only. The fact that Ang. II loses no biological activity during passage through the lung does not rule out the possibility that some hydrolysis of the peptide occurs, coupled with the release of a second biologically active substance by the perfused organ. RYAN et al. (1970) and TÜRKER et al. (1971) have both reported the presence of substances in effluent from isolated rat lung preparations that support this possibility. These results are not conclusive. Before studying the metabolism of Ang. II in the lung TÜRKER et al. (1971) excised the organ and perfused it for 30 to 40 min. This prolonged period of perfusion might have damaged the lung with release of intracellular enzymes not normally active in vivo. RYAN et al. (1970) perfused lung by the method of LEARY and SMITH (1970) which causes negligible tissue damage as assessed by electron microscopy, but used highly unphysiological concentrations of peptide in their experiments.

Conclusions. Peptidases capable of hydrolysing Ang. II are present in lung tissue. Evidence from *in-vivo* lung perfusion studies indicates that only negligible inactivation of the peptide occurs during passage through the lung. Recent studies suggest that peptidase activity coupled with more complex changes, including the release of a hitherto unidentified vaso-active substance, may occur within the lung.

Cardiac Angiotensinases

Homogenates of cardiac tissue are capable of hydrolyzing Ang. II, but the angiotensinase content is low compared with homogenates of intestinal mucosa, kidney and liver (Fasciolo et al., 1940; Itskovitz and Miller, 1967). Rat coronary vascular bed perfused with modified Kreb's solution is active in extracting [Asp^1-Val^5]-Ang. II, hydrolyzing some 30 to 70% of injected peptide in a single circulation (Biron, 1969; Osborne et al., 1970; Türker et al., 1971).

Hind Quarter Angiotensinases

The metabolism of Ang. II in isolated hindquarter preparations has been studied in dogs (Hodge et al., 1967; Biron et al., 1968), rats (Biron et al., 1968; Bakhle et al., 1969; Biron and Campeau, 1971), and sheep (Osborn et al., 1969). Hind limbs were perfused through femoral artery catheters and peptide in the effluent blood or Krebs' solution was assayed by superfusion (Hodge et al., 1967; Bakhle et al., 1969) rat colon bioassay (Biron and Campeau, 1971) or monitoring of the pressor response in the experimental animal (Biron et al., 1968; Osborn et al., 1969). All groups reported that 50 to 70% of the peptide was inactivated during a single passage through the limbs.

Changes in Angiotensinase Activity in Various States

Changes in Plasma and Serum

There have been many attempts to correlate changes in plasma angiotensinase activity with disease or altered physiology, but results have been largely inconsistent (Page and McCubbin, 1968). The lack of a standardized method of angiotensinase assay and the relative unimportance of plasma enzymes in angiotensin catabolism (Hodge et al., 1967), lessen the significance of the findings reviewed below.

1. *Hypertension.* There is no consistent evidence of altered angiotensinase activity in plasma in various forms of spontaneous and experimental hypertension in man or animals (Dexter, 1942; Haynes and Dexter, 1945, 1947; Wolf et al., 1961; Wood, 1962; Klaus, 1962; Hickler et al., 1963; Lagrue and Meyer, 1963; Biron et al., 1964; Nagatsu et al., 1965; Itskovitz et al., 1957; Lubash et al., 1967).

2. *Liver Disorders.* Marked increases in plasma angiotensinase activity in patients with cirrhosis and other hepatic disorders have been reported from many laboratories (Hickler et al., 1963; Klaus et al., 1963; Biron et al., 1964; Kokubu et al., 1965; Hirasawa, 1968; Page and McCubbin, 1968). It has been suggested that such increased activity might contribute to the improvement of arterial blood pressure in some hypertensive patients who have developed liver disease. (Kokubu et al., 1965; Hirasawa, 1968; Page and McCubbin, 1968). More recent studies comparing α-[Asn^1-Val^5]-Ang. II and α-L-aspartyl-β-naphthalmide (α-ANA) as substrates in serum from normal and cirrhotic patients, suggest the increased enzyme activity found in liver disease may relate only to angiotensinase A_1 and not to the peptidase more specific to naturally occurring Ang. II [Nagatsu et al., 1965 (2); Lubash et al., 1968). A physiologically important change in plasma angiotensinase activity in cirrhosis remains unproven.

3. *Pregnancy.* Klaus et al. (1963) and Landesman et al. (1963) were unable to find differences in the rates of destruction of [Asn^1-Val^5]-Ang. II in the plasma of pregnant women compared with non-pregnant controls. Nagatsu et al. [1965 (2)]

also found inactivation of [Asn^1-Val^5]-Ang. II to be normal, while α-ANA was more rapidly destroyed in the serum of pregnant women in the third trimester, suggesting a selective increase in Angiotensinase A_2 activity. LUBASH et al. (1969) differ from NAGATSU et al., claiming not only increased hydrolysis rates of α-ANA but also of [Asn^1-Val^5]-Ang. II in the third trimester sera. It is unlikely that such changes, even if consistently detected, are of serious importance.

4. *Shock.* ITSKOVITZ et al. (1969) have reported enhanced inactivation of both [Asn^1] and [Asp^1]-Ang. II analogues by plasma from dogs shocked by haemorrhage or by E. coli endotoxin.

Effects of Changes in Sodium Balance and Other Conditions on Angiotensin-II Catabolism

Sodium balance. LEARY and LEDINGHAM [1970 (1 and 2)] have perfused isolated rat liver and kidney from animals previously fed on low- and high-sodium diets. [Asn^1-Val^5]-Ang. II or its Asp^1-Val^5- and Asp^1-Ileu^5-analogues were added to the perfusion medium, and the time course of destruction of peptide determined by bioassay of serial samples over a 6-min period. Hepatic inactivation was not altered by changes in sodium balance. In contrast the capacity of the isolated kidney for to inactivation of Ang. II analogues was markedly reduced after sodium loading and significantly increased after depletion of salt. BIRON and CAMPEAU (1971), using a different technique, have found a 20% increase in inactivation of Ang. II analogues in kidneys from adrenalectomized salt-depleted rats, and a 20% decrease when the animals had previously been salt loaded, but their results were not statistically significant.

There is other evidence that changes in sodium balance may alter angiotensin catabolism. JOHNSTON et al. (1972) have examined the metabolic clearance rate (MCR) of Ang. II in hypertensive and normotensive man in states of sodium loading and depletion. In normotensives, the MCR increased significantly from 2.23 ± 0.36 L/min in repletion, to 3.19 ± 0.34 L/min in depletion of sodium. MCR in hypertensive patients on a high salt intake was significantly higher than in normotensives and did not increase with sodium depletion. BRUNNER et al. (1972) infused rabbit Ang. II anti-body intravenously in anaesthetized rats, and determined the amount required to prevent the pressor response to exogenous Ang. II. This amount was found to be 8-fold higher in sodium-loaded animals than in those on a low-salt diet. Although BRUNNER et al. (1972) have suggested their data may indicate to changes in the affinity of vascular receptors to Ang. II, with changes in sodium balance, their results, and those of JOHNSTON et al. (1972), are also compatible with a reduction of tissue-angiotensin degradation in sodium-loaded animals. LUBASH et al. (1971) report results differing from those of JOHNSTON et al. The latter found plasma Ang. II levels to rise less in sodium depletion than in sodium repletion, during infusion of Ang. II amide: LUBASH et al. report the opposite, and found the half-life of Ang. II prolonged in the presence of salt depletion.

JELINEK et al. (1971) have recently reported that the angiotensinase content of rat kidney homogenates is lower than normal in both sodium-depleted and sodium-loaded animals. These results are difficult to interpret, since the capacity of organs to destroy angiotensin in vivo is unlikely to be reflected accurately by work using tissue homogenates [JOHNSON and RYAN, 1968; LEARY and LEDINGHAM, 1970 (1); OSBORNE et al., 1970; BIRON and CAMPEAU, 1971)].

Conclusion. There is now suggestive but not conclusive evidence that renal angiotensin catabolism may be reduced in salt loading and increased in salt

depletion, perhaps selectively in the renal vascular bed. This hypothesis is compatible with the long recognized variation in pressor sensitively to angiotensin with changes in salt balance in man and animals (REID and LARAGH, 1965; OSTROVSKY and GORNALL, 1964; KUCHEL et al., 1964; LARAGH, 1962; KAPLAN and SILAH, 1964; AMES et al., 1965).

Experimental Hypertension. LEARY and LEDINGHAM [1970 (1)] examined the effects of unilateral renal artery clips with or without contralateral nephrectomy on the capacity of isolated perfused rat kidneys to inactivate [Asn^1-Val^5]-Ang. II. In rats with one renal artery constricted and the other intact, considerably less peptide was removed by the intact kidney than the ischaemic kidney: in this situation the ischaemic kidney inactivated angiotensin at the same rate as control normal organs. In rats with one kidney removed and the remaining renal artery constricted by a silver clip, the ischaemic kidney removed less peptide from perfusate than its controls. Thus the overall capacity of the renal vascular beds to inactivate angiotensin is reduced in both experimental hypertensive situations, perhaps relating to sodium retention at least in the uninephrectomized rats. BRUNNER et al. (1972) also studied renovascular hypertension with or without contralateral nephrectomy in rats, and showed a 2-fold increase in the amount of rabbit anti-angiotensin II antibody required to block the pressor effects of exogeneous angiotensin. Taken together, the data suggest the possibility that a decreased catabolism of Ang. II may contribute to GOLDBLATT hypertension in the rat.

Experiments relating kidney homogenate angiotensinase to experimental renal hypertension have not produced consistent results (BLAQUIER et al., 1961; BING, 1962; HIRASAWA, 1969; JELINEK et al., 1971).

Effects of Carbon Tetrachloride in Rat Liver. WERNZE and FUJII (1966) found the pressor sensitivity to [Asn^1]-Ang. II of rats was reduced if they had previously been given carbon tetrachloride (CCl_4), but the response to portal vein versus femoral vein injections indicated that the capacity of the liver to inactivate angiotensin was not impaired. BIRON and MEYER (1968) confirmed reduced pressor sensitivity in CCl--treated rats, but also reported a partial or total loss of the capacity of the poisoned livers to destroy either α-Asp^1 or β-[Asp_4]-Ang. II. HIRASAWA (1968) in contrast found evidence of increased angiotensinase activity in liver homogenates, intact infused livers, and the plasma of rats previously given CCl_4. Data so far is therefore conflicting: the different doses of carbon tetrachloride used, and the poor and variable state of health of the animals may account for these discrepancies.

Angiotensinase Assays

In ideal circumstances, enzymes are assayed after purification, in conditions of constant pH, temperature, ionic strength, specific ionic concentration and amount of substrate present. The rate of the reaction may then be taken as an index of the activity of the enzyme, and such activity may be expressed as units per mg of protein or per mg nitrogen. The kinetics of the reaction between the enzymes and its substrate should be determined by a series of estimations of substrate or reaction product concentrations, with time, in the presence of a standard amount of enzyme. In zero-order kinetics, the reaction is linear with time and rate is simply estimated. In first-order kinetics, when the reaction rate depends on the substrate concentration, the equation describing the first -order reaction may be used to express enzyme activity. When higher-order or more complex reactions

occur the empirical device often used to establish an assay is the measurement of the *initial* rate of the reaction, which tends to approach zero order.

In the assay of angiotensinases in vitro or in vivo, these simple principles of enzyme assay (MEHLER, 1957) either cannot be, or too often have not been, observed. Most estimates of angiotensinase activity in vitro have depended upon the incubation under standard conditions of a known concentration of Ang. II (or an analogue), with plasma, serum or tissue extract, and assay of residual pressor activity after variable time intervals. It cannot be over-emphasized that single-point assay systems are valueless in the assessment of enzyme activity, unless the reaction kinetics have been studied, and it is known whether zero-, first- or higher-order kinetics pertain. Estimates of enzyme activity based on single-point assay systems occur all too frequently in the literature on angiotensinases. This kind of error is easily avoided but there are other difficulties which are not so easily solved.

In-Vitro Assays: plasma, serum and tissues contain a variety of peptidases, so that more than one enzyme is always present, and pure preparations of angiotensinase enzymes have only very rarely been achieved (NAGATSU et al., 1970). In spite of these difficulties some useful estimate of angiotensinase activity in vitro can be achieved provided samples are assayed at frequent intervals after incubation and rates are calculated from the linear part of the curve relating angiotensin survival with time. The time taken for a 50-percent loss of substrate (T50%) may be the best index, and has been successfully used by JOHNSON (1967), JOHNSON and RYAN (1968), YANG et al. (1968) and JELINEK et al. (1971). Substrates other than Ang. II may occasionally be of value in the assay of individual angiotensinases [NAGATSU et al., 1965 (2), 1970] and reaction rates may be estimated from rate of yield of individual amino acids [NAGATSU et al., 1965 (1)].

In-Vivo Assays: The single-pass superfusion assay of angiotensinase activity (HODGE et al., 1967; BAHKLE et al., 1969; BIRON et al., 1968; BIRON and CAMPEAU, 1971; OSBORNE et al., 1970) whether applied to anaesthetized animals, or to isolated perfused organs does not allow any estimate of rate of loss of angiotensin with time. Activity of angiotensinases in organ vascular beds can therefore only be assessed in terms of percentage loss of angiotensins in a single circulation. Change of activity in altered physiological or in pathological states may therefore be difficult or impossible to quantitate.

The isolated perfused organ techniques of LEARY and LEDINGHAM [1961, 1970 (1 and 2)] allow recirculating medium to be assayed for residual angiotensin at regular intervals over relatively prolonged periods. [Asn^1-Val^5], [Asp^1-Val^5] and [Asp^1-Ileu^5]-Ang. II in concentrations of 66 or 133 ng/ml were destroyed by the perfused rat liver or kidney at a rate linear with time up to 6 min. Between 6 and 14 min there was evidence of substrate concentration becoming rate-limiting (LEARY and LEDINGHAM — unpublished observations). This technique therefore allows rates of inactivation to be compared in various physiological and pathological states, with or without the addition of enzyme inhibitors to the medium. Application of immunoassay methods to this technique, so that more physiological concentrations of angiotensin can be used, will offer obvious advantages over the bioassay methods so far employed.

Metabolic clearance rates of angiotensin. JOHNSTON et al. (1972) have devised a method of estimating the metabolic clearance rate (MCR) of Ang. II in man. [Asn^1-Val-]-Ang. II (Hypertensin Ciba) was infused intravenously at a subpressor dose of 100 ng per min for 2 h. Plasma renin and angiotensin levels were measured before and after the infusion and the clearance rate calculated with a correction applied for the renin-suppressing effect of the infusion. The MCR was shown to be

constant over infusion rates of 100 to 1000 ng/min and was not therefore altered by the infusion itself.

Difficulties particular to Ang. II metabolism arise in the accurate estimation of MCR, and these have been discussed by JOHNSTON et al. Moreover clearance data data do not distinguish receptor uptake (LIN and GOODFRIEND, 1970; GOODFRIEND and LIN, 1970) from tissue degradation of angiotensin.

Summary

The term angiotensinase is used to describe a number of enzymes capable of hydrolyzing Ang. II, but not proven to be specific to it.

Tissue extract and homogenate experiments have been helpful in the detection of separate aminopeptidases, endopeptidases and carboxypeptidases, but cannot be taken to reflect angiotensin catabolism in vivo.

The techniques devised over the last 5 years for the study of angiotensin inactivation by intact organs have been productive. There is now more than a hint that angiotensin catabolism in tissue vascular beds may be as important as its rate of formation in determining physiological activity at receptor sites.

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PAPER A8

Impaired Prostaglandin Release from the Kidneys of Salt-Loaded and Hypertensive Rats

This study was based on an idea which emerged from discussions between the authors. All experiments were carried out by me and the paper was written with Dr. J.G. Ledingham. Dr. J.R. Vane edited the final draft.

IMPAIRED PROSTAGLANDIN RELEASE FROM THE KIDNEYS OF SALT-LOADED AND HYPERTENSIVE RATS.

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ABSTRACT.

Prostaglandin (PG) release by the isolated perfused kidney of the rat has been stimulated by nor-adrenaline infusion and measured by bioassay. There was no basal output of PGE_2-like activity, but stimulated release reached mean concentrations of 9.1 ng/g kidney/ml perfusate in kidneys from female albino rats drinking water and 2.9 ng/g/ml in those from animals given 1.5% NaCl to drink. Kidneys from uninephrectomised animals with mock-clipped renal arteries released 7.3 ng PG/g/ml and those from rats with uninephrectomy and constricted renal arteries 3.3 ng/g/ml.

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INTRODUCTION.

Prostaglandins (PG) are released continuously by the kidneys of anaesthetised dogs and may be detected in their renal venous blood(1). PG release increases after several different stimuli to the kidney including ischaemia, renal sympathetic nerve stimulation, and intra-arterial infusion of nor-adrenaline, bradykinin or angiotensin(2). The main PG detected is PGE_2, or like PGE_2, and probably acts intrarenally as a vasodilator and natriuretic(3). Abolition of PG release by indomethacin, a potent inhibitor of PG synthesis, greatly augments the renal vasoconstrictor effects of angiotensin II and interferes with the autoregulation of renal bloodflow(4,5). Thus, PGs probably play an important role in the regulation of renal blood flow and because of this, we have studied the capacity of rat kidneys to release PGs under conditions known to induce hypertension. PG release by the isolated perfused rat kidney was stimulated by nor-adrenaline infusion in various groups of normotensive and hypertensive albino rats and measured by bioassay.

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MATERIALS AND METHODS.

Female albino rats were kept on a standard diet and given tap water to drink *ad lib*. Sodium chloride was added (1.5% w/v) to the drinking water of some rats ("high salt") and a unilateral nephrectomy performed on others. The remaining kidney in these rats was either manipulated ("mock clip") or mobilised, after which the renal artery was constricted with a small silver clip.

Each week animals were weighed and arterial blood pressure was measured by the tail-cuff method. Experimental conditions were maintained for at least one month before kidney perfusion was carried out. Mean blood pressures (mm Hg ± SEM) in the four groups were then: 119 ± 2.4 for controls, 126 ± 4.5 for the "high salt" group, 134 ± 3.8 for "mock clip" uninephrectomised animals, and 163 ± 11.2 for uninephrectomised rats with a constricted renal artery.

Rats were selected in random order and a single kidney was perfused *in situ* using the method previously described(6). Oxygenated Krebs' solution (pH 7.4) was warmed to 37°C and delivered to the renal artery from a constant output pump at 5 ml/min. A second roller pump took half of the renal outflow to superfuse a series of isolated assay tissues, the excess effluent running to waste against a hydrostatic pressure of 4 - 8 cm. Three assay tissues were used, usually including a rat stomach strip, rat colon, and chick rectum to allow differentiation between prostaglandins of the E and F series(7). Assay tissues were rendered insensitive to catecholamines, acetylcholine, histamine and 5-hydroxytryptamine by infusing a mixture of antagonists(7) into the Krebs' solution distal to the perfused kidney. Indomethacin (4 µg/ml) was added to the solution superfusing these tissues to inhibit any synthesis of prostaglandins by them and to make them somewhat more sensitive to PGs(8). The perfusion rate of 5 ml/min gave a renal perfusion pressure of 100-140 mm Hg.

When perfusion of the kidney was first started, the assay tissues contracted, representing a PG-like activity of 0.2 - 3.0 ng/ml (assayed as PGE_2). The assay tissues then relaxed and returned to their previous baseline within a few minutes, as blood washed out of the kidney. At this time the PG precursor arachidonic acid (40 ng/ml) was infused into the renal perfusion inflow; there was no increase in PG output in response to arachidonic acid alone. After a further 5 minutes, nor-adrenaline was infused intra-arterially for 5 min at a rate (6 - 10 ng/ml) which gave a rise in arterial pressure of 100 mm Hg. Renal perfusion pressure was measured on a mercury manometer and recorded manually. It returned to previous values within two minutes of discontinuing the infusion. A substantial release of PG-like activity into the renal effluent took place and this was estimated by bracketing the effects on the assay tissues between contractions induced by

PGE_2 infusions given directly to the assay tissues immediately before and after each nor-adrenaline infusion.

RESULTS.

The PG-like activity released had the same pattern of activity as PGE_2 on the three assay tissues. Any appreciable release of PGF would have caused a much greater relative contraction of the rat colon preparation than that recorded in these experiments.

A second infusion of nor-adrenaline induced a similar release of PG-like activity, except after infusion of indomethacin (4 µg/ml) through the kidney, when release of PG-like activity by nor-adrenaline was abolished.

The results of the experiments in the four different groups of rats are shown in Tables I and II. After nor-adrenaline stimulation, the PG-like activity of renal effluent reached a mean peak concentration of 10.4 ng/ml in control rats with both kidneys intact and 10.9 ng/ml in the uninephrectomy "mock clip" group. Expressed as output per g kidney, release was 9.1 and 7.3 ng/g/ml. PG-like activity in renal effluent of the two other groups was much less. Kidneys from salt-loaded animals released a mean of 3.5 ng/ml (2.9 ng/g/ml) and those from animals with constricted renal arteries, 5.2 ng/ml (3.3 ng/g/ml). Compared with the relevant control groups, each of these reductions was highly significant ($p < 0.001$).

DISCUSSION.

No single cause for the development and maintenence of raised arterial pressure has been found in experimentally induced hypertension or in human benign essential hypertension, although the probable aetiological importance of the kidney has been repeatedly stressed[9].

The discovery of renomedullary PGs with marked hypotensive and natriuretic properties has renewed interest in the antihypertensive properties of the kidney and in the possibility that humoral vasodilators and constrictors are both of importance in the control of arterial pressure[10].

There are at least two general ways in which release of a vasodilator PG can contribute to a lowering of blood pressure. The first is by a local vasodilation within the kidney, thereby tending to reverse a reduction in oxygen supply caused by a reduction in blood flow or in oxygen availability. Such a local effect may modify renin release. The second is for the released PG to have a circulating function, inducing

vasodilatation not only in the kidney but also in other parts of the body.

Prostaglandins of the A series are more likely candidates for a circulating role, for they escape prodigious inactivation in the pulmonary circulation(11), as occurs with PGs of the E series(12).

All the stimuli so far tested increase release of PGEs from the kidney and increased PGA release has not yet been detected(4,5,13,14). Thus, it is likely that renal PG release has a local, rather than a circulating role. This conclusion is supported by experiments in which PG synthesis and release are abolished by indomethacin; blood flow and autoregulation are decreased, and the effects of vasoconstrictor agents are increased selectively in the kidney(4,5).

Our experiments show that chronic sodium loading or renal arterial constriction impair the ability of the rat kidney to release PGs. Zusman *et al* (15) found low PG levels in the plasma and kidney of salt-loaded rats and Nekrasova *et al* (16) reported a decrease in the PG content of ischaemic kidneys in uninephrectomised rabbits. These results may also reflect an impaired PG biosynthesis.

Leary & Ledingham(6) showed that kidneys from rats subjected to similar experimental procedures had a reduced capacity to inactivate angiotensin II. Thus, a reduced availability of renal PGs and an increased activity of angiotensin II could both contribute to the pathogenesis of experimental hypertension.

Certainly, our results show that interference with a prostaglandin mechanism cannot by itself account for hypertension. Thus, the rats with uninephrectomy and manipulation of the other kidney in the "mock clip" control group also showed a raised blood pressure, possibly due to slight inflammation around the remaining kidney, with some compromise of the renal circulation. However, in this group, there was no significant fall in PG release and no clear correlation between arterial pressure and PG release in individual animals. The failure of sodium loading to produce hypertension, despite the reduction in PG release is another indication that more than one mechanism must be involved, as is the fact that chronic treatment with prostaglandin synthetase inhibitors, such as aspirin and indomethacin, has never been reported to cause hypertension.

TABLE I.

PROSTAGLANDIN RELEASE BY ISOLATED PERFUSED KIDNEY FROM THE RAT: EFFECT OF SODIUM LOADING

Experimental Group	Weight (g ± SEM)	No. of rats	Arterial pressure (mm Hg ± SEM)	Kidney weight (g ± SEM)	Stimulated PG output per kidney (ng/ml perfusate ± SEM)	PG output (ng/g kidney/ml perfusate ± SEM)
Controls	279.0 ± 13.7	7	119.4 ± 2.4	1.14 ±0.05	10.4 ± 1.18	9.1 ±0.98
High salt intake	250.3 ± 5.7	6	126.6 ± 5.6	1.18 ±0.05	3.5 ± 0.61	2.9 ±0.49
Signif-cance	ns		ns	ns	P <0.001	P <0.001

TABLE II:

PROSTAGLANDIN RELEASE BY ISOLATED PERFUSED KIDNEY FROM THE RAT, EFFECT OF A CLIP ON RENAL ARTERY.

Experimental Group	Weight (g ± SEM)	No of rats	Arterial pressure (mm Hg ± SEM)	Kidney weight (g ± SEM)	PG output per kidney (ng/ml perfusate ± SEM)	PG output (ng/g kidney/ml perfusate ± SEM)
Uninephrectomy "Mock Clip"	260.1 ± 11.9	7	134.1 ± 3.9	1.67 ±0.09	10.9 ± 1.16	7.3 ±0.71
Uninephrectomy Clip	244.7 ± 14.7	7	163.3 ± 11.2	1.83 ±0.11	5.2 ± 0.62	3.3 ±0.52
Significance	ns		P <0.05	ns	P <0.001	P <0.001

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PAPER A9

Pulmonary Inactivation of Prostaglandin by Hypertensive Rats

This was entirely my own work. The co-authors provided editorial comment only.

PULMONARY INACTIVATION OF PROSTAGLANDIN BY HYPERTENSIVE RATS.

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ABSTRACT

Lungs of normotensive and genetically hypertensive (GH) albino rats were perfused in situ with 5 ml/min Krebs solution containing 100 ng/ml PGE_2 or $F_2\alpha$. Inactivation rates of 76,0 ng $PGF_2\alpha$ and 146,3 ng PGE_2/g dry wt. lung/min were recorded for GH rats. Values for normotensive controls were 191,2 and 226,1 ng/g/min.

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INTRODUCTION

Differences in systemic arterial pressure observed between genetically hypertensive (GH) albino rats and their controls may be related to a variation in the metabolism of vasoactive substances including catacholamines, angiotensin and prostaglandins. This paper reports experiments in which the lungs of GH and normotensive rats were perfused in situ and the pulmonary inactivation of PGE_2 and $F_2\alpha$ measured.

MATERIALS AND METHODS

Forty six male Wistar rats were studied. Twenty three were of the New Zealand genetically hypertensive (GH) strain and the remainder their normotensive controls. All weighed 220-350 g and were housed and fed identically with tap water ad libitum for a minimum of 10 weeks before any experiment. Systemic arterial pressures were measured in conscious animals every 14 - 21 days by the tail-cuff method and, in anaesthetised rats, by carotid cannulation immediately before thoracotomy. Normotensive and GH rats were perfused in random order, but experiments with PGE_2 were completed 12 months after $PGF_2\alpha$ studies.

Rats were weighed immediately before study and anaesthetised with intraperitoneal pentobarbitone 60 mg/kg. A fine plastic cannula was introduced into the left carotid artery and blood pressure measured by Statham strain-gauge transducer and traced on a Unicorder U400 pen recorder. The thorax was then opened and the great vessels, heart

and lungs prepared for perfusion in situ as described by Leary and Smith (1). The inferior vena cava was tied below its junction with the right atrium and the heart rotated anti-clockwise so that the left precaval and azygos veins could be mobilised and tied. Cannulae were placed in the left and right ventricles through the aorta and right precaval vessels which were then tied to secure the cannulae and prevent back perfusion.

Perfusion commenced at a pressure of 10-12 mm.Hg with 5ml/min unoxygenated Krebs Ringer solution (pH 7,4, 37°C), containing 100 ng/ml PGE_2 or $F_2\alpha$. The initial 2-5 ml blood-stained pulmonary venous effluent was discarded and subsequent perfusate collected for 15 minutes and assayed for PG-like activity by superfusion, of a rat stomach strip, chick rectum and rat colon using the method of Ferreira and Vane (2). Responsiveness of these tissues to PG was increased by continuous superfusion with a solution of pharmacological antagonists and indomethacin in normal saline at 37°C (3). Percentage PG activity was measured by bracket assay of pulmonary venous effluent with perfusate collected at the right ventricular cannula. Heart and lungs were excised after perfusion and dessicated over calcium chloride at room temperature so that dry weight of these tissues could be determined. In 4 additional experiments the lungs of 2 normotensive and 2 GH rats were perfused without adding PG to the Krebs solution. Effluent was assayed for the presence of endogenous PG.

The mean of 3 bracket assays for each perfusion was used to calculate PG inactivation as ng/g dry weight of lung. Mean data for control and GH rats were compared by an unpaired t test for sample means (Hewlett Packard 65, programme 1-38A).

RESULTS

The results of this study are shown in Table 1. GH rats have significantly heavier hearts and higher arterial pressures than their controls. Endogenous PG was not detected in the 4 samples assayed.

Pulmonary inactivation of PGE_2 was slightly greater than that of $PGF_2\alpha$ for both GH and control rats although this difference was not statistically significant except when percentages inactivated by GH rats were compared ($P = < 0,025$). Both prostaglandins were metabolised to a significantly greater extent in normotensive than GH animals.

DISCUSSION

Intravenous infusion of $PGF_2\alpha$ elevates the systemic arterial pressure in man and various animals including the rat, dog, monkey and baboon (4). Pressor activity is not mediated through the sympathetic nervous system

TABLE I

Pulmonary perfusion of normotensive and GH male rats with prostaglandins.
Data expressed as Mean ± S.E.M.

GROUP	DRY HEART MASS (g)	DRY LUNG MASS (g)	SYSTOLIC B.P. (mm.Hg)	$PGF_2\alpha$ INACTIVATED %	$PGF_2\alpha$ INACTIVATED ng/g DRY LUNG WT.
Normotensive n = 14	0,17 ±0,01	0,34 ±0,02	108,8 ± 3,00	60,8 ± 8,12	191,2 ± 29,24
Hypertensive n = 11	0,22 ±0,01	0,29 ±0,01	157,2 ± 3,89	23,0 ± 8,70	76,0 ±30,86
P value	$<$ 0,005	$<$ 0,025	$<$ 0,0005	$<$ 0,0025	$<$ 0,01
GROUP	DRY HEART MASS (g)	DRY LUNG MASS (g)	SYSTOLIC B.P. (mm.Hg)	PGE_2 INACTIVATED %	PGE_2 INACTIVATED ng/g DRY LUNG WT.
Normotensive n = 9	0,19 ±0,01	0,37 ±0,05	102,2 ± 6,19	73,3 ± 6,22	226,1 ± 33,43
Hypertensive n = 12	0,27 ±0,01	0,39 ±0,03	173,3 ± 6,20	53,7 ± 9,17	146,3 ± 27,53
P value	$<$ 0,0005	$>$ 0,3	$<$ 0,0005	$>$ 0,05	$<$ 0,05

in rats, but probably results from peripheral vasoconstriction leading to an increased venous return and cardiac output. Prostaglandins of the E series lower arterial pressures in most laboratory animals, (4) but may produce a biphasic response in the rat with a resultant nett increase in blood pressure (5). The part played by endogenous PG in regulating arterial pressure is uncertain, although the cardiovascular and renal effects of prostaglandins may be as important to homeostasis as those of angiotensin, aldosterone and the kinins. Abnormal synthesis or inactivation of PG might contribute to the pathogenesis of hypertension in some circumstances.

Leary, Ledingham and Vane (6) have shown that chronic sodium loading or renal arterial constriction impair the ability of the rat kidney to release PGs and similar findings have also been reported by Zusman et al (7) and Nekrasova et al (8). The experiments reported in this paper indicate that pulmonary degradation of PGE_2 and $PGF_{2\alpha}$ is reduced in albino rats of the New Zealand GH strain. This is consistent with the findings of Armstrong et al (5) who have described a similar defect in the kidneys of GH rats. Unpublished work by the same group, using elegant in vitro techniques, confirm that lung tissue of GH rats is relatively deficient in PG 15-hydroxy-dehydrogenase. Whether this deficiency is limited to the GH strain of albino rats or whether it occurs in other forms of experimental and naturally occurring hypertension remains to be seen.

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PAPER A10

Enhanced Release of "PGI_2-like" Substance in Experimental Hypertension

This work was based on my ideas and followed a protocol which I designed. Dissections were carried out largely by me and platelet aggregation was measured by Dr. J. Botha. The publication was prepared in consultation with Dr. Botha. Professor A.C. Asmal's name was included as a courtesy and in recognition of his final editorial comments.

Prostaglandins and Medicine 3: 251-252, 1979. Short note

ENHANCED RELEASE OF 'PGI_2-LIKE' SUBSTANCE IN EXPERIMENTAL HYPERTENSION

J.H. Botha, W.P. Leary and A.C. Asmal. Pharmacology Department, University of Natal, Box 17039, Congella 4013, South Africa. (Reprint requests to JHB).

Blood vessels of spontaneously hypertensive rats increase conversion of arachidonic acid to PGI_2 (1) and generate increased endogenous PGI_2 (2,3). This short communication describes experiments which measured release of PGI_2-like substance from aortic strips of rats with surgically induced hypertension and their age, sex and weight matched normotensive controls.

Materials and Methods. Blood from a healthy human volunteer who had taken no drugs during the previous 2 weeks was processed as described by Sagel et al (4) using plastic apparatus to yield platelet rich (prp) and platelet poor plasma (ppp). Plasma was stored at room temperature under carbogen and used within 2h of venepuncture. Platelet aggregation was determined by the method of Born (5) in a dual channel model 340 Chronolog Aggregometer. Threshold aggregation was induced by ADP (1,6μM), (Sigma Ltd., St. Louis, U.S.A.). Calculations of percentage aggregation were made as described by Sagel et al(4) and two parameters were used for quantitation and comparison; a) the initial rate of aggregation, as indicated by the extent of aggregation in the first 0,5 minutes, and b) the extent of permanent aggregation at 4 minutes.

A group of male, weanling Wistar rats (University of Natal inbred strain), ±150g weight, were anaesthetised with pentobarbital (6mg/100g). A left uninephrectomy was performed in every animal through a midline abdominal incision. The right kidney was mobilised and wrapped with Mersilk in half the rats in order to induce hypertension (6). After 10 weeks systemic arterial pressures were measured in conscious animals, using the tail cuff method. Paired control and hypertensive rats were given water ad libitum and sacrificed by a blow to the neck after an overnight fast. The abdominal aorta was removed, cleared and rinsed with ice cold tris buffer (0,5 mmol/l pH 7,5). After adjustment to an approximate wet weight of 16 mg, the tissue was stored in 1,5 ml of buffer on ice until tested (within 30 minutes). Using a slight modification of the method of Moncada et al (7), each aorta was cut longitudinally into 2 strips which were then incubated in 700μl tris buffer at room temperature. PGI_2 generation was stimulated mechanically using a small plastic covered magnetic stirrer bar. At various times after incubation began, 20μl of supernatant were added to prp, 1 minute before the addition of ADP. The percentage inhibition produced by the incubate was assessed by comparing the two parameters a) and b) with those of control curves obtained by adding 20μl tris, at room temperature, to prp 1 minute prior to addition of ADP. Values for percentage inhibition were converted into absolute units, (ng PGI_2/wet weight of aorta), by comparison with standard curves obtained when synthetic PGI_2 sodium salt (Upjohn Ltd., Kalamazoo), was added to samples of the human donor's prp.

Results and Discussion. The results which are summarised in Table I show that generation of PGI_2-like activity by hypertensive rat aortas was significantly greater at each sampling time than that of their matched controls. The fact that the same ability to generate increased levels of PGI_2 has been found in induced hypertension as has been reported for spontaneous hypertension (1,2, 3) adds further credibility to the suggestion that this may be an adaptive response to raised arterial pressure.

	NORMOTENSIVE RATS (n=5)	HYPERTENSIVE RATS (n=5)	
	AGE: 19 weeks WT: 287,00 ± 10,68g BP: 109,80 ± 1,50mmHg	AGE: 19 weeks WT: 229,60 ± 15,39g BP: 144,00 ± 7,31mmHg	
INCUBATION TIME (MIN)	ng PGI_2/mg WET WEIGHT AORTA		LEVEL OF SIGNIFICANCE p
2	3,04 ± 0,38	4,90 ± 0,81	< 0,05
8	5,89 ± 0,34	7,56 ± 0,44	< 0,0025
18	6,19 ± 0,35	8,04 ± 0,29	< 0,01

Table I : ng 'PGI_2-like' activity produced by normotensive and hypertensive rat aortas. Results expressed as mean value ± SEM.

Acknowledgement: Miss A. von Middelkoop gave statistical advice; Upjohn Ltd., PGI_2; and M.R.C. (S.A.) financial support.

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PAPER A11

Enhanced Release of a "Prostacyclin-Like" Substance from Aortic Strips of Spontaneously Hypertensive Rats

This work was based on my ideas and followed a protocol which I designed. Dissections were carried out largely by me and platelet aggregation was measured by Dr. J. Botha. The publication was prepared in consultation with Dr. Botha. Professor A.C. Asmal assisted with editorial comment.

ENHANCED RELEASE OF A 'PROSTACYCLIN-LIKE' SUBSTANCE FROM AORTIC STRIPS OF SPONTANEOUSLY HYPERTENSIVE RATS

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ABSTRACT

The release of an endogenous 'prostacyclin-like' substance from aortic strips of 8 male Wistar rats of the New Zealand genetically hypertensive strain (GH) was compared with that of 8 weight, age and sex matched normotensive Wistar control rats. The amount of 'prostacyclin-like' substance released by the aortic strips into tris buffer, under the influence of mechanical stimulation, was measured by its ability to inhibit human platelet aggregation as compared to the inhibitory effect of standard prostacyclin sodium salt. It was shown that generation of this substance increased with incubation time and that a significantly greater amount was produced by GH rats.

INTRODUCTION

The isolation of prostacyclin (PGX later PGI_2) from vascular tissue by Moncada et al (1) and the suggestion that prostacyclin generation induced vasodilation and could inhibit intravascular platelet aggregation aroused interest in the production of this substance in pathological conditions associated with vascular disease, such as hypertension. Spontaneously hypertensive rats provide a useful experimental model for human essential hypertension (2) and have been widely studied, although the aetiology of the condition remains obscure. Pace-Asciak et al (3) have reported increased conversion of arachidonic acid to prostacyclin by intact rings and homogenates of aorta from spontaneously hypertensive rats of the Wistar Aoki-Okamoto strain, compared with their controls, and Minoru Okuma et al have shown significantly increased generation of prostacyclin by vessels of both stroke-resistant and stroke-prone spontaneously hypertensive rats of the Wistar-Kyoto colony prior to stroke (4).

In this paper we demonstrate that aortas of male rats of the New Zealand genetically hypertensive strain (GH) produce significantly more 'prostacyclin-like' substance than those of their age, weight and sex matched controls.

MATERIALS AND METHODS

Blood was withdrawn from the antecubial vein of a healthy human volunteer who had taken no drugs during the previous 2 weeks. The blood was processed as described by Sagel et al (5) using plastic syringes, containers and pipettes to yield platelet rich (prp) and platelet poor plasma (ppp). Plasma was stored at room temperature, under carbogen and

used within approximately 2 h of venepuncture. Platelet aggregation was determined by the method of Born (6) in a dual channel model 340 Chronolog Aggregometer. Threshold aggregation was induced by ADP 1,2μM, (Sigma Ltd., St. Louis, U.S.A.). Calculations of percentage aggregation were made as described by Sagel et al (5) and two parameters were used for quantitation and comparison :

a) The initial rate of aggregation, as indicated by the extent of aggregation in the first 0,5 minutes
b) The extent of permanent aggregation at 4 minutes.

Systemic arterial pressures were measured in conscious animals prior to the study, using an adaptation of the tail cuff method (7). Rats were given water ad libitum and sacrificed by a blow to the neck after an overnight fast. The abdominal aorta was removed, cleared and rinsed with ice cold tris buffer (0,5 mmol/l pH 7,5). After adjustment to an approximate wet weight of 21 mg, the tissue was stored in 1,5 ml of buffer on ice until tested (within 30 minutes). Using a slight modification of the method of Moncada et al (8), each aorta was cut longitudinally into 2 strips which were then incubated in 700μl tris buffer at room temperature. Prostacyclin generation was stimulated by mechanical means, using a small plastic covered magnetic stirrer bar. At various times after incubation began, 20μl of supernatant were added to the prp, 1 minute before the addition of ADP. The percentage inhibition produced by the incubate was assessed by comparing the two parameters a) and b) with those of control curves obtained by adding 20μl tris, at room temperature, to prp 1 minute prior to addition of ADP. Values for percentage inhibition were converted into absolute units, (ng prostacyclin/mg wet weight of aorta), by comparison with standard curves obtained when synthetic prostacyclin sodium salt (Upjohn Ltd., Kalamazoo) was added to samples of the human donor's prp (9).

RESULTS & DISCUSSION

The results which are summarised in Table I (and illustrated in Figure I), show that under the influence of mechanical stimulation generation of'prostacyclin-like' activity of GH rat aortas was significantly greater at each sampling time than that of their matched controls. This was consistent with the results of preliminary experiments with 10 pairs of matched rats in which mechanical stimulation was not used and 'prostacyclin-like' substance was produced at a slower rate.

These results are consistent with the findings of Pace-Asciak et al (3), who demonstrated increased prostacyclin synthesis from arachidonic acid by aortas of Aoki-Okamoto GH rats and with Minoru Okuma et al (4) who showed that, prior to stroke, elevated amounts of 'prostacyclin-like' substance were produced by spontaneously hypertensive rats (stroke-prone and stroke-resistant), of the Wistar-Kyoto colony.

Our findings support the suggestion that elevation in prostacyclin levels could be an adaptive response to hypertension and associated platelet activation and vascular injury although it remains to be seen whether such increases occur in all forms of experimental hypertension and if they can be reversed by hypotensive agents.

NORMOTENSIVE RATS		HYPERTENSIVE RATS	
AGE : 34-36 weeks		AGE : 34-36 weeks	
WT : 350,25 ± 6,10 g		WT : 303,75 ± 3,24 g	
BP : 121,25 ± 3,79 mmHg		BP : 198,75 ± 4,30 mmHg	
INCUBATION TIME (MIN)	ng PGI_2/mg WET WEIGHT AORTA		LEVEL OF SIGNIFICANCE p
2	1,66 ± 0,30	2,30 ± 0,23	< 0,025
8	3,17 ± 0,25	3,94 ± 0,20	< 0,025
14	3,80 ± 0,15	4,37 ± 0,13	< 0,005

TABLE I. ng 'PROSTACYCLIN (PGI_2)-LIKE' ACTIVITY PRODUCED BY NORMOTENSIVE AND HYPERTENSIVE RAT AORTAS.

The assumption that prostacyclin caused the inhibition of platelet aggregation is supported by the fact that aliquots of supernatant injected intravenously into pentobarbitol anaesthetised rats lowered the blood pressure (10). This fall in pressure was greater in response to extracts from GH rats than to samples of equal volume from their controls. The 'prostacyclin-like' activity generated was transient, which further supports the argument that the effects noted were not due to another PG. However, the 'prostacyclin-like' substance responsible for the biological changes observed requires positive identification and further studies are in progress to determine whether similar differences can be found between normotensive rats and others with secondary hypertension.

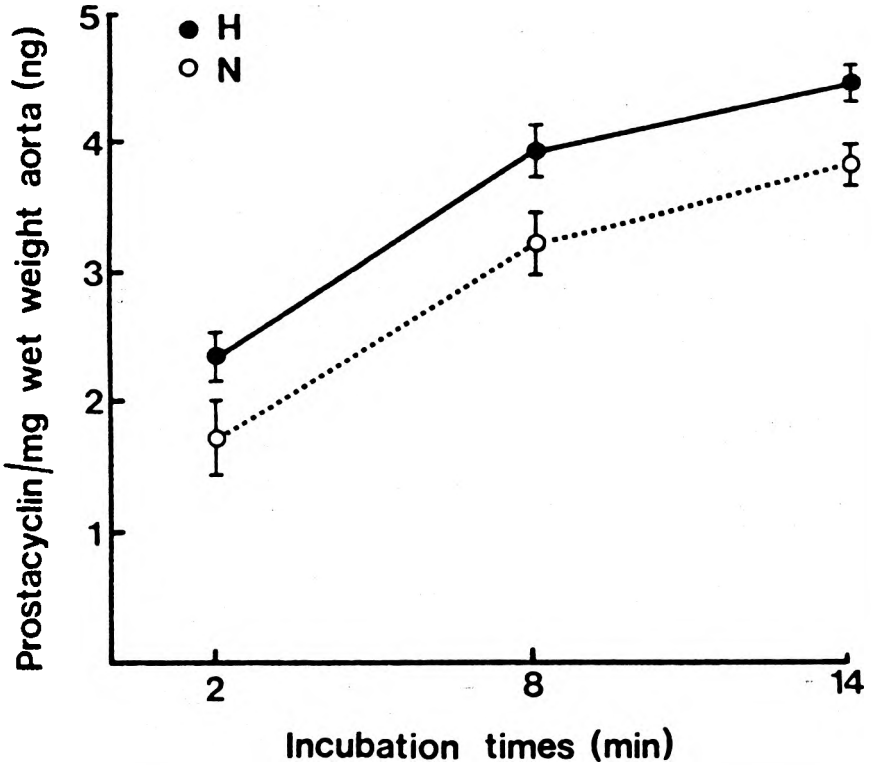

Figure 1. Apparent prostacyclin production by incubates of aortic strips from normotensive (N) and hypertensive (H) rats.

ACKNOWLEDGEMENTS

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PAPER A12

Mechanical Reduction in Pressure and Pulse Pressure Decreases the Ability of Hypertensive Rat Aortas to Produce "PGI_2-Like" Activity

This work was based on my ideas and followed a protocol which I designed. Dissections were carried out largely by me and platelet aggregation was measured by Dr. J. Botha. The publication was prepared in consultation with Dr. Botha and with editorial assistance from Professor A.C. Asmal.

Prostaglandins and Medicine 6: 267-268, 1981 Short Note

MECHANICAL REDUCTION IN PRESSURE AND PULSE PRESSURE DECREASES THE ABILITY OF HYPERTENSIVE RAT AORTAS TO PRODUCE 'PGI_2-LIKE' ACTIVITY

J.H. Botha and W.P. Leary. Pharmacology Dept., University of Natal, Box 17039, Congella 4013, South Africa. (Reprint requests to JHB.)

Blood vessels from rats with various forms of hypertension (spontaneous, induced renovascular and DOCA saline) have been shown to produce increased amounts of PGI_2 (1 - 5). To investigate whether this ability may be related to a local pressure effect we have measured production of a 'PGI_2-like' substance by rat abdominal aortas in which pressure had been lowered mechanically.

Materials and Methods. The systemic arterial blood pressures of a group of 16 male Wistar rats of the New Zealand genetically hypertensive strain (± 300g in weight and 12 - 16 weeks old) were measured in conscious animals using the tail cuff method. Pairs of rats were then anaesthetised with pentobarbital (6mg/100g). The aorta just below the diaphragm was mobilised in both animals through a midline abdominal incision. One animal acted as a sham control. The aorta of the other was constricted above the hepatic artery and as near to the diaphragm as possible by a silver clip (Scoville Lewis JN 1300IW) thus producing hypotension distally (6). Eighteen hours after the operation distal aortic pressures were measured by the tail cuff method in both clipped (C) and sham (S) rats which had been fasted overnight. Paired animals were then sacrificed by a blow to the neck. The abdominal aortas were removed, cleared and rinsed with ice cold tris buffer (0,5mmol/l pH 7,5). After adjustment to an approximate wet weight of 10mg, the tissue was stored in buffer on ice until tested (within 30min). Each aorta was cut once longitudinally and thrice transversely to produce eight approximately equal pieces which were then incubated in 700 µl tris buffer at room temperature. PGI_2 generation was stimulated mechanically using a small plastic covered magnetic stirrer bar. 'PGI_2-like' activity produced by the vessel at various incubation times was assessed as described previously (3,4).

Results. As indicated in Table 1 application of the clip caused a significant reduction in tail pressure. Abdominal aortas in which pressures had been reduced produced decreased amounts of 'PGI_2-like' activity. (Table 2) This tendency was consistent at the 8 and 14 minute sampling times and when analysed by the Wilcoxon matched-pairs signed-ranks test reached a level of significance of $p \leq 0,02$.

	BLOOD PRESSURE (mm Hg)			
	MEAN		DIFFERENCE WITHIN PAIRS	
	C	S	MINIMUM	MAXIMUM
Prior to operation	178	180	0	12
Post operation	95	160	34	93

Table 1 : Tail pressures of conscious rats

	ng PGI_2 PRODUCTION/mg WET WT. AORTA			
INCUBATION TIME (MIN)	MEAN		MEAN DIFFERENCE WITHIN PAIRS (NUMBER OF PAIRS IN BRACKETS)	
	C	S	C > S	S ≥ C
2	1,50	1,58	0,26 (4)	0,41 (4)
8 *	3,14	3,75	0,14 (1)	0,72 (7)
14 *	3,54	4,05	(0)	0,50 (8)

Table 2 : ng 'PGI_2-like' activity produced by rat abdominal aortas
*Comparing C to S $p \leq 0,02$

Discussion. The results indicate that the ability to produce PGI_2 may be influenced by prior local changes in pressure and/or pulse pressure. Elevated PGI_2 production by aortas of hypertensive rats may be a mechanical effect related to raised pressure/pulse pressure; vena cavae from spontaneously hypertensive rats do not show an increased ability to produce PGI_2 when compared with controls (7).

Acknowledgements. Miss A. van Middelkoop gave statistical advice; Prof. J. van Dellen silver clips; Upjohn Ltd. PGI_2 and MRC (SA) finance.

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PAPER A13

Platelets of Spontaneously Hypertensive Rats are not Abnormally Sensitive to PGI_2

This paper resulted from discussions between the authors. Dr. Botha carried out all the laboratory work and we collaborated in writing this paper.

Prostaglandins and Medicine 7: 499-500, 1981 Short note

PLATELETS OF SPONTANEOUSLY HYPERTENSIVE RATS ARE NOT ABNORMALLY SENSITIVE TO PGI_2

J.H. Botha and W.P. Leary. Pharmacology Dept., University of Natal, Box 17039, Congella 4013, South Africa. (Reprint requests to JHB.)

It has been suggested that haemostatic balance in various pathological conditions is influenced not only by vascular PGI_2 generation but also by changes in platelet sensitivity to PGI_2 (1). For example; when atherosclerosis is induced in rabbits, decreased PGI_2 generation occurs together with increased sensitivity of platelets to PGI_2 (2,3). This communication describes the effect of PGI_2 on ADP-induced aggregation of platelets from Wistar rats of the New Zealand genetically hypertensive strain (GH) and on those of age, weight and sex matched normotensive Wistar control rats (N). These two groups have previously been shown to differ significantly in their aortic generation of PGI_2 (4).

Materials and Methods. Using the tail cuff method, systemic arterial blood pressures were measured prior to the study in 21 conscious, 3 month old male animals from each group (approximate wt. 270g). In each of 7 experiments three animals from each group were anaesthetised with intraperitoneal pentobarbitone (6mg/100g). Blood was collected from the abdominal aorta into disposable plastic syringes containing heparin (10 U/ml blood). Platelet rich plasma (prp) was prepared by centrifugation of the blood at 160g for 18 minutes. Further centrifugation of the remaining blood at 2000g for 15 minutes yielded platelet poor plasma (ppp). Aggregation of pooled prp from the 3 rats in each group was carried out in a dual channel model 340 Chronolog Aggrometer using ADP (final conc. 1μM) added to prp 1 minute after the addition of tris buffer (pH 9,7) or the buffer containing various concentrations of PGI_2 as sodium salt (Upjohn). Maximum aggregation was measured as a percentage of the distance between prp and ppp.

Results. Systemic arterial pressures of the two groups differed significantly : mean 115, (range 80 - 125)mmHg and mean 172, (range 158 - 190)mmHg for N and GH rats respectively. Platelet counts in prp differed by no more than 4% in the two groups. As illustrated in Figure 1 the response of the platelets to ADP did not differ significantly between the 2 groups whether ADP followed tris buffer alone or buffer containing various concentrations of PGI_2.

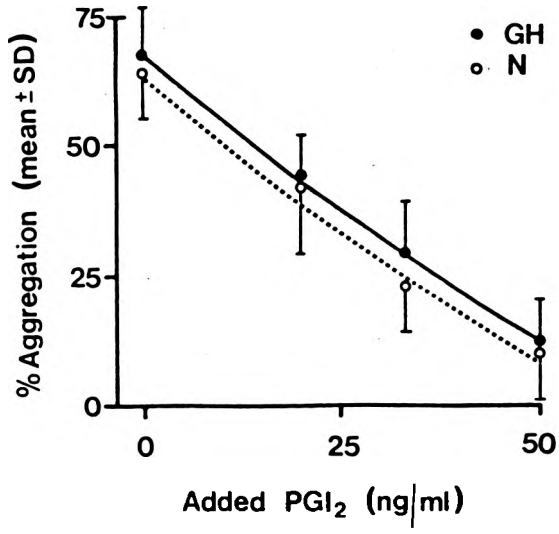

Fig. 1: Effect of PGI_2 on ADP induced rat platelet aggregation.

Discussion. Recent work has shown decreased sensitivity of human platelets to PGI_2 during long-term intraarterial infusion in peripheral vascular disease (5). The possibility therefore existed that increased aortic generation of PGI_2 in hypertension (4) might be associated with reduced platelet sensitivity. However, the results indicate that platelets from both GH and N rats are equally sensitive to the anti-aggregatory effect of PGI_2. The significance of this is not clear at this stage. It is interesting that in experimental atherosclerosis and hypertension, in which arterial thromboses appear superficially similar, changes in vascular PGI_2 generation and platelet sensitivity are not consistent.

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SECTION B : CLINICAL TRIALS IN HYPERTENSION

These papers represent a small percentage of clinical trials published during the last 10 years. They are typical examples however and all have similar shortcomings. Many open or only partially blind trials are initiated in my department at an early stage in the development of new antihypertensive drugs. Other studies may involve innovative approaches to the use of established medications and are also conducted in an open fashion since double-blind trials would be unethical. Generally, such preliminary work is followed by double-blind, controlled studies elsewhere if justified by the results of the pilot study.

PAPER B1

Treatment of Uncomplicated Essential Hypertension with Xipamide

I carried out all the work reported here with the assistance of two nurses. Professor A.C. Asmal provided editorial help.

CURRENT
THERAPEUTIC
RESEARCH
VOL. 24, NO. 8, DECEMBER, 1978

TREATMENT OF UNCOMPLICATED ESSENTIAL HYPERTENSION WITH XIPAMIDE

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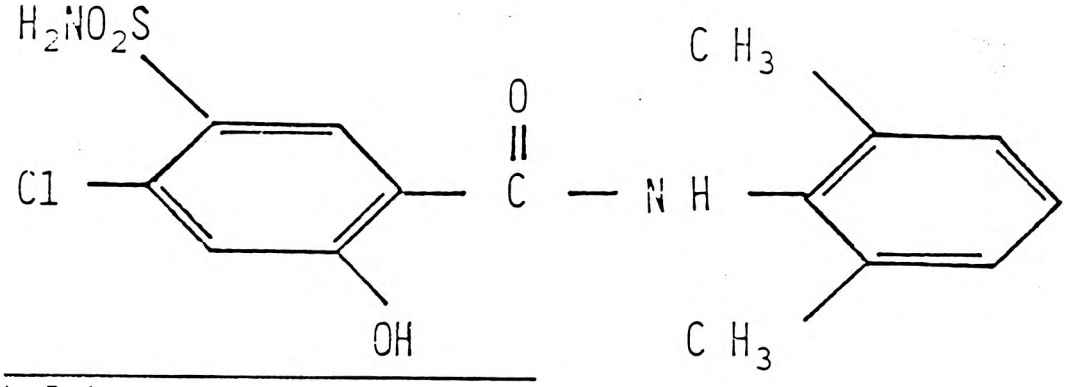
ABSTRACT

Xipamide, a relatively new non-thiazide diuretic was used as sole therapy in 21 patients with mild to moderate elevations of blood pressure. Supine and erect systolic and diastolic pressures were significantly reduced. Changes recorded in arterial pressure, plasma urate, potassium and glucose were similar to those associated with established diuretics.

INTRODUCTION

Saliuretics of the benzothiazine type are commonly used, alone or as adjunctive therapy, in the management of hypertension and oedema of cardiac, hepatic or renal origin. These medicines vary slightly in potency and duration of action, and are generally effective and safe, although associated with a tendency to cause hypokalaemia, hyperuricaemia and hyperglycaemia, particularly when used for prolonged periods. Since

Figure 1 —*Structural formula of xipamide.*



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these adverse effects occasionally have serious consequences, attempts to synthesise improved diuretics continue.

Xipamide (Fig. 1) is a new non-thiazide diuretic antihypertensive compound with marked saliuretic effects in animals and man.[1] Preliminary studies in healthy adults indicate that xipamide 20 mg is approximately equipotent with furosemide 40 mg and hydrochlorothiazide 100 mg.[2]

This paper reports the results of an early double-blind study designed to determine the effects of xipamide upon arterial blood pressure in patients with uncomplicated mild to moderate essential hypertension.

PATIENTS AND METHODS

The blood pressures of 200 clerks and semi-skilled workers employed at a Durban factory were measured by nursing staff attached to a clinic within the building. Those with raised pressures were referred to a medical practitioner for possible treatment.

On referral, a medical history was recorded and general clinical examination carried out. Arterial pressures were measured in the right arm by standard techniques, using the first appearance and final disappearance of Korotkov sounds to define systolic and diastolic pressures, respectively. Patients rested supine for 3 minutes and stood upright for 2 minutes before measurement of supine and erect pressures, which were each measured twice. Lowest systolic and diastolic pressures recorded were adjusted upwards to the nearest 2 mmHg. Blood pressure was measured again, at the same time of day, 2 weeks later. Patients who gave their consent, and with supine diastolic pressures of 90 to 120 mmHg, entered the clinical trial, unless disqualified by any exclusion criterion.

Patients who fell within one or more of the following categories were excluded from the trial and given alternative therapy:

1. Clinical evidence or history of gout.
2. History of severe congestive cardiac failure.
3. History of severe renal or hepatic impairment.
4. Gross obesity.
5. Pregnant women and women likely to become pregnant.
6. Abnormal pretreatment laboratory tests.
7. Patients taking cardiac glycosides, psychotropics, antihypertensives or oral contraceptives.
8. Retinopathy more severe than slight irregularity or narrowing of vessels.

Patients were examined by the same physician every week for 6 weeks, and monthly thereafter for a further 5 months. Weight and blood pressure were recorded at the same time of day at each visit, (1200-1400 hr). Xipamide was administered double-blind as a single daily dose taken at breakfast (0600-0700 hr). Patients were given 20 mg placebo tablets for two weeks, followed by 20 mg active xipamide for 4 weeks. This was increased to 40 mg daily if an adequate blood pressure response was not obtained after 4 weeks at the lower dose. They were then maintained on 20 or 40 mg xipamide per day to the end of the study. Patients with untreated supine diastolic pressure of 90 to 109 mmHg were classified as having mild hypertension; those with a pressure of 110 to 120 mmHg were classified as

suffering from moderate hypertension. A controlled response was regarded as a diastolic pressure below 90 mmHg in mild and below 96 mmHg in moderate hypertension.

Serum urate, potassium and random glucose estimations were carried out at each visit and a 12-lead EKG was recorded at the beginning and at the end of the study. Patients were questioned about adverse reactions at each examination.

Results were analysed for statistical significance of responses to treatment using Student 't' test for comparison of paired data.

RESULTS

Twenty-one patients entered the study, but 2 were excluded before Week 6 because of non-compliance with prescribed treatment. Two of the remaining patients were female and 17 male; all were Indian with the exception of 3 Zulu males. Ages ranged from 25 to 60 years (mean 47). No evidence of a primary cause for hypertension was noted in any case. Patients took 20 mg (13) or 40 mg (6) xipamide daily.

Detailed results are presented in Tables I and II. Thirteen patients had "mild" and 6 "moderate" hypertension, as defined above. Significant falls in systolic and diastolic pressures occurred during both

Table I — *Effect of xipamide on arterial pressure.*

	SUPINE ARTERIAL PRESSURE (Systolic)	(Diastolic)	STANDING ARTERIAL PRESSURE (Systolic)	(Diastolic)
PRE-THERAPY	168.6 5.14	104.4 2.40	165.7 4.89	106.5 2.39
INITIAL THERAPY	149.8* 4.71	93.8* 2.24	146.6* 4.19	99.3* 1.88
STABILISED THERAPY	139.6* 4.00	90.3* 1.91	139.8* 3.88	98.1* 1.97

Results expressed in mmHg as mean ± SEM
Significant differences: Pre-therapy vs Initial and Stabilised Therapy; * P = $<$0.005

Table II — *Effect of xipamide on serum potassium, urate and glucose levels.*

	POTASSIUM	URATE	GLUCOSE
PRE-THERAPY	3.9 0.13	0.40 0.03	7.0 0.65
INITIAL THERAPY	3.9 0.13	0.42 0.03	6.8 0.48
STABILISED THERAPY	3.6* 0.13	0.45* 0.02	7.0 0.43

Results expressed in m.mol/l as mean ± SEM
Significant differences: Pre-therapy vs Stabilised Therapy; * P = $<$0.025

initial and stabilised periods of therapy. Mean supine systolic pressure fell from 168.6 to levels of 149.8 and 139.6 mmHg.

Mean supine diastolic pressure fell from 104.4 to 93.8 and 90.3 mmHg. These changes were similar to the responses in standing blood pressure: Systolic pressure fell from 165.7 to 146.6 during initial therapy and 149.8 mmHg in the stabilised period. Equivalent diastolic pressure readings were 106.5, 99.3 and 98.1 mmHg respectively. These responses to initial and stabilised therapies all represented values recorded during the placebo phase ($P = < 0.005$).

Supine diastolic pressure was reduced by xipamide in all 6 patients with moderately severe hypertension and fell below 96 mmHg in 4. Both patients who responded relatively poorly were given 40 mg xipamide daily during the period of stabilised therapy. Systolic pressure also fell in this group of hypertensives from an initial mean level of 187.7 to 145.0 mmHg ($P = < 0.0025$).

Thirteen patients were classified as "mild" hypertensives. Supine diastolic pressure fell below 90 mmHg in 9 cases during stabilised therapy and was effectively reduced during initial therapy in 2 others. The cause of the slight pressure rise noted during stabilised therapy in these 2 cases is uncertain, but might be due to non-compliance. In the remaining patients only slight changes in diastolic pressure occurred. Similar changes in systolic pressure were noted and overall control during stabilised treatment was significantly better than that during placebo therapy ($P = < 0.0005$).

No change in pulse rate occurred. Mean serum potassium level fell from 3.9 to 3.6 m mol/l in the course of this clinical trial. Though relatively unimpressive, this change was statistically significant ($P = < 0.025$), reflecting falls of 0.1-1.2 m mol/l in all but 5 patients. Plasma urate rose slightly from a mean of 0.40 m mol/l during placebo therapy to 0.45 on stabilised treatment. This increase, which ranged from 0.01 to 0.17 m mol/l in the 14 patients so affected, was statistically significant ($P = < 0.025$). No change in glucose level was detected despite slight differences in time elapsed since the meal preceding each clinic visit.

DISCUSSION

The preliminary study described in this paper was designed to answer 2 relatively simple questions. These concerned the capacity of xipamide 20 to 40 mg to reduce raised arterial pressures in man and whether hypokalaemia, hyperuricaemia or hyperglycaemia would occur in a small group of patients taking the diuretic daily for 6 months without potassium supplements.

Xipamide lowered systolic and diastolic pressures to a significant

degree whether patients were standing or supine during measurement. This resembled the response to other diuretics of the thiazide type[3] and suggests that zipamide 20 to 40 mg daily may be used in hypertension as an alternative to compounds such as hydrochlorothiazide, chlorthalidone, chlorexolone and cyclopenthiazide. Blood pressure control over 24 hours was not studied, although the relatively prolonged diuretic effect of xipamide suggests that it might be effective if prescribed once daily.[2]

The changes in urate and potassium levels observed in this clinical trial were slight, but consistent with the findings of other investigators.[1,4] Increases in fasting blood glucose levels have been reported in patients given xipamide,[4] though no change in random levels was detected in the present study.

Xipamide administered once a day is an effective new saluretic with some of the clinical characteristics of the benzothiadiazine diuretics. It remains to be seen whether this preparation has any advantage over alternative diuretics in the management of essential hypertension.

Acknowledgements

Xipamide was supplied by Drs. A. Groesbeek and B. Friedman of Bristol Myers Ltd., to whom the authors express their thanks.

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PAPER B2

Evaluation of the Efficacy and Safety of Guanabenz versus Clonidine

I carried out all the work reported here with the assistance of one nurse. Professor Asmal provided editorial help.

Evaluation of the Efficacy and Safety of Guanabenz versus Clonidine

W. P. LEARY, A. C. ASMAL, P. C. WILLIAMS

SUMMARY

The antihypertensive efficacy and safety of guanabenz were evaluated against clonidine in two groups of 18 patients with uncomplicated essential hypertension. Both compounds reduced systolic and diastolic blood pressure at the doses used, whether pressures were measured in the supine or standing positions. Side-effects, such as dry mouth and drowsiness, were similar in both groups of patients. No postural hypotension occurred.

S. Afr. med. J., **55**, 83 (1979).

Guanabenz (2,6-dichlorobenzylidine amino guanidine acetate) (Fig. 1), is a potent hypotensive agent structurally related to clonidine and thought to act by stimulating sympathetic vasoconstrictor inhibitory centres and peripheral adrenergic outflow.[1] Preliminary studies in laboratory animals and man demonstrated no contraindication to the use of guanabenz in hypertensive patients and showed that arterial blood pressure could be reduced in most patients by doses of 4 - 24 mg twice daily.[2,3]

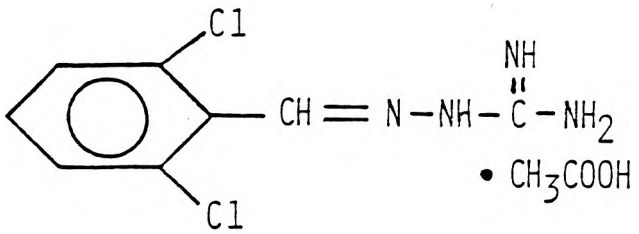

Fig. 1. Guanabenz (2,6-dichlorobenzylidine amino guanidine acetate).

The purpose of the study reported here was to evaluate the antihypertensive efficacy and the safety of guanabenz against clonidine in two groups of patients with uncomplicated essential hypertension.

PATIENTS AND METHODS

The arterial pressures of clerks and metal-workers employed at a Durban factory were measured by trained nursing staff at a casualty clinic on the factory premises. Those with a supine diastolic pressure above 90 mmHg

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Date received: 4 August 1978.

were referred to a medical practitioner (A.C.A.) for assessment and possible treatment.

On referral, a medical history was recorded and a general clinical examination was carried out. Patients rested supine for 3 minutes or stood upright for 2 minutes before measurement of supine and erect pressures by standard sphygmomanometry, using the first appearance and final disappearance of Korotkoff sounds to define systolic and diastolic pressures respectively. Blood pressures were measured again, at the same time of day, 7 and 14 days later. Lowest systolic and diastolic pressures recorded were adjusted upwards to the nearest 2 mmHg.

Patients who had not been previously treated for hypertension, and who had supine diastolic pressures consistently between 90 and 130 mmHg, entered the trial after giving their consent, unless disqualified by any exclusion criteria. Venous blood was drawn from these patients for measurement of creatinine and urea nitrogen levels, and a 12-lead ECG was recorded at rest.

Patients with any of the following disorders were excluded from the study: (*i*) severe cardiac disease including cardiac failure, angina pectoris and arrhythmias other than sinus arrhythmia; (*ii*) malignant hypertension or retinopathy more severe than grade II/IV; (*iii*) a recent history of stroke; or (*iv*) a severe concomitant pathological state, including malignancy, overt psychosis, drug dependence, endocrine disorders, severe infection, renal or hepatic disease.

The grades of essential hypertension and criteria of effective management were arbitrarily defined as follows:

Mild hypertension. Supine diastolic pressure 90 - 104 mmHg. Effective reduction: below 90 mmHg.

Moderate hypertension. Supine diastolic pressure 105 - 114 mmHg. Effective reduction: below 95 mmHg.

Moderately severe hypertension. Supine diastolic pressure 115 - 129 mmHg. Effective reduction: below 105 mmHg.

Patients were assigned randomly to groups given guanabenz 8 - 24 mg twice a day (group A) or clonidine 0,05 - 0,30 mg 3 times a day (group B). Medicines were taken in equal divided doses with initial doses at the lower limit of the above ranges. Blindness was achieved by separating dispensing and recording of blood pressure assessments.

Clinical symptoms, pulse rate, blood pressure and weight were recorded twice a week during the 1st and 2nd week, and once a week from the 3rd to the 8th week. Arterial pressures were measured by the same observer and at the same time of day. Doses of both medicines were increased at weekly intervals in relation to response and side-effects.

An ECG, funduscopy and laboratory data were recorded at the end of the 4th and 8th weeks. A physical examination was carried out at the end of the 8th week when the trial was completed.

Results were analysed for antihypertensive efficacy and side-effects of both compounds within and between patients of the same group and between groups.

RESULTS

Forty patients were selected for study (Table I); 36 completed the trial and 4 were disqualified for non-compliance with the protocol. Each treatment group included 18 patients. No significant changes in funduscopy and laboratory or ECG data were recorded.

TABLE I. DETAILS OF PATIENTS IN GUANABENZ AND CLONIDINE GROUPS (DATA EXPRESSED AS MEAN ± SD)

	Guanabenz	Clonidine
Number of patients	**21**	**19**
Age (yrs)	**44,9* ± 7,6**	**50,9 ± 5,9**
Weight (kg)	**71,68 ± 12,8**	**72,18 ± 11,7**
Baseline severity of hypertension		
Moderate/severe	**7**	**5**
Moderate	**5**	**7**
Mild	**9**	**7**

* Comparison significant ($P<0,01$).

The mean daily dose of each medication was gradually increased over the study period. The average starting dose was 16,0 mg guanabenz or 0,45 mg clonidine. Titration increments were larger for patients with a relatively severe degree of initial hypertension. The overall mean daily dose of guanabenz was 22,8 mg (range 12,0 - 48,0 mg) and 0,56 mg for clonidine (range 0,45 - 0,90 mg).

Eleven patients in group A (guanabenz) and 13 in group B (clonidine) experienced at least one adverse effect (Table II), but these responses did not discriminate between the two treatment groups. Dry mouth and drowsiness were the most frequent adverse responses to both medications.

TABLE II. DRUG-RELATED ADVERSE EFFECTS

Adverse response	Guanabenz	Clonidine
Dry mouth	**7**	**10**
Dizziness	**2**	**5**
Drowsiness	**10**	**10**
Weakness	**0**	**1**
Disorders of micturition	**0**	**1**
Disturbed sexual function	**0**	**1**
Total affected	**11**	**13**

Systolic and diastolic blood pressures in both standing and supine positions were significantly decreased ($P<0,01$) from the baseline measurements in each treatment group (Tables III and IV). Pulse rate in both positions was usually reduced by both guanabenz and clonidine. Systolic and diastolic blood pressure averages were generally lower in the guanabenz group throughout the study. In the supine position, this difference was significant at weeks 5 and 8 (systolic pressure). Average standing systolic pressure was significantly lower in the guanabenz than in the clonidine group at week 5. Postural hypotension did not occur with either compound.

At the end of the study 10 patients in group A and 7 in group B were rated as having effective reductions in supine diastolic pressure. However, the difference between the groups was not statistically significant.

TABLE III. MEAN BLOOD PRESSURE AND PULSE RATE IN SUPINE POSITION

	Systolic pressure (mmHg)		Diastolic pressure (mmHg)		Pulse rate (beats/min)	
Trial period	Guanabenz	Clonidine	Guanabenz	Clonidine	Guanabenz	Clonidine
Control	**163,9**	**163,6**	**104,8**	**106,9**	**76,5**	**76,9**
Treatment week						
5	**133,0***	**144,6**	**88,3**	**88,9**	**68,5***	**76,1**
6	**135,1**	**139,1**	**90,1**	**90,8**	**66,6**	**72,6**
7	**137,7**	**142,6**	**91,0**	**89,2**	**71,6**	**68,4**
8	**134,1***	**146,7**	**90,7**	**94,0**	**66,5**	**70,3**

* Difference between treatment groups significant ($P<0,05$).

TABLE IV. MEAN BLOOD PRESSURE AND PULSE RATE IN STANDING POSITION

	Systolic pressure (mmHg)		Diastolic pressure (mmHg)		Pulse rate (beats/min)	
Trial period	Guanabenz	Clonidine	Guanabenz	Clonidine	Guanabenz	Clonidine
Control	**159,9**	**162,9**	**107,9**	**110.1**	**79,9**	**80,2**
Treatment week						
5	**126,6***	**140,5**	**93,1**	**98,2**	**73,0***	**80,7**
6	**130,5**	**134,5**	**92,4**	**95,0**	**72,9**	**77,9**
7	**133,1**	**129,4**	**93,2**	**90,2**	**76,4**	**73,6**
8	**125,8**	**133,4**	**93,3**	**95,1**	**72,6**	**74,4**

* Difference between treatment groups significant ($P<0,05$).

DISCUSSION

The structural and pharmacological similarities of clonidine and guanabenz are reflected by results of the present clinical trial. Guanabenz was at least as effective as clonidine in the patients treated and within the dosage ranges used, and similar adverse responses were associated with both preparations. Care should be exercised in selecting patients for treatment with guanabenz since serious adverse reactions to clonidine have been reported.[4] These may logically be expected when related preparations are given, although rebound hypertension has not yet been reported in man after withdrawal of guanabenz therapy.

Clonidine-like compounds should be used cautiously in patients with coronary or cerebral insufficiency and in depressives. Patients should not drive or operate machines since drowsiness and inattention could have serious consequences. Withdrawal of treatment must be gradual to minimize the danger of a hypertensive crisis occurring 8 - 24 hours after withdrawal.[5]

Further clinical experience with guanabenz is required before its properties and potential uses in hypertension can be clarified. Present experience indicates that guanabenz is effective when administered twice daily, and may be used as an alternative to clonidine in the treatment of patients resistant to other medication or unable to tolerate adrenergic neuron-blocking drugs because of severe postural hypotension.

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PAPER B3

Treatment of Hypertension with Verapamil

I carried out all the work reported here with the assistance of 2 nurses and a technician. Professor Asmal provided editorial help.

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TREATMENT OF HYPERTENSION WITH VERAPAMIL

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ABSTRACT

The effects of verapamil upon arterial pressure were assessed in 16 Negro and 24 Caucasian patients, with mild to moderate hypertension. Verapamil 80 mg given thrice daily in combination with reserpine 0.1 mg significantly reduced systolic and diastolic pressures, producing mean falls of 8.2, 6.5, 8.4 and 9.8 mmHg in supine and standing systolic and diastolic pressures, respectively. When verapamil 160 mg was given twice daily as sole medication, falls of 21.0, 8.9, 17.6 and 8.4 mmHg were recorded. It is concluded that verapamil has a mild hypotensive effect useful in managing hypertension of mild to moderate degree.

INTRODUCTION

Verapamil is a synthetic papaverine derivative which has proved useful in the management of angina pectoris and supraventricular tachyarrhythmias.[1] It blocks the uptake of ionised calcium by myocardial cell membranes thus limiting energy release from ATP by the action of calcium dependent ATPase and reducing myocardial contractility and oxygen consumption. The increased coronary vascular resistance noted during treatment with beta adrenergic blockers does not occur[2] in response to verapamil.

Verapamil induces a degree of relaxation in vascular smooth muscle presumably by an effect on calcium ions similar to that which is obtained in the heart. The pharmacological responses to this drug are vasodilation and a slight negative inotropic effect which result in a fall in arterial blood pressure, an effect which has proved useful in managing patients with hypertensive crises.[3]

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Whereas the efficacy of intravenous verapamil in the treatment of emergency situations has been established, relatively few studies on its oral use in hypertension have been carried out.[4,5,9,10,11] Verapamil disappears rapidly from plasma after oral administration, as a result of intensive hepatic first pass metabolism, indicating that doses of 120 to 160 mg must be given at least 2 or 3 times daily to maintain therapeutic concentrations in man. Unsubstantiated independent reports[6,7] suggest that verapamil acts synergistically with methyldopa or reserpine to reduce raised arterial pressures in man; this effect may allow dosage reductions in reserpine and methyldopa with resultant decreases in their side effects. This paper describes a double-blind clinical trial designed to establish the effects upon arterial pressure of verapamil given alone or in combination with small doses of reserpine. A subsequent open extension of the trial examined the effect of verapamil given twice daily as sole medication to the same patients.

MATERIALS AND METHODS

Forty male patients (16 Zulus and 24 Caucasians) with uncomplicated essential hypertension of mild to moderate severity were studied. All were clerks or semi-skilled workers employed at a Durban factory and were selected from personnel referred for medical assessment when raised pressures were detected at routine examinations made by trained nursing staff attached to the factory clinic.

On referral, a medical history was recorded and general clinical examination carried out. Patients rested in the supine position for 3 minutes or stood upright for 2 minutes before measurement of supine and erect pressures by standard sphygmomanometry, using the first appearance and final disappearance of Korotkov sounds to define systolic and diastolic pressures, respectively. Blood pressures were measured again, at the same time of day, 7 days later. Lowest systolic and diastolic pressures recorded were adjusted upwards to the nearest 2 mmHg. Patients who gave their consent and had supine diastolic pressures consistently between 94 and 134 mmHg entered the trial unless disqualified by any exclusion criterion. Venous blood was drawn for measurement of urea nitrogen levels, routine urinalysis was carried out and a 12-lead EKG recorded at rest before entry into the trial.

Patients with any of the following disorders were excluded from the study:

(a) Severe cardiac disease including cardiac failure, angina pectoris and arrhythmias other than sinus arrhythmia or occasional extrasystoles.
(b) Malignant hypertension or retinopathy more severe than grade II/IV.
(c) Recent history of stroke.
(d) Severe concomitant pathology, endocrine disorders, severe infection, renal or hepatic disease.

Each patient was given placeboes for reserpine 0.1 mg and verapamil 80 mg thrice daily for 2 weeks. Thereafter they were randomly assigned to groups taking active reserpine 0.1 mg and active verapamil 80 mg *t.d.s.* (Group A) or active reserpine 0.1 mg and verapamil placebo *t.d.s.* (Group B). After 6 weeks of therapy, active medications were again replaced by placeboes and 2 weeks later regimens were crossed over between Groups A and B. Blindness was achieved by the use of placeboes identical in appearance to active medicines and by separating dispensing and recording of blood pressure assessments.

Clinical symptoms, pulse rate, standing and supine blood pressure were measured every 14 days throughout the trial. Arterial pressures were measured by the same observer and at the same time of day. Serum urea and electrolytes were measured and a 6-lead EKG recorded at the end of each treatment period.

On completion of the double-blind portion of the trial, reserpine was withdrawn and replaced by verapamil 160 mg given twice daily for 8 weeks. Active therapy was then replaced with verapamil placebo taken for 4 weeks before reintroduction of any active medication. Patients were held blind during this section of the clinical trial.

Results were examined for antihypertensive efficacy of the regimens used. Data collected during the final 4 weeks of each treatment period were analysed using a Students 't' test programme for comparison of paired data prepared for the Hewlett-Packard H-P 67 computer. Responses during the initial and "washout" placebo periods were pooled, but data collected during the final placebo period were treated separately for statistical purposes since they formed no part of the initial double-blind study.

RESULTS

Results are set out in Tables I and II. Forty patients, including 16 Black Africans and 24 Caucasians completed the double-blind study. Mean

Table I — *Effects of thrice-daily reserpine and verapamil on blood pressure (mmHg) and heart rate, (beats/min.). Results expressed as mean ± SEM (n = 40).*

		PLACEBO	RESERPINE 0.1 mg t.d.s.	RESERPINE 0.1 mg + VERAPAMIL 80 mg t.d.s.
SUPINE				
Pressure	Systolic	158.7 ± 2.87	162.2 ± 3.15	150.5 ± 3.20
	Diastolic	102.8 ± 1.46	100.9 ± 1.64	96.3 ± 1.54
STANDING				
Pressure	Systolic	153.5 ± 2.43	156.8 ± 3.01	145.1 ± 3.05
	Diastolic	106.9 ± 1.47	104.1 ± 1.98	97.1 ± 1.38
Heart rate		77.7(± 1.66)	71.0(± 1.23)	68.4(± 1.48)

Table II — *Effects of twice-daily verapamil on blood pressure (mmHg) and heart rate (beats/min.). Results expressed as mean ± SEM (n = 40).*

		PLACEBO	VERAPAMIL 160 mg b.d.
SUPINE			
Pressure	Systolic	178.3 ± 4.04	157.3 ± 2.83
	Diastolic	110.4 ± 2.22	101.5 ± 1.54
STANDING			
Pressure	Systolic	171.5 ± 2.77	153.9 ± 2.91
	Diastolic	114.5 ± 1.39	106.1 ± 1.40
Heart rate		77.0 ± 2.03	79.0 ± 1.86

supine systolic pressure ± standard error of mean fell from 158.7 (± 2.87) to 150.5 (± 3.20) mmHg in response to reserpine 0.1 mg and verapamil 80 mg thrice daily. This fall in pressure was statistically significant ($p = 0.001$). Mean pressure was 162.1 (± 3.15) mmHg when reserpine was given alone. Supine diastolic pressure was reduced from a mean of 102.8 (± 1.46) to 100.9 (± 1.64) mmHg by reserpine and fell to 93.6 (± 1.54) mmHg when verapamil was added. This pressure change was significant when compared with placebo ($p = 0.001$) or treatment with reserpine alone ($p = 0.005$).

Similar changes in standing pressures were recorded. Systolic pressure fell from 153.5 (± 2.43) to 145.1 (± 3.05) mmHg in response to the reserpine-verapamil regimen ($p = 0.001$), but actually rose slightly to 156.8 (± 3.01) mmHg when reserpine was the only medicine used ($p = 0.001$). Standing diastolic pressure was reduced from a mean level of 106.9 (± 1.47) mmHg to 104.1 (± 1.98) mmHg when placebo was replaced by active reserpine ($p = 0.05$). A further significant reduction to 97.1 (± 1.38) mmHg followed the addition of active verapamil to the regimen ($p = 0.001$). Extended analysis of these data demonstrated no significant differences in response to therapy by these patients when grouped by race or the order in which therapeutic regimens were followed. Mean heart rate fell slightly from 77.7 (± 1.66) to 71.0 (± 1.23) and 68.4 (± 1.48) beats/minute in response to reserpine and the verapamil reserpine regimen, respectively. These changes were statistically significant (p 0.0005 and 0.025).

In the course of the open extension to the trial which followed, patients were given verapamil 160 mg twice daily for 8 weeks followed by verapamil placebo for 4 weeks. Responses to verapamil resembled those to combined therapy with reserpine and verapamil although pressures actually rose slightly when the verapamil dosage was adjusted and reserpine withdrawn. However, pressures increased significantly when active medication was replaced by placebo. Thus mean standing pressures were 171.5 (± 2.77) and 153.9 (± 2.91) mmHg in response to placebo, and verapamil, respectively ($p = 0.005$). Diastolic pressures were 110.4 (± 2.22) and 101.5 (± 1.54) mmHg and these changes were also significant ($p = 0.025$). Supine pressures were similarly affected, mean systolic pressure falling from 178.3 (± 4.04) to 157.3 (± 2.38) mmHg ($p = 0.005$) and diastolic from 114.5 (± 1.39) to 106.1 (± 1.40) mmHg ($p = 0.0025$). An additional finding emerged on further analysis. The blood pressures of Black Africans were significantly reduced by verapamil 160 mg twice daily whether standing or supine, systolic or diastolic pressures were considered ($p = 0.0025$). No such change occurred in response to verapamil in Caucasian patients, although the overall effect on the whole group was significant, as noted above.

No changes in serum urea, electrolytes or EKG were recorded

during the studies reported here, and no side effects were reported except for 1 case of impotence which developed during a placebo phase of management and responded to the intramuscular injection of sterile saline 1 ml.

DISCUSSION

The protocol followed in this study may be criticized on several grounds. Thus, the fixed doses of reserpine and verapamil were used throughout the double-blind portion of the trial thereby limiting its value since the clinical effects of increasing doses of either medication were not assessed. In addition, a 2-week washout period between treatments was at least theoretically inadequate, since the effects of verapamil or reserpine might persist for the first weeks of the therapeutic regimen. This was not borne out by the results obtained, however; combined therapy with reserpine and verapamil was superior to treatment with reserpine alone, irrespective of the order in which the 2 regimens were followed.

Despite these reservations a number of useful points have emerged from this study:

Reserpine in a dose of 0.3 mg daily resulted in no clinically significant fall in systolic or diastolic pressures whether measured with patients in the supine or erect posture. The addition of verapamil 80 mg t.d.s. to this regimen reduced arterial pressure significantly, when compared with placebo or reserpine alone. No racial difference in these results was observed.

In a subsequent open extension to the trial, Caucasian patients responded poorly to verapamil 160 mg given twice daily as sole medication. In black (Zulu) hypertensives, however, significant reductions in arterial pressure were obtained when this regimen was used. This apparent racial difference, though unexplained at present, is not unprecedented. Racial differences in the response of raised arterial pressures to beta blockers have been described elsewhere.[8]

The response in both groups to verapamil given in combination with reserpine appears to support the conception that these compounds may act synergistically to reduce blood pressure, although the lack of response to reserpine alone suggests that verapamil is of greater importance in the regimen used.

The results of this study confirm that verapamil has a mild hypotensive effect in the doses tested, and that it offers a rational alternative to other treatments currently used in managing hypertension of mild to moderate degree. Further studies are required in greater depth to determine the efficacy of higher doses of verapamil given alone, at different intervals, and in combination with various other hypotensive agents.

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PAPER B4

Aldactone and Acebutolol in Treatment of Hypertension

I carried out all the work reported here with the assistance of two nurses. Professor Asmal provided editorial help.

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Aldactone and Acebutolol in Treatment of Hypertension

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Twenty-one essential hypertensives were randomly treated with Aldactone, acebutolol or a combination of the two drugs. Statistically significant falls in systolic and diastolic pressures were demonstrated during treatment with Aldactone 100 mg daily, also when acebutolol and Aldactone 200 mg daily were administered. When the combination of Aldactone and acebutolol was given, diastolic pressures were lowered to a greater degree than seen with individual treatments.

Introduction

Their efficacy and low cost have placed thiazide diuretics in an important position in the therapy of hypertension (Dollery 1977). In milder grades of hypertension they are employed alone; in the moderate and severer grades they are used in combination with agents such as reserpine, β-blockers and methyldopa. Potassium depletion, hyperglycaemia and hyperuricaemia are important adverse effects accompanying their long-term use.

The potassium-sparing diuretics such as Aldactone® are free of these adverse effects but their use has not been widespread, probably because of their greater expense. They are primarily indicated in hypertensive states if associated with hypokalaemia, and possibly in situations when thiazides are contraindicated (Simpson 1976). In the present paper the efficacy of Aldactone in combination with a β-blocker has been investigated in the treatment of mild to moderate hypertension.

Methods

Twenty-one patients with uncomplicated essential hypertension were studied. Ten were Zulus and eleven Indians. All were males aged 28 – 63 years, who attended a clinic held at the factory where they were employed in various capacities. Of the twenty-one patients, nine had been on no previous anti-hypertensive therapy and twelve had taken a thiazide diuretic either alone or in combination with acebutolol. All medication was discontinued 2

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weeks before this study. Informed consent was obtained.

Patients attended the clinic every 14 days and were given the following treatments, in random order, single-blind. Placebo was substituted for the relevant active medication as patients were moved between regimens 3 – 5.

1. Placebo alone
2. Aldactone 100 mg daily plus acebutolol placebo
3. Aldactone 200 mg daily plus acebutolol placebo
4. Acebutolol 300 – 400 mg daily plus Aldactone placebo
5. Acebutolol 300 – 400 mg daily plus Aldactone 100 mg daily

Arterial pressures were measured at 0900 hours by the same observer throughout the trial using the standard indirect auscultatory method. Patients rested for 5 minutes and stood for 3 minutes before measurement of supine and erect pressures respectively. The lowest of three measurements in each position was recorded at every examination, diastolic pressure being taken at the complete disappearance of sounds.

Medicines were taken at 0700 hours as a single dose. Aldactone was given as a fixed dose, either 100 mg or 200 mg daily. Acebutolol dosage was chosen on the basis of previous experience with this β-blocker in individual patients, where this was available, or in accordance with the clinical response monitored by the observer. Acebutolol dosage was increased by 100 mg daily until supine diastolic pressure was less than 100 mm Hg on two separate occasions or pulse rate fell below 50 per minute. Each treatment was administered for 8 weeks or until the maximum acebutolol dosage had been in effect for a month.

To minimize the 'carry-over' effects of each preceding medication, the only data recorded at Weeks 6 and 8 were analyzed for purposes of comparison between the regimens followed.

Resting ECGs were recorded and venous blood samples drawn for measurements of plasma urea and electrolytes during treatment with placebo, and acebutolol plus Aldactone.

Data collected from each patient were analyzed for statistical significance of responses to therapy using Student's t test for paired comparisons.

Results

Twenty-one patients were studied. Three did not complete therapy. Mean acebutolol dose was 399 mg when taken as sole medication and 365 mg in combination with Aldactone 100 mg.

The mean supine systolic and diastolic pressures (Table 1) fell from 154/103 during placebo therapy to 144/98 when Aldactone 100 mg was given. This change was significant for both systolic and diastolic pressures ($p < 0{\cdot}025$; $p < 0{\cdot}01$) respectively. Significant falls in pressure also occurred when acebutolol or Aldactone 200 mg were given alone. These pressure changes, however, were not statistically different to those seen with Aldactone 100 mg. Combination of Aldactone 100 mg with acebutolol lowered blood pressure to 144/92. The diastolic response was significantly better than of either Aldactone ($p < 0{\cdot}005$) or acebutolol ($p < 0{\cdot}0025$) given alone.

The blood pressure responses in the erect posture were similar to those described above (Table 2). Mean systolic and diastolic pressures fell from 144/105 during placebo treatment to 140/100 in response to Aldactone 100 mg. Aldactone 200 mg daily and acebutolol given singly reduced pressure to 141/101 and 143/98 respectively. All three regimens produced significant lowering of diastolic pressures only. The combination of acebutolol and Aldactone 100 mg, however, lowered both systolic and diastolic pressures in relation to the other schedules of therapy ($p < 0{\cdot}05$).

No significant change in ECG, resting pulse, urea or electrolyte levels was detected (Table 3), although in seven patients serum potassium rose slightly during therapy.

Discussion

The aim in treating uncomplicated hypertension is to lower pressure, thereby forestalling the development of complications and improving prognosis, without causing major discomfort or inconvenience to the patient (Simpson 1974). This was achieved to some degree by the regimens used in this study, although prolonged trials might reveal shortcomings in therapy not apparent here.

Small but significant falls in both systolic and diastolic arterial pressures were produced

Table 1

Effects of Aldactone and acebutolol on supine arterial pressures

Therapy	*Systolic pressure*	*Diastolic pressure*
1. Placebo	154·3 5·28	103·4 1·51
2. Aldactone 100 mg	144·3**** 3·75	97·5** 2·26
3. Aldactone 200 mg	148·1 4·21	99·2* 1·87
4. Acebutolol	149·8** 5·08	98·5*** 1·86
5. Acebutolol and Aldactone 100 mg	144·4 4·37	91·8= 1·86

Pressures expressed in mm Hg, as mean ± SEM

Significance of treatment cf. placebo data (paired comparison):

* $p < 0.025$
** $p < 0.01$
*** $p < 0.005$
**** $p < 0.0025$

Treatment 5 compared to 4 and 2:

= $p < 0.005$

Table 2

Effects of Aldactone and acebutolol on erect arterial pressure

Therapy	*Systolic pressure*	*Diastolic pressure*
1. Placebo	143·9 4·23	104·8 1·97
2. Aldactone 100 mg	140·4 4·66	100·4*** 2·21
3. Aldactone 200 mg	140·0 3·03	101·3* 1·58
4. Acebutolol	143·2 5·14	98·4** 2·13
5. Acebutolol and Aldactone 100 mg	134·8 3·59	95·2= 1·66

Pressures expressed in mm Hg, as mean ± SEM

Significance of treatment cf. placebo data (paired comparison):

* $p < 0.05$
** $p < 0.025$
*** $p < 0.01$

Treatment 5 cf. 2 and 4:

= $p < 0.05$

Table 3

Effects of Aldactone and acebutolol on pulse beat, urea and electolytes

	Pulse	*Urea*	*Na^+*	*K^+*
Placebo	74·3 ± 2·81	4·1 ±0·39	140·7 ± 0·84	4·4 ±0·13
Aldactone 100 mg	78·8 ± 3·63	—	—	—
Aldactone 100 mg and Acebutolol	72·7 ± 3·38	4·5 ±0·44	141·2 ± 0·91	4·5 ±0·07

Values expressed as group mean ± SEM. Pulse, beats/min, urea, Na^+, K^+, mmol/l.

No statistically significant changes occurred.

by Aldactone or acebutolol given as sole medication. The hypotensive effect of Aldactone was not dose-related as indicated by the fact that responses to 100 and 200 mg doses were indistinguishable.

Increase in daily dosage above 100 mg is therefore not warranted, particularly since larger doses of this steroid often cause gynaecomastia in men and menstrual irregularities in women.

Combination of Aldactone with acebutolol produced a marked improvement in both supine and erect blood pressure consistent with results of studies in which β-blockers have been prescribed with amiloride, an unrelated potassium-sparing diuretic (A.M.A. Drug Evaluations, 1977).

Satisfactory control of arterial pressure may be obtained by acebutolol administered once daily (Fournier *et al* 1976). Though pressures were not measured throughout the day during the present study, the fact that control at 0900 hours was enhanced by Aldactone which has a relatively prolonged action suggests that improved blood pressure control might also be obtained by this combination over 24 hours. This approach to management with Aldactone requires further detailed investigations.

Over the short period of observation of this trial the combination of acebutolol and Aldactone has demonstrated efficacy and relative safety. However, it is worth remembering that the management of hypertension is that of chronic disease. Chronic injudicious use of Aldactone may cause gynaecomastia, hyponatraemia and hyperkalaemia. The frequency of these problems as well as the sustained efficacy of this drug over prolonged periods has been investigated by a long-term study in hypertensive patients, however, and it appears that adverse responses to Aldactone should be relatively uncommon (Cangiano *et al* 1977).

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PAPER B5

Antihypertensive Effects of Sotalol and Atenolol Given Once Daily

I carried out all the work reported here with the assistance of two nurses. Professor Asmal provided editorial help.

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Antihypertensive Effects of Sotalol and Atenolol Given Once Daily

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SUMMARY

Twenty-five male patients aged 21 - 65 years and with supine diastolic arterial pressures of 96 - 120 mmHg entered an open, controlled cross-over study, in which the effects of single daily doses of sotalol and atenolol were compared. Data collected indicate that moderately elevated blood pressure is effectively controlled for 24 hours by single daily doses of both β-blockers (sotalol 160 - 800 mg and atenolol 100 - 500 mg). No important side-effects occurred.

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The management of raised arterial pressure in man poses various problems for both medical attendant and patient. Despite attempts at patient education and motivation, non-compliance with prescribed therapy is common[1] and may reflect the patients' difficulty in remembering to take multiple doses of medicine in the course of a busy day, or reluctance to use medicines which cause unpleasant side-effects. Since improved patient compliance may be expected if simple treatment regimens are followed for chronic conditions such as hypertension, it is important to establish whether the medicines prescribed are effective for 24 hours when given in single daily doses.

Sotalol and atenolol are β-adrenergic blocking agents, both of which effectively reduce raised arterial pressure in man despite slightly different pharmacological characteristics.[2,3] Sotalol is a non-selective β-blocker with no intrinsic sympathomimetic or membrane-stabilizing activity, and a plasma half-life of 15 - 17 hours. Atenolol, like sotalol, lacks intrinsic sympathomimetic and membrane-stabilizing activity, but is relatively cardioselective and has a shorter half-life in man — only 8 hours. Both these components may provide satisfactory control of raised blood pressure in man for up to 24 hours, but their relative efficacies within a single group of patients have not been established.

The controlled, open, cross-over study reported in this article was designed to compare the effects of single daily doses of sotalol and atenolol given to patients with moderately elevated arterial pressures. Particular attention was paid to side-effects and the relative potencies of the two agents concerned.

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PATIENTS AND METHODS

The arterial pressures of clerical and manual workers employed at a local factory were measured by trained nursing staff at a casualty clinic on the factory premises. Subjects with supine diastolic pressures above 95 mmHg were referred to a medical practitioner for assessment and possible treatment. On referral a medical history was obtained and a general clinical examination carried out. Arterial pressure was measured at this examination and again 7 days later. Patients rested supine for 5 minutes or stood upright for 2 minutes before measurement of arterial pressures by standard sphygmomanometry. The first appearance and final disappearance of Korotkov sounds were used to define systolic and diastolic pressures respectively. Pulse rate was counted over 30 seconds in both postures. The protocol and aims of the study were explained to those patients whose supine diastolic pressures remained consistently above 95 mmHg, so that informed consent for further studies could be obtained. Venous blood samples were collected from each patient and evaluated for serum bilirubin, cholesterol, triglyceride, transaminase, uric acid, urea, creatinine, sodium and potassium concentrations. A full blood count, standard urinalysis and a 12-lead ECG were also performed.

A total of 25 male patients in the age group 21 - 65 years and with supine diastolic pressures between 96 and 120 mmHg entered the study. Patients were excluded from the clinical trial and given alternative treatment if they suffered from congestive cardiac failure, secondary hypertension, atrioventricular block, active hepatic or renal disease, diabetes uncontrolled by diet alone, major respiratory disease, asthma, oedema of any cause, or obesity. Patients with a history of adverse reaction to β-blockers, laboratory or clinical evidence of significant disease unrelated to hypertension, or receiving concomitant medication for any other ailment were also excluded from the trial.

Previous antihypertensive therapy was discontinued and patients were randomly allocated to one of two groups: placebo tablets for sotalol 160 mg (group A) or for atenolol 100 mg (group B), with the instruction that one should be taken every morning at 08h00. After 4 weeks this was replaced by active sotalol 160 mg (group A), or atenolol 100 mg (group B). Patients attended the clinic weekly for the first 4 weeks and every 2 weeks thereafter for the duration of the clinical trial. Throughout the trial blood pressure recordings were taken in the early morning before drug administration (24 hours after the previous medication), and in the late afternoon (8 hours after previous medication).

If the supine diastolic blood pressure of any patient remained above 90 mmHg at the end of the first 2 weeks of active therapy, the dose of sotalol was increased to 320 mg or that of atenolol to 200 mg daily. Further adjustments to 480, 640 and 800 mg sotalol or 300, 400 and 500 mg atenolol per day were made every 2 weeks if arterial pressure remained inadequately controlled. Active medication was given for a total of 14 weeks, followed by a 4-week wash-out period during which atenolol placebo was given to patients in group A and sotalol placebo to patients in group B. This was followed by dosage with active medication, following the protocol used during the previous 14-week treatment period. Thus patients in group A were given sotalol and atenolol in turn, and patients in group B took the same medicines but in reverse order. Laboratory investigations and ECGs were repeated during the final week of each treatment period.

Means of pulse rates and supine and standing systolic and diastolic pressures recorded for each patient during placebo therapy and at each treatment stage were noted and used for statistical analysis. Significance of responses to placebo, atenolol (100 - 200 mg and 100 - 500 mg) and sotalol (160 mg, 160 - 320 mg and 160 - 800 mg) was determined by Student's t test for paired data using a programme prepared for the Hewlett-Packard 637 computer, after pooling of data collected from groups A and B.

RESULTS

Results are set out in Tables I - IV. A total of 23 patients of whom 12 were Blacks and 11 Whites completed the study. Two patients were excluded during placebo therapy. No significant differences emerged relating to the order in which the drugs were given.

Effect of Sotalol on Blood Pressure

Supine systolic and diastolic pressures were reduced 8 hours after the maximum dosage of sotalol (Table I). These changes were statistically significant for both systolic and diastolic pressures (P = 0,05). Diastolic pressure remained significantly reduced after 24 hours (P = 0,005). The mean maximum dose of sotalol given was 459,2 mg per day (range 160 - 800 mg). Each patient took sotalol 160 mg, 17 were given 320 mg daily and 13 graduated to higher doses.

Similar changes were noted for erect systolic and diastolic pressures 8 hours after sotalol. These changes were statistically significant for both systolic (P = 0,0025) and diastolic pressures (P = 0,0025) and were maintained after 24 hours (P = 0,005).

Analysis of responses to lower doses of sotalol given early in the trial showed no significant differences between the means of supine pressures recorded during treatment with sotalol 160 or 320 mg and the maximum dose given. In 6 patients sotalol 160 mg was the highest dose used; arterial pressure fell slightly in a further 7 after sotalol 320 mg and 480 - 800 mg caused further reductions in another 8 patients. These changes were not statistically significant, however. Standing arterial pressure responded similarly, although control at 8 hours was significantly improved by doses above 160 mg. Thus diastolic pressures were reduced by sotalol 320 mg (P = 0,025) and a further significant reduction in both systolic and diastolic pressures was recorded at the maximum dose (P = 0,05).

Effect of Atenolol on Blood Pressure

The mean dosage of atenolol was 282,6 mg daily (range 100 - 500 mg). Every patient took atenolol 100 mg, 17 were given 200 mg daily and 11 eventually took higher doses. Supine systolic and diastolic pressures were reduced

TABLE I. EFFECTS OF SOTALOL ON ARTERIAL BLOOD PRESSURE (mmHg)

	Placebo (24 h)		160 mg (8 h)		160 mg (24 h)		160 - 320 mg (8 h)		160 - 320 mg (24 h)		160 - 800 mg (8 h)		160 - 800 mg (24 h)	
	Sys	Dias	Sys	Dias	Sys	Dias	Sys	Dias	Sys	Dias	Sys	Dias	Sys	Dias
Supine														
Mean	151,5	104,3	148,6	97,0	145,8	96,6	147,4	94,1	147,2	93,0	145,7	92,6	147,7	91,2
SEM	1,95	1,15	3,25	2,22	2,27	2,55	3,54	2,62	3,41	2,27	3,68	2,28	4,16	2,29
Erect														
Mean	151,7	109,5	149,0	105,9	145,1	99,5	147,4	100,0	145,1	100,0	142,1	97,5	144,7	99,4
SEM	2,01	1,39	3,63	2,24	3,49	1,94	3,07	2,00	2,89	2,41	2,61	2,80	2,69	2,01

TABLE II. EFFECTS OF ATENOLOL ON ARTERIAL BLOOD PRESSURE (mmHg)

	Placebo (24 h)		100 mg (8 h)		100 mg (24 h)		100 - 200 mg (8 h)		100 - 200 mg (24 h)		100 - 500 mg (8 h)		100 - 500 mg (24 h)	
	Sys	Dias	Sys	Dias	Sys	Dias	Sys	Dias	Sys	Dias	Sys	Dias	Sys	Dias
Supine														
Mean	153,1	98,2	146,4	93,8	146,6	96,2	144,4	91,4	144,8	92,7	145,8	90,9	141,5	87,4
SEM	3,43	2,49	4,24	3,36	4,16	3,75	4,17	2,85	3,93	3,19	4,65	3,00	3,86	2,25
Erect														
Mean	152,4	107,9	148,7	102,8	148,7	103,5	146,6	99,4	145,4	98,5	147,3	100,6	144,7	97,4
SEM	3,70	2,27	3,71	2,97	4,14	3,34	3,61	2,26	3,12	2,55	3,55	2,12	2,77	1,98

8 hours after dosage with atenolol (Table II). These changes were statistically significant for both systolic (P = 0,025) and diastolic (P = 0,0025) pressures. The lowered pressures were maintained 24 hours after the last dose of atenolol and remained significantly lower than those recorded during placebo therapy (P = 0,0025).

Erect arterial pressures were reduced 8 hours after dosage with atenolol. The slight fall in systolic pressure noted was unimportant but the change in diastolic pressure was statistically significant (P = 0,005). The changes were maintained 24 hours after the last dose of atenolol (P = 0,005).

Responses to atenolol 100 and 200 mg daily were also analysed. Mean supine pressures were reduced 8 and 24 hours after atenolol 100 mg (Table II). These pressure changes were significant when compared with responses to placebo (P = 0,01), but systolic and diastolic pressures were slightly higher 24 hours after atenolol 100 mg than at the same time after maximum dosage (P = 0,05). Responses to atenolol 200 mg were indistinguishable from those to the maximum dose used, although 11 patients required more than 200 mg atenolol daily. As in the case of sotalol, 6 patients responded satisfactorily to the lowest dose used.

No clinically important differences were found between responses to maximal doses of the two compounds tested. Erect systolic and diastolic pressures were slightly lower after sotalol than after atenolol at the 8-hour examination, whereas 24-hour supine systolic pressure was best controlled by atenolol (P = 0,05).

Three patients responded poorly to both these compounds and their supine diastolic pressures remained above 100 mmHg, despite doses of sotalol 800 mg or atenolol 500 mg daily. Two patients were relatively resistant to sotalol 800 mg and 3 to atenolol 500 mg. Six of these patients were Blacks (Zulu) and 2 were of Indian descent.

Effect on Pulse Rates

Supine and erect pulse rates were reduced 8 hours after maximum doses of sotalol and were maintained after 24 hours. These changes were highly significant (P = 0,0005). Pulse rates were also markedly reduced, below the levels produced by placebo, by sotalol 160 and 320 mg (P = 0,005), but were slightly higher at 24 hours than those recorded at maximum dosage (P = 0,025).

Responses to atenolol were similar. Supine and erect pulse rates were reduced 8 and 24 hours after maximum doses (Table IV). These changes were highly significant (P = 0,0005) with the exception of that in the erect pulse rate at 24 hours, which barely achieved acceptable significance levels (P = 0,05). Pulse rate was also reduced by atenolol 100 or 200 mg daily, although supine and erect pulse rates were higher at 8 hours after 100 mg than after maximum doses of atenolol (P = 0,025). Pulse rates were significantly lower 24 hours after maximum doses of sotalol than after similar doses of atenolol, although in practical terms the difference was slight (P = <0,005). On detailed analysis only 3 patients appeared relatively resistant to the negative chronotropic effects of the two compounds tested. All 3 showed satisfactory responses in arterial pressure, however. No major side-effects were reported in this study although three Blacks complained of relative impotence while on placebo therapy. No important changes in electrocardiographic or laboratory values were recorded.

TABLE III. EFFECTS OF SOTALOL ON PULSE RATE (PER MINUTE)

	Placebo (24 h)		160 mg (8 h)		160 mg (24 h)		160 - 320 mg (8 h)		160 - 320 mg (24 h)		160 - 800 mg (8 h)		160 - 800 mg (24 h)	
	S	E	S	E	S	E	S	E	S	E	S	E	S	E
Mean	**77,7**	**84,0**	**68,6**	**75,0**	**70,7**	**76,3**	**68,7**	**73,4**	**70,7**	**77,3**	**67,1**	**72,2**	**66,1**	**71,4**
SEM	**2,25**	**2,29**	**2,28**	**2,50**	**2,62**	**2,66**	**2,59**	**2,59**	**2,47**	**2,72**	**2,68**	**3,25**	**2,39**	**2,59**

S = supine, E = erect.

TABLE IV. EFFECTS OF ATENOLOL ON PULSE RATE (PER MINUTE)

	Placebo (24 h)		100 mg (8 h)		100 mg (24 h)		100 - 200 mg (8 h)		100 - 200 mg (24 h)		100 - 500 mg (8 h)		100 - 500 mg (24 h)	
	S	E	S	E	S	E	S	E	S	E	S	E	S	E
Mean	**74,2**	**80,5**	**70,1**	**74,3**	**70,4**	**76,0**	**68,0**	**70,8**	**70,2**	**77,1**	**65,7**	**70,2**	**68,3**	**76,4**
SEM	**2,39**	**2,77**	**2,39**	**2,62**	**2,08**	**2,11**	**2,03**	**2,23**	**2,45**	**2,71**	**1,86**	**2,24**	**2,22**	**2,63**

S = supine, E = erect.

DISCUSSION

The data presented in this article indicate that moderately elevated arterial blood pressure may be effectively controlled for 24 hours by single daily doses of sotalol or atenolol, as is the case with other β-adrenoceptor antagonists, including acebutolol and timolol.[4,5] Atenolol proved slightly more potent than sotalol by a factor of about 1,6, but patients required an average of 2 - 3 tablets daily, irrespective of the preparation in use. Single doses of both compounds reduced resting pulse rates, but sotalol was more effective than atenolol at 24 hours, possibly indicating slightly superior maintenance of anti-anginal and anti-arrhythmic effects.

No significant haematological or biochemical changes were observed during the study and very few adverse reactions were reported. Three patients developed impotence during placebo therapy; 2 were excluded from further studies and 1 responded to reassurance and was able to complete the trial. No patient complained of excessive fatigue, and bronchospasm did not occur. Thus the relative cardioselectivity of atenolol offered no obvious advantage over sotalol, in terms of either blood pressure control or freedom from side-effects, in this group of otherwise healthy hypertensives. Cardioselectivity might nevertheless be important in the management of hypertensives with bronchial asthma or insulin-dependent diabetes mellitus, although therapists would be well advised to use all β-adrenoreceptor antagonists cautiously in the presence of these conditions.

Compliance with prescribed treatment was not investigated by measurement of serum drug levels in this study, although the fall in heart rate which was recorded in almost 90% of patients taking either medication suggests that it was remarkably good. Certainly there is no reason to suspect that compliance was inferior to that obtained with multiple-dose regimens.

Taken as a group, these patients fared almost as well on low doses of sotalol or atenolol as on the maximum doses given. This suggests that the practice of increasing doses of expensive β-antagonists above levels which reduce the pulse rate by 10 - 20% may be wasteful and that a diuretic should probably be added to the regimen at an early stage when hypertension is treated with β-antagonists.

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PIONEER 43380

PAPER B6

Antihypertensive and Metabolic Effects of a Combination of Hydrochlorothiazide and Amiloride

This work was carried out by me and analysed and prepared for publication in collaboration with Professor A.J. Reyes.

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Antihypertensive and metabolic effects of a combination of hydrochlorothiazide and amiloride

W. P. LEARY, A. J. REYES

Summary

A combination of hydrochlorothiazide 50 mg and amiloride 5 mg (HTZ + AMI) was administered twice daily for 12 weeks to 18 hypertensive patients, as a monotherapy. There was a statistically and clinically significant decrease in the mean blood pressure level throughout the treatment period.

Significant steady or random changes in blood variables included decreases in chloride, magnesium and bilirubin levels and increases in sodium, calcium, phosphorus, creatinine, triglycerides, total protein, albumin, alkaline phosphatase and SGPT levels. Blood urea nitrogen values changed biphasically. Most of these statistically significant metabolic changes had no clinical relevance.

The dosage problem with HTZ + AMI is discussed.

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Diuretics are the drugs of first choice in the treatment of hypertension.[1] A combination of hydrochlorothiazide 50 mg and the potassium-sparing diuretic amiloride 5 mg (HTZ + AMI) has been demonstrated to be an effective antihypertensive medication when used as monotherapy.[2-4] However, published observations on the metabolic effects of this drug combination do not provide comprehensive cover of biochemical variables in blood and are not consistent with each other.[2-4]

The principal objective of this study was to evaluate the metabolic effects of HTZ + AMI administered to hypertensive patients at its highest recommended dose of 2 tablets per day.

Patients and methods

Patients

Eighteen ambulatory male patients aged 21 - 65 years consented to participate in the trial after they had been fully informed about its objectives and implications. Patients had mild-to-moderately severe hypertension with supine diastolic blood pressure readings between 90 and 120 mmHg. Fourteen were Blacks and 4 were Whites.

Patients who fell within one or more of the following categories were not included in the trial: secondary or renal hypertension, congestive cardiac failure, a history of severe cerebrovascular or hepatic impairment, impaired renal function (creatinine > 2 mg/dl), hyper- (> 5,5 mEq/l) or hypokalaemia (< 3,5 mEq/l), clinical evidence or history of gout, abnormal pretreatment laboratory tests (except high blood glucose which was not an exclusion criterion), or retinopathy more severe than slight irregularity or narrowing of vessels. Patients taking cardiac glycosides or psychotropic drugs were also excluded.

Measurements

Arterial pressures were measured in the working arm by the standard indirect technique, using the first appearance and final disappearance of Korotkoff's sounds to define systolic and diastolic pressures, respectively. The mean arterial blood pressure was defined as the diastolic pressure plus one-third of the pulse pressure. Patients rested supine for 10 minutes and stood upright for 3 minutes before measurement of supine and erect pressures, which were each measured three times, the average values being recorded. All blood pressure measurements were done between 08h00 and 09h30. The sphygmomanometer and stethoscope used for blood pressure measurements were the same throughout the trial. Clinical evaluations were carried out by the same observer physician throughout the trial.

The following variables were measured in blood or serum samples by standard laboratory techniques in 14 patients: chloride, sodium, potassium, calcium, phosphorus, magnesium, osmolality, blood urea nitrogen, creatinine, uric acid, glucose, cholesterol, triglycerides, total protein, albumin, bilirubin, alkaline phosphatase, γ-glutaryltransferase, SGOT and SGPT.

Procedure

All diuretic and/or antihypertensive medication being taken by the patients was discontinued and 1 placebo capsule was prescribed twice daily for 4 weeks. HTZ + AMI was then substituted for 12 weeks, and thereafter placebo was prescribed as in the run-in period for 4 more weeks.

Diet was not restricted, and contained approximately 2 g sodium per day. Complete clinical evaluations were carried out every 4 weeks. Laboratory analyses were done at the beginning of the trial, at the end of the run-in placebo period (referred to as week 0 hereafter) and at the end of the 4th, 8th and 12th weeks of active treatment.

Statistics

Results are expressed as mean values ± standard errors of the means (SEM). Values during treatment and after treatment were contrasted with pretreatment (week 0) values. Variance homogeneities were tested through the F ratio. When variances were homogeneous, a paired t test was used to compare mean

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values and, when variances were not homogeneous, Wilcoxon's signed rank test was used instead. All tests were two-tailed and $P = 0,05$ was considered the upper limit of significance.

Results

Effects of HTZ + AMI on arterial blood pressure and heart rate

HTZ + AMI decreased mean blood pressure significantly compared with control values (Fig. 1). These changes were of clinical significance but 4 weeks after treatment was discontinued mean blood pressure did not differ significantly from control (week 0) levels (Fig. 1).

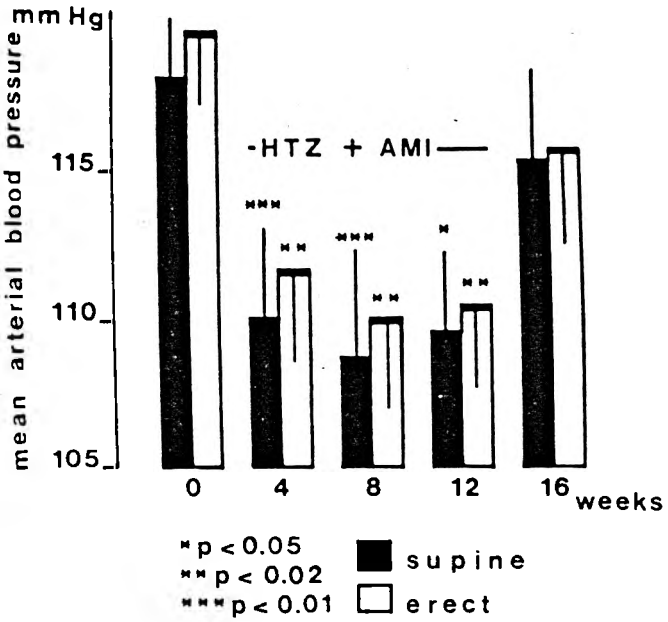

Fig. 1. Mean blood pressure values (± SEM) in 18 hypertensive patients before (week 0), during (weeks 4 - 8) and after (week 16) treatment with HTZ + AMI twice daily.

A significant increase ($P<0,05$) in heart rate was observed at the end of week 8. However, it had no clinical importance, ranging from 77,8 ± 2,6/min in week 0 to 83,1 ± 2,0/min in week 8.

Effects of HTZ + AMI on serum electrolytes

The results are shown in Table I. Serum chloride decreased significantly throughout the treatment period, its lowest level being found at the end of week 4; it recovered, but was still below control levels thereafter. Serum sodium had increased significantly by the end of week 8. Serum potassium did not change significantly during treatment. Serum calcium was significantly increased at the end of week 8. Serum phosphorus was significantly increased at the end of weeks 4 and 8 and returned to control levels at the end of week 12. Serum magnesium was significantly below control level at the end of week 12.

Effects of HTZ + AMI on biochemical blood variables other than electrolytes

The results are shown in Table II. Plasma osmolality did not change significantly during HTZ +AMI treatment. Blood urea nitrogen increased significantly by the end of week 4, decreasing to control values at the end of week 8 and finally decreasing significantly compared with pretreatment levels at the end of week 12. Serum creatinine was found to be significantly increased at the end of weeks 4 and 8 and returned to normal values at the end of week 12. It followed a decreasing trend throughout treatment that paralleled that of blood urea nitrogen. Serum uric acid did not change significantly during HTZ + AMI treatment.

Blood glucose did not change significantly during HTZ + AMI treatment. However, as 4 patients were diabetics under dietary treatment, two different biological populations as regards blood glucose should be considered; one group, consisting of 10 patients, was a population of non-diabetics whose blood glucose values did not change significantly during treatment (Table II). The other population consisted of 4 diabetics who were not fully compensated and whose management was not changed during HTZ + AMI treatment; blood glucose did not change significantly in this group either during treatment with HTZ +AMI.

Plasma cholesterol did not change significantly during HTZ +AMI treatment and serum triglycerides were found to be increased compared with week 0 only at the end of week 8 of treatment.

Total plasma protein was significantly increased at the ends of week 8 and week 12 of treatment, whereas plasma albumin was significantly increased throughout treatment. Serum bilirubin was found to be significantly decreased at the end of weeks 4 and 12. Serum alkaline phosphatase was significantly increased at the end of weeks 8 and 12 of HTZ + AMI treatment. Serum γ-glutaryltransferase and SGOT did not change significantly during treatment. SGPT increased significantly at the end of week 12 of treatment, but remained well within the normal range.

TABLE I. SERUM ELECTROLYTE CONCENTRATIONS (MEAN ± SEM) IN 14 HYPERTENSIVE PATIENTS BEFORE AND DURING TREATMENT WITH A COMBINATION OF HTZ + AMI

Electrolyte	Pretreatment (last on placebo)	At the end of treatment week: 4	8	12
Chloride (mEq/l)	102,6 ± 0,8	97,7 ± 0,8§	100,2 ± 0,6*	100,8 ± 0,7
Sodium (mEq/l)	145,1 ± 0,6	146,5 ± 0,9	147,0 ± 0,8*	100,8 ± 0,7*
Potassium (mEq/l)	4,11 ± 0,06	4,21 ± 0,01	4,23 ± 0,10	3,93 ± 0,08
Calcium (mmol/l)	2,44 ± 0,04	2,53 ± 0,03	2,64 ± 0,03‡	2,47 ± 0,04
Phosphorus (mmol/l)	1,12 ± 0,03	1,33 ± 0,05‡	1,26 ± 0,04†	1,04 ± 0,07
Magnesium (mmol/l)	0,85 ± 0,02	0,86 ± 0,02	0,85 ± 0,02	0,79 ± 0,02†

Significances of differences with respect to pretreatment means:
*$P<0,05$.
†$P<0,02$.
‡$P<0,01$.
§$P<0,001$.

TABLE II. HAEMATOLOGICAL VARIABLES (MEAN ± SEM) OTHER THAN SERUM ELECTROLYTES IN 14 HYPERTENSIVE PATIENTS BEFORE AND DURING TREATMENT WITH HTZ + AMI TWICE DAILY

Variable	Pretreatment (last on placebo)	At the end of treatment week: 4	8	12
Osmolality (mOsm/kg)	281,3 ± 1,8	283,9 ± 3,9	277,1 ± 1,4	276,5 ± 3,4
BUN (mmol/l)	4,05 ± 0,33	5,48 ± 0,42†	3,61 ± 0,29	2,12 ± 0,26*
Creatinine (mmol/l)	101,4 ± 3,7	120,5 ± 5,7§	113,0 ± 4,4*	111,4 ± 3,3
Uric acid (mmol/l)	0,31 ± 0,02	0,32 ± 0,02	0,33 ± 0,03¶	0,33 ± 0,02
Glucose (N = 14) (mg/dl)	120,7 ± 16,2	140,5 ± 20,3¶	120,8 ± 12,5$	131,2 ± 17,1
Glucose (N = 10) (mg/dl)	95,5 ± 5,0	103,3 ± 6,2¶	102,0 ± 6,8	107,8 ± 11,6
Cholesterol (mmol/l)	4,69 ± 0,27	4,70 ± 0,30	4,96 ± 0,24	4,97 ± 0,24
Triglycerides (mmol/l)	2,07 ± 0,25	2,31 ± 0,24	2,85 ± 0,34*	2,67 ± 0,42
Total protein (g/l)	76,6 ± 1,4	77,0 ± 1,3	81,6 ± 1,4*	80,6 ± 1,7
Albumin (g/l)	43,5 ± 1,2	46,3 ± 0,6	49,1 ± 1,0‡	47,9 ± 1,2¶§
Bilirubin (μmol/l)	9,69 ± 1,23¶	5,27 ± 0,98	9,33 ± 0,94¶	5,30 ± 0,91*
Alkaline phosphatase (U/l)	120,1 ± 7,9	135,0 ± 10,6	148,5 ± 13,3‡	138,1 ± 8,9‡
γ-glutaryltransferase (U/l)	51,4 ± 10,2	59,8 ± 11,5	56,7 ± 14,2	51,2 ± 12,6
SGOT (U/l)	21,1 ± 1,9	23,4 ± 2,2	22,8 ± 3,5	25,0 ± 3,8
SGPT (U/l)	14,1 ± 1,3	16,2 ± 1,9$	14,9 ± 2,3	19,9 ± 2,4†

Significances of differences with respect to pretreatment means:
*$P<0.05$.
†$P<0.02$.
‡$P<0.01$.
§$P<0.001$.
¶Data from 13 patients.
$Data from 12 patients.

No change occurred in biochemical variables in individual patients that could indicate a toxic effect of the drug combination. HTZ + AMI was well tolerated and no clinical signs of side-effects were observed.

Discussion

Effects of HTZ + AMI on serum electrolytes

The significant decrease found in serum chloride values is a well-recognized effect of thiazide diuretics. The trend whereby serum chloride levels returned toward reference levels as treatment progressed indicates that homeostatic adjustments take place during prolonged diuretic treatment.[5]

The transient significant increase in serum sodium found at the end of week 8 of treatment was irrelevant in clinical terms.

Serum potassium values did not change significantly during HTZ + AMI treatment, thus demonstrating that 10 mg amiloride daily can prevent the development of hypokalaemia without raising serum potassium to dangerous, abnormally high levels, when 100 mg hydrochlorothiazide is administered conjointly. However, these results should not be considered as the end-point of the potassium problem during diuretic treatment, since what actually matters, both physiologically and clinically, is the intracellular potassium level.[1] Normal serum potassium levels do not necessarily reflect normal intracellular levels since potassium has to be actively pumped into the cells in order to keep its high intracellular concentration; the activity of the potassium pump is critically determined by the intracellular level of magnesium.[1] Diuretics increase renal magnesium excretion and may provoke magnesium depletion, which is usually observed as a decrease in serum magnesium occurring rather late in treatment,[5] as was the case in this trial, in which serum magnesium was found to be significantly decreased only at the end of week 12. A thorough assessment of these complicated interactions and of the effects of HTZ + AMI in this respect requires more detailed long-term studies. However, serum potassium is one of the determinants of intracellular potassium[6] and during hydrochlorothiazide treatment it is preferable to keep it within the normal range by using a potassium-sparing diuretic like amiloride, since potassium supplements induce a higher percentage of side-effects.[7]

The transient increase in serum calcium levels that occurred at the end of week 8 is indicative of an increase in calcium reabsorption in the proximal tubule which is provoked by hydrochlorothiazide and may be related to the proximal tubular increase in sodium and water reabsorption that occurs during treatment with thiazide diuretics; these changes might in turn be secondary to plasma volume contraction provoked by these diuretics.[7] A regulatory effect of the increased calcium reabsorption elicited by hydrochlorothiazide could be an increased secretion of thyrocalcitonin, whose augmented activity could explain the significant increases in serum phosphorus levels that were observed at the end of weeks 4 and 8 of HTZ + AMI treatment. Thyrocalcitonin is known to decrease renal excretion of magnesium through an increased reabsorption of this cation at the ascending limb of the loop of Henle[8] and would then tend to raise serum magnesium levels. Therefore, the decrease in serum magnesium observed at the end of week 12 of HTZ + AMI treatment should not have been expected. However, such a decrease also occurs with loop diuretics that increase calcium excretion and are supposed consequently to decrease thyrocalcitonin secretion.[6] The renal loss of magnesium provoked by diuretics therefore does not appear to be related to the altered renal handling of calcium induced by these drugs.

Effects of HTZ + AMI on biochemical blood variables other than electrolytes

The significant increase in blood urea nitrogen and serum creatinine levels at the end of week 4 of HTZ + AMI treatment tended to return towards control values, surpassing them in the case of blood urea nitrogen. The mechanisms whereby these changes occur are similar to those discussed for calcium.

Serum uric acid and blood glucose are usually increased during treatment with a thiazide diuretic. However, neither of them was found to be significantly increased during HTZ + AMI treatment. The reason serum uric acid levels did not rise significantly is far from clear, although the intimate renal handling of uric acid is obscure, despite extensive

research.[9] The reason for the lack of any significant change in blood glucose during HTZ + AMI treatment is also uncertain. Amiloride does not increase serum uric acid and blood glucose *per se*, whereas hydrochlorothiazide does, and the lack of change during HTZ + AMI treatment could be particularly important when diabetes-prone populations are being treated.

Plasma cholesterol levels did not change significantly and triglycerides were found to be significantly increased only at the end of week 8 of HTZ + AMI treatment. Serum lipids are increased by thiazide and loop diuretics,[10-14] because of an augmented lipolysis in adipose tissue and a subsequent augmented synthesis of mainly very-low-density lipoprotein in the liver. Whether the increase in lipolysis is elicited by an increase in the sympathetic drive, secondary to plasma volume contraction provoked by diuretics, or is due to the fact that diuretics inhibit phosphodiesterase activity, thus increasing intracellular levels of cyclic adenosine monophosphate, is not known. For a better description of the effects of HTZ + AMI on serum lipids, it would be necessary to study the different lipoprotein fractions. The ultimate significance of the serum lipid-raising effect of diuretics on overall cardiovascular prognosis is not known.

The significant increases found in total serum protein and albumin levels during HTZ + AMI treatment could be due to haemoconcentration secondary to the volume depletion provoked by diuretics.

The significant increases in serum alkaline phosphatase levels during HTZ + AMI treatment are not obviously due to intrahepatic obstruction as assessed by the lack of significant increases in plasma cholesterol and albumin; they cannot be explained on the basis of the changes in calcium either. Nevertheless, these increases in serum alkaline phosphatase were of no clinical relevance.

Serum γ-glutaryltransferase and SGOT levels did not change significantly during treatment and SGPT only increased significantly at the end of week 12 of treatment; this change was of no clinical importance, thus indicating that HTZ + AMI is a safe medication from the point of view of the liver.

HTZ + AMI dosage in hypertension

As the principal aim of this study was to evaluate the metabolic safety of HTZ + AMI, high doses were used. However, it is now known that effective antihypertensive diuretics exert their maximal hypotensive effect at a dose lower than their standard diuretic dose. Therefore, even though HTZ + AMI was found to be safe at the dosage studied, it would be reasonable to prescribe a dose of $^1/_2$ - 1 tablet per day in the initial treatment of hypertension. Lower doses may spare magnesium and potassium[6] and, in addition, cause less long-term derangement of metabolism.

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PIONEER

PAPER B7

Effects of Low Doses of Xipamide Given as Monotherapy in Essential Hypertension

All the clinical work was carried out under my direction. The study was designed in consultation with Professor A.J. Reyes who developed the mathematical model used and was co-author.

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EFFECTS OF LOW DOSES OF XIPAMIDE GIVEN AS MONOTHERAPY IN ESSENTIAL HYPERTENSION

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ABSTRACT

Twenty-three ambulant male patients, including Blacks and Caucasions with mild or moderate uncomplicated essential hypertension, were treated with xipamide at the substandard diuretic doses of 5 and 10 mg daily, each formulation given as monotherapy for randomly allocated periods of 9 weeks.

Both dosage regimens caused statistically and clinically significant decreases in systolic and diastolic blood pressures which, from the first week of treatment onwards, evolved as decreasing power functions of time. The 5 mg/day dosage was less effective than the 10 mg/day regime in Black patients, although 5 and 10 mg/day were equally effective in Caucasians.

Laboratory evidence suggesting magnesium depletion in Black patients was found after 8 weeks of treatment with xipamide 5 mg/day.

Xipamide 10 mg/day should be regarded as a maximal dose for the treatment of uncomplicated essential hypertension. Higher doses do not reduce blood pressure further and are more likely to have adverse effects upon potassium, magnesium and zinc turnovers and carbohydrate, lipid and purine metabolisms. However, even when xipamide is used chronically at a dose of 5 mg/day, it may induce somatic potassium and magnesium depletions and therefore dangerous cardiac arrhythmias. Consequently, clinical and laboratory monitoring and prophylactic measures are necessary whenever xipamide is prescribed, at any dose, in long-term treatments.

INTRODUCTION

Xipamide, a high potency distal tubular diuretic,[1-3] effectively lowers the

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blood pressure of patients with mild or moderately severe hypertension when administered as monotherapy in standard diuretic doses of 20 to 40 mg daily.[4,5] Most antihypertensive diuretics cause untoward urinary potassium, magnesium and zinc losses and deleterious effects on carbohydrate, lipid and purine metabolisms when taken in standard diuretic doses, but there is evidence that some of them exert their maximal effect upon high blood pressure at doses as low as one-fourth or one-fifth of usually prescribed diuretic doses.[6-8]

This study was undertaken to determine whether low doses of xipamide are effectively antihypertensive, to describe any changes in blood pressure induced by such low doses mathematically and to assess their effects upon serum potassium and magnesium levels.

PATIENTS AND METHODS

Patients

Twenty-five males, clerical or manual workers aged 35 to 56 years, were selected for the study after the prognostic significance of hypertension, importance of medical supervision and objectives of therapy were explained to them, using the vernacular where necessary to ensure that informed oral consent was obtained.

Thirteen were Black and twelve Caucasian. All had morning supine diastolic pressures of 96 to 120 mmHg recorded after 3 and 4 weeks of treatment with placebo. Individuals with any of the following abnormalities were excluded from the study and given appropriate alternative treatment: secondary hypertension, congestive cardiac failure, history of severe cerebrovascular or hepatic impairment, abnormal renal function (serum creatinine > 2 mg/dl), serum potassium above 5.5 mEq/l or below 3.5 mEq/l, clinical evidence or history of gout, retinopathy more severe than slight irregularity or narrowing of the arterial vessels, electrocardiographic abnormality other than supraventricular extrasystoles or any systemic disease requiring treatment with other drugs.

Operational Procedures

Any medicine taken by the patients was discontinued and replaced by a single placebo tablet administered once daily for four weeks. Thereafter, patients were randomly allocated to treatment with 5 or 10 mg xipamide, presented as identical tablets and taken once daily at 0700 hours for nine weeks of single-blind active treatment. This period was followed by a 4-week washout, when placebo was administered. After this period, active treatment with xipamide at the dose not received during the first part of active treatment was reinstituted for 9 weeks.

Arterial pressures were measured in the working arm by the standard indirect technique, using the first appearance and the final disappearance of Korotkoff's sounds, or their muffling when they persisted down to 0 mmHg, to evaluate systolic and diastolic pressures, respectively. Patients rested supine for 10 minutes before blood pressure measurement, which was repeated three times in both the erect and the supine bodily postures. Average values were recorded.

Pressures were measured each week, 1.5 to 3.5 hours after the usual daily dose of xipamide. The observer and instruments used for recording blood pressure remained the same throughout the study.

Serum sodium, potassium, magnesium, uric acid and glucose concentrations were measured by standard laboratory techniques (atomic absorption was used for the evaluation of magnesium

before and during the active treatment period, on a weekly basis.

Compliance with treatment was assessed by counting the number of tablets returned at each visit. Complete clinical evaluations were carried out at the end of the placebo period and after the fourth and eighth weeks of active treatment. No other medications were prescribed or permitted during the trial period.

Data Analysis

Mean blood pressure is defined as diastolic blood pressure plus one-third of the difference between systolic and diastolic blood pressure.

Results are expressed as means and standard errors of means (S.E.M.). Acceptable levels of normality of frequency distributions and homoscedasticity of variances were assessed directly or in transformed data, as appropriate, before carrying out statistical analysis. The paired t-test was used to assess the significance of differences between any two means. Data were linearised as reported previously,[5] the standard linear correlation and regression techniques were applied to their analysis and the t-test for analysing the significance of the difference between two slopes was evaluated as necessary. All tests were two-tailed and $p = 0.05$ was taken as the limit of significance.

RESULTS

Compliance with treatment was estimated to be excellent since more than 90% of prescribed tablets were taken in all cases.

Effects of Xipamide Treatment on Blood Pressure

One patient (Black) missed three weekly controls of blood pressure during the 5 mg/day treatment period and another patient (Caucasian) missed two weekly controls during the 10 mg/day treatment period. For these reasons, neither was included in the results.

The systolic and diastolic blood pressure mean values before and during treatment with xipamide 5 and 10 mg/day are shown in Tables I to IV. The last measurement on placebo was taken as pre-treatment or untreated blood pressure. Xipamide 5 and 10 mg/day reduced supine blood pressure in a statistically significant manner from the first week of treatment. In Black patients, responses to 5 mg/day were not consistently significant until week 3 of treatment; here the sample size was small and the standard deviations evaluated were correspondingly large (Tables I and II).

Erect blood pressure was significantly reduced by xipamide 5 mg/day from week 2 of treatment. This dosage did not reduce blood pressure consistently in the Black subgroup. The 10 mg/day dose significantly reduced erect blood pressures throughout the study whether patients were ethnically subdivided or considered as a single group (Tables III and IV).

The changes in the ethnically undivided mean supine systolic, diastolic and mean blood pressure, P, values as functions of time, t, were satisfactorily accounted for by decreasing power functions from the end of the first to the end of the ninth weeks of treatment:

$$P = P_f - (P_u - P_f - P_s) \exp(-k.\ln t),$$

Table I — *Supine blood pressure (mmHg) changes during treatment with xipamide 5 mg/day. Mean ± S.E.M.*

Patients	Blood Pressure	Pre-treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
All 23	Systolic	168.9 ±3.2	156.4*** ±4.3	162.0(NS) ±5.0	150.1**** ±3.1(n=22)	152.9**** ±3.3	149.0**** ±4.0	150.8**** ±4.0(n=22)	151.9**** ±4.0(n=22)	147.6**** ±3.9(n=21)	145.8**** ±4.1(n=21)
All 23	Diastolic	104.0 ±1.6	94.8*** ±2.5	96.3** ±3.1	90.0**** ±1.6(n=22)	93.1*** ±2.6	87.6**** ±2.4	91.7**** ±2.8(n=22)	87.9**** ±2.1(n=22)	90.7**** ±2.7(n=21)	88.8**** ±2.6(n=21)
11 Caucasians	Systolic	166.5 ±4.6	154.9(NS) ±6.3	159.6(NS) ±6.3	151.8** ±4.9	148.2*** ±4.3	148.7** ±6.7	148.4*** ±5.5	149.3*** ±5.0(n=10)	142.9*** ±5.7(n=9)	143.0**** ±5.7(n=10)
11 Caucasians	Diastolic	102.7 ±1.5	93.6*** ±2.9	90.2*** ±2.6	92.5**** ±1.4	89.8*** ±2.2	86.4**** ±2.5	87.8**** ±3.1	86.2**** ±2.5(n=10)	86.0**** ±2.8(=9)	88.0**** ±3.1(n=10)
12 Blacks	Systolic	171.0 ±4.6	157.8* ±5.6	164.4(NS) ±7.9	148.6*** ±4.1(n=11)	157.3* ±4.8	149.3**** ±5.1	153.3** ±6.0(n=11)	154.5* ±6.4	151.2*** ±5.4	148.2**** ±6.0(n=11)
12 Blacks	Diastolic	105.2 ±2.9	95.8(NS) ±4.1	102.4(NS) ±5.2	87.2**** ±2.7(n=11)	96.2(NS) ±4.4	88.7**** ±4.1	95.6* ±4.5(n=11)	89.6**** ±3.5	94.2* ±4.0	89.5**** ±4.1(n=11)

n =number of patients.

Significances of the differences with respect to pre-treatment mean values: (NS) = non significant; *$p < 0.05$; **$p < 0.025$; ***$p < 0.01$; ****$p < 0.001$.

Table II — *Erect blood pressure (mmHg) changes during treatment with xipamide 5 mg/day. Mean ± S.E.M.*

Patients	Blood Pressure	Pre-treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
All 23	Systolic	163.7 ±3.0	160.6(NS) ±4.2	154.0* ±4.4	146.4**** ±3.1(n=22)	149.2*** ±3.6	148.9*** ±3.0	150.0** ±4.4(n=22)	147.8**** ±3.9(n=22)	148.4**** ±3.9(n=21)	144.5**** ±4.0(n=21)
All 23	Diastolic	106.8 ±1.7	98.4(NS) ±2.6	97.5*** ±2.1	93.0**** ±1.9(n=22)	97.3**** ±2.2	94.3**** ±2.2	97.4**** ±2.8(n=22)	93.1**** ±1.9(n=22)	94.5**** ±2.8(n=21)	95.6**** ±2.5(n=21)
11 Caucasians	Systolic	159.1 ±4.4	151.3(NS) ±6.0	150.4(NS) ±6.5	141.6*** ±4.2	140.5*** ±3.1	142.5*** ±3.8	145.4(NS) ±6.2	140.5** ±4.4(n=10)	141.8(NS) ±5.3(n=9)	136.0*** ±5.6(n=10)
11 Caucasians	Diastolic	105.1 ±2.0	96.0** ±3.2	94.7*** ±2.4	91.8**** ±1.8	94.4**** ±2.0	94.2**** ±2.8	93.6*** ±3.6	91.6*** ±3.1(n=10)	87.5*** ±2.4(n=9)	90.4**** ±2.1(n=10)
12 Blacks	Systolic	168.0 ±3.9	156.2(NS) ±6.2	157.6(NS) ±6.1	150.7** ±4.4(n=11)	157.2(NS) ±5.4	154.8(NS) ±4.0	154.5(NS) ±6.3(n=11)	155.1(NS) ±5.9	153.3*** ±5.2	151.2** ±5.0(n=11)
12 Blacks	Diastolic	108.3 ±2.6	100.7(NS) ±4.2	100.4(NS) ±3.5	94.0**** ±3.2(n=11)	99.3* ±3.7	94.3**** ±3.4	101.1(NS) ±4.2(n=11)	94.5*** ±2.4	99.7(NS) ±4.0	100.0* ±3.9(n=11)

n =number of patients.

Significances of the differences with respect to pre-treatment mean values: (NS) = non significant; *$p < 0.05$; **$p < 0.025$; ***$p < 0.01$; ****$p < 0.001$.

Table III — *Supine blood pressure (mmHg) changes during treatment with xipamide 10 mg/day. Mean ± S.E.M.*

Patients	Blood Pressure	Pre-treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
All 23	Systolic	169.6 ±3.2	159.7** ±3.3	154.7**** ±3.8	155.2**** ±4.6	154.5**** ±4.3	153.7**** ±4.3(n=22)	151.5**** ±4.2	148.8**** ±4.6(n=20)	147.6**** ±4.8	146.6**** ±4.6(n=22)
All 23	Diastolic	103.0 ±1.0	94.4** ±3.2	90.6**** ±3.0	89.0**** ±2.9	93.2*** ±3.1	92.2*** ±3.1(n=22)	85.9**** ±2.8	86.9**** ±2.9(n=20)	89.0**** ±2.9	87.4**** ±2.8(n=22)
10 Caucasians	Systolic	164.4 ±5.5	157.8(NS) ±6.4	147.6**** ±6.5	146.8*** ±7.5	146.6* ±8.2	157.1(NS) ±7.8(n=9)	150.2* ±8.1	140.0**** ±7.0	145.8** ±8.6	141.2*** ±7.9
10 Caucasians	Diastolic	103.6 ±1.3	95.6* ±2.8	85.8*** ±4.3	88.2*** ±3.5	86.8*** ±3.6	90.0**** ±3.2(n=9)	80.8**** ±3.8	81.2**** ±3.2	89.2*** ±2.2	87.4*** ±4.3
13 Blacks	Systolic	173.5 ±3.5	161.2*** ±3.4	160.5*** ±4.1	161.7* ±5.1	160.6*** ±3.9	151.4**** ±5.1	152.5**** ±4.2	157.6*** ±4.8(n=10)	149.1**** ±5.5	151.2**** ±5.2(n=12)
13 Blacks	Diastolic	102.5 ±1.5	93.5*** ±3.2	94.3*** ±2.1	89.5** ±4.0	98.1(NS) ±3.3	93.8** ±2.8	89.8*** ±3.5	92.6*** ±2.5(n=10)	88.9**** ±3.6	87.3*** ±2.8(n=12)

n =number of patients.

Significances of the differences with respect to pre-treatment mean values: (NS) = non significant; *$p < 0.05$; **$p < 0.025$; ***$p < 0.01$; ****$p < 0.001$.

Table IV — *Erect blood pressure (mmHg) changes during treatment with xipamide 10 mg/day. Mean ± S.E.M.*

Patients	Blood Pressure	Pre-treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
All 23	Systolic	168.9 ±3.8	154.8**** ±3.7	148.9**** ±3.4	152.3**** ±3.7	151.9**** ±4.5	149.7**** ±3.5(n=22)	151.2**** ±3.7	147.8**** ±4.1(n=20)	146.1**** ±4.6	143.8**** ±4.1(n=22)
All 23	Diastolic	106.8 ±1.1	96.9**** ±2.3	96.2**** ±1.4	95.7*** ±2.0	96.7*** ±2.4	96.4**** ±1.5(n=22)	92.7**** ±2.3	92.3**** ±1.5(n=20)	93.4**** ±2.2	91.4**** ±1.5(n=22)
10 Caucasians	Systolic	163.6 ±7.7	149.1* ±6.9	142.2*** ±5.6	142.8*** ±5.4	143.0*** ±7.4	154.2(NS) ±6.2(n=9)	146.8** ±5.7	137.2*** ±4.2	143.0*** ±7.2	144.0** ±6.4
10 Caucasians	Diastolic	107.0 ±2.1	97.2* ±3.2	96.8*** ±2.5	96.4** ±3.6	91.8*** ±3.5	95.1*** ±2.2(n=9)	91.2*** ±4.3	90.8*** ±2.3	96.2** ±2.2	90.4**** ±2.4
13 Blacks	Systolic	172.9 ±3.1	159.2*** ±3.8	154.1*** ±3.8	159.7*** ±4.2	158.8*** ±5.0	146.6**** ±4.0	154.6*** ±4.8	158.4** ±5.3(n=10)	148.5**** ±6.2	147.0**** ±5.5(n=12)
13 Blacks	Diastolic	106.7 ±1.1	96.7*** ±3.3	95.8*** ±1.8	95.2**** ±2.3	100.4(NS) ±3.1	97.4**** ±2.0	93.8**** ±2.6	93.8**** ±2.0(n=10)	91.2*** ±3.4	92.2**** ±2.1(n=12)

n =number of patients.

Significances of the differences with respect to pre-treatment mean values: (NS) = non significant; *$p < 0.05$; **$p < 0.025$; ***$p < 0.01$; ****$p < 0.001$.

where P_f is a theoretical final limit value to which blood pressure tends upon indefinite prolongation of treatment, P_u is untreated blood pressure, P_s is the difference between P_u and the theoretical P value after the first week of treatment and k is a dimensionless constant that governs the slope of the function. The statistical and parametrical features of the functions for the supine, systolic and diastolic pressures are shown in Table V. Changes in supine mean blood pressure in the entire group of patients during the 5 and 10 mg/day dosage regimens are shown in Figures 1 and 2, respectively, where the corresponding functions are plotted. No significant differences were found between the slopes of the systolic, diastolic and mean blood pressures derived from the xipamide 5 mg/day and xipamide 10 mg/day treatments.

Table V — *Statistical and parametrical features of the linearised functions pressure (time) for the supine blood pressures. Entire group of patients.*

Variable and Treatment	r	p	P_u (mmHg)	P_f (mmHg)	P_s (mmHg)	k
Systolic blood pressure xipamide 5 mg/day	-0.786	< 0.05	168.9	135	9.0	0.3003
Diastolic blood pressure xipamide 5 mg/day	-0.706	< 0.05	104.0	80	8.2	0.2648
Systolic blood pressure xipamide 10 mg/day	-0.901	< 0.01	169.6	135	8.0	0.3129
Diastolic blood pressure xipamide 10 mg/day	-0.675	< 0.05	103.0	80	8.8	0.2879

Effects of Xipamide Treatment on Heart Rate

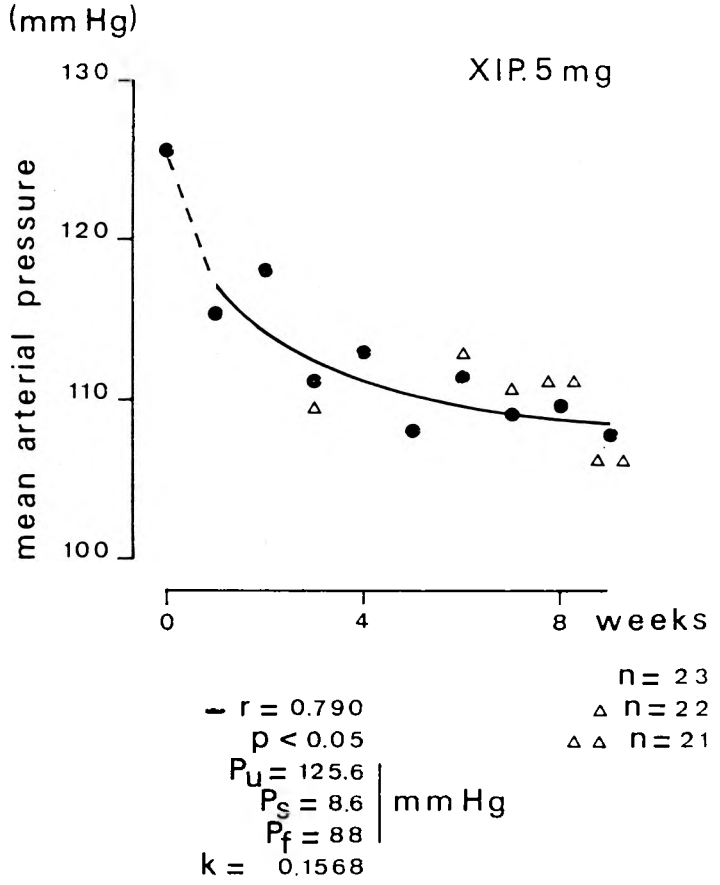
Heart rate did not significantly change in response to treatment with xipamide except during week 1, when 5 mg caused an increase from 79.1 ± 1.2 to 81.6 ± 1.1 beats/min ($p < 0.005$). This was of no clinical significance.

Effects of Xipamide Treatment on Serum Variables

Voluntary compliance with weekly blood sampling was poor and it was considered unethical to insist that patients cooperated in this regard.

No statistically significant change in the serum sodium, potassium, uric acid or glucose levels was observed during the trial with either dose of xipamide, although serum potassium concentration fell below the arbitrary lower limit of normality (3.5 mEq/l) in one Black patient taking 10 mg of the diuretic after 8 weeks of treatment. The only statistically significant change found in serum magnesium levels was a decrease from 0.80 ± 0.01 at week 0 to

Figure 1 — *Mean values of supine mean arterial blood pressure before (week 0) and during (weeks from 0 to 9) treatment with xipamide 5 mg day. The P(t) function has been fitted to the experimental means. n = number of patients; P_u : pre-treatment blood pressure; P_s : difference between P_u and the blood pressure value at week 1; P_f : final limit value to which blood pressure tends upon indefinite prolongation of treatment; k : dimensionless constant.*



0.74 ± 0.02 mmol/l at week 8 in eight Black patients taking 5 mg daily ($p < 0.05$).

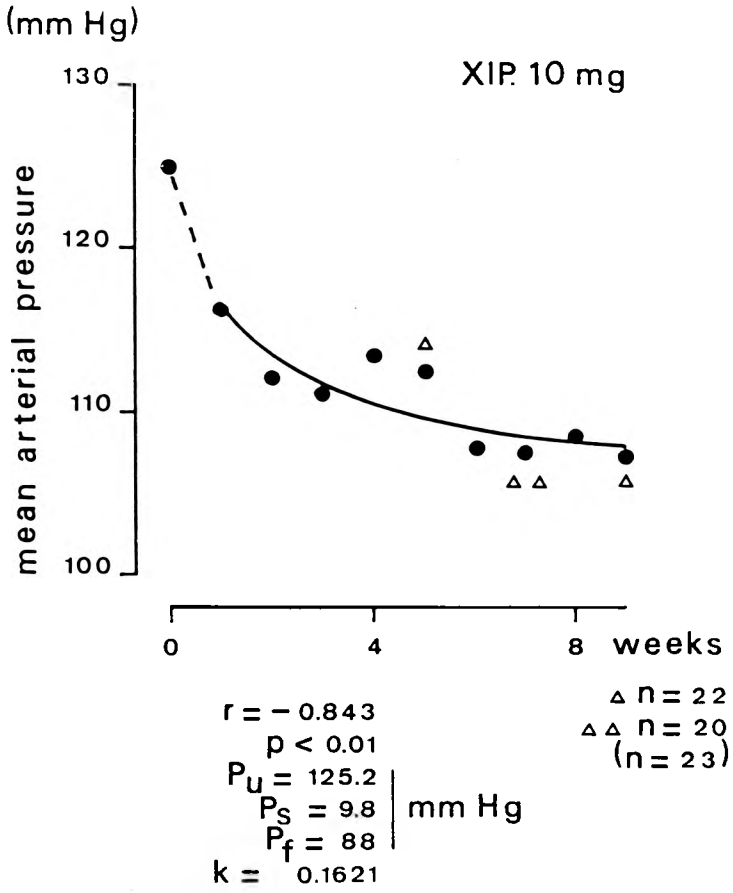
Clinical Effects of Xipamide Treatment

No relevant clinical effects of xipamide, except for increases in urinary outputs reported by some patients, were detected during the clinical assessments.

DISCUSSION

Xipamide, given as monotherapy in doses of 5 or 10 mg daily, effectively reduced blood pressure in the ethnically undivided groups of patients and in Caucasoid patients with uncomplicated essential hypertension of mild to moderate degree. As judged by the parameter values set out in Table V,

Figure 2 — *Mean values of supine mean arterial blood pressure before (week 0) and during (weeks from 0 to 9) treatment with xipamide 10 mg/day. The P(t) function has been fitted to the experimental means. n = number of patients. P_u : pre-treatment blood pressure; P_s : difference between P_u and the blood pressure value at week 1; P_f : final limit value to which blood pressure tends upon indefinite prolongation of treatment; k : dimensionless constant.*



responses to these doses were identical and should have the same effects in clinical practice. In the case of Black patients, however, 10 mg appeared to be the more effective dose. The reason for this ethnic difference is not immediately apparent but could reflect a difference in the dose-response curves of the two groups studied.

Doses of xipamide which are currently prescribed for the treatment of arterial hypertension, congestive cardiac failure, hepatic oedema and nephrotic syndrome (20-40 mg/day)[2,3] may be excessive for the satisfactory treatment of most patients with uncomplicated hypertension. Moreover, it is noteworthy that xipamide 5 or 10 mg/day induced changes in blood pressure with time that were described by power functions, as was the case when the antihypertensive effects of xipamide 20 or 40 mg/day were studied.[4,5] This characteristic is shared by indapamide[8] and differentiates these drugs from diuretics such as hydrochlorothiazide,[9] cyclothiazide[5] and tizolemide,[10] that

lower blood pressure following smoothly decreasing linear function during the first weeks of treatment, even when administered at standard diuretic doses.

The finding that xipamide has marked antihypertensive effects at dosages well below the conventional diuretic dosage is consistent with reports indicating that other antihypertensive diuretics, including hydrochlorothiazide,[6] chlorthalidone[7] and indapamide[8] share this characteristic. The lack of side effects or significant changes in most plasma variables, especially in serum potassium, glucose and uric acid concentrations, suggests that metabolic disruption and its concomitant risks of sudden death and myocardial infarction can be diminished to some extent by strict limitation of xipamide dosage to a maximum of 10 mg/day in hypertension. Magnesium depletion provoked by diuretics appears to be the principal mechanism whereby these drugs provoke cardiac arrhythmias and sudden death when chronically administered.[11-14] The fact that a significant decrease in the mean serum magnesium concentration value occurred in Black patients after 8 weeks of treatment with xipamide 5 mg/day indicates that magnesium depletion may result from that treatment. Moreover, serum magnesium concentration is a tardy revealer of magnesium depletion and usually falls significantly after three months of treatment with a diuretic.[15] In addition, xipamide 5 or 10 mg have significant hyperkaliuretic and hypermagnesiuretic effects when administered as monodoses to healthy probands (A.J. Reyes and W.P. Leary, unpublished). Therefore, it remains a mandatory practice to monitor relevant plasma variables, principally potassium and magnesium concentrations, during long-term treatment with xipamide, irrespective of the dose prescribed. Appropriate manoeuvres for the prophylaxis of magnesium and potassium depletion, including restriction of dietary sodium, supplementation of potassium and magnesium intake[16] and the judicious use of the potassium-retaining and magnesium-sparing diuretic amiloride[17] should be taken when necessary. The effects of prolonged therapy with low doses of xipamide upon magnesium balance requires careful assessment, since magnesium depletion may ultimately be responsible for the development of arrhythmias during chronic diuretic treatment.

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PAPER B8

Effects of a Hydrochlorothiazide and Amiloride Combination on Plasma Magnesium in Patients with Essential Hypertension

This trial was conceived by the two principal authors and the work carried out under my direction. Computer technology was provided by Mr. K. van der Byl and the paper written and edited by the first two authors.

Effects of a hydrochlorothiazide and amiloride combination on plasma magnesium in patients with essential hypertension

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Zusammenfassung

Bei 21 ambulanten Patienten wurde der Einfluß von 50 mg Hydrochlorothiazid allein (H) und in Kombination mit 5 mg Amilorid (H+A) auf das Verhalten des Plasma-Mg untersucht. Die Patienten wurden zufällig und unter Doppelblindbedingungen den beiden Behandlungsarten, die 23 Wochen lang durchgeführt wurden, zugeordnet und erhielten täglich je eine Tablette.

Beide Therapieformen waren am Ende der Beobachtungszeit gleichermaßen antihypertensiv wirksam, obwohl die hypotensive Wirkung der Kombinationsbehandlung (H + A) schneller einsetzte.

Nach durchschnittlich 22 Wochen fiel das Plasma-Mg in der (H)-Gruppe von 0,77 auf 0,70 mmol/l ($p < 0,05$) und in der (H + A)-Gruppe von 0,82 auf 0,75 mmol/l ($p < 0,05$). Sieben von neun Patienten der (H)-Gruppe und sechs von zehn der (H + A)-Gruppe hatten Plasma-Mg-Konzentrationen unter 0,75 mmol/l nach durchschnittlich 22 Behandlungswochen. Hinsichtlich des Plasma-Mg bestehen zwischen der (H)- bzw. (H + A)-Gruppe keine signifikanten Unterschiede.

Es wird gefolgert, daß bei Langzeitbehandlung die zusätzliche Gabe von 5 mg Amilorid nicht die durch 50 mg Hydrochlorothiazid verursachte Abnahme des Plasma-Mg verhindert.

Summary

The effects on plasma Mg of 50 mg hydrochlorothiazide (HCTZ) and those of a 50 mg hydrochlorothiazide and 5 mg amiloride combination (HCTZ + AMI) were studied in twenty-one ambulant patients suffering from essential hypertension. Patients were double-blind and randomly allocated to treatment with one daily tablet of either formulation, for a 23-week period.

Both formulations exerted equally efficacious final antihypertensive effects, though the hypotensive action of HCTZ + AMI proceeded at a higher rate than that of HCTZ.

After an average of 22 weeks of therapy, plasma Mg fell from a pretreatment mean value of 0.77 to 0.70 mmol.L^{-1} ($p < 0.05$) in the HCTZ-treated group, and from 0.82 to 0.74 mmol.L^{-1} ($p < 0.05$) in the HCTZ + AMI-treated group. Seven out of the nine patients in the HCTZ-treated group and six out of ten of the patients in the HCTZ + AMI-treated group exhibited plasma Mg concentrations below 0.75 mmol.L^{-1}, after a mean duration of treatment of 22 weeks. No significant differences existed between the effects the studied formulations had on plasma Mg.

It is concluded that the addition of 5 mg amiloride to 50 mg hydrochlorothiazide does not help preventing the fall in plasma Mg induced by 50 mg hydrochlorothiazide upon prolonged administration.

Resumé

L'effet de 50 mg d'hydrochlorothiazide (HCTZ) et celui d'une combinaison de 50 mg d'hydrochlorothiazide et 5 mg d'amiloride (HCTZ + AMI) sur le Mg plasmatique étaient étudiés chez vingt-et-un malades ambulatoires avec hypertension artérielle essentielle. Les malades étaient assignés, par hasard et double-aveugle, au traitement avec HCTZ ou HCTZ + AMI une fois par jour, pendant 23 semaines.

Les deux formulations montrèrent des efficacies antihypertensives de la même magnitude finale, bien que l'effet de l'HCTZ + AMI était plus rapide que celui de l'HCTZ. La concentration plasmatique du Mg tomba d'une moyenne de 0.77 mmol.L^{-1} avant le traitement à 0.70 mmol.L^{-1} ($p < 0.05$) après une moyenne de 22 semaines de thérapie avec HCTZ. Pendant le meme période, le magnesium plasmatique descendu de 0.82 à 0.74 mmol.L^{-1} ($p < 0.05$) chez les malades traités avec HCTZ + AMI. Sept des neuf malades traités avec HCTZ et six des dix des malades traités avec HCTZ + AMI eurent chiffres de Mg plasmatique inférieures au limite normal de 0.75 mmol.L^{-1}, après une moyenne de 22 semaines de traitement.

On conclut que l'addition de 5 mg d'amiloride ne prévient pas la chute du Mg plasmatique provoqué par l'administration journalière de 50 mg d'hydrochlorothiazide.

Introduction

Diuretics are widely used in the treatment of essential hypertension. However, the chronic deployment of these substances is not devoid of important side-effects [26, 27], amongst which bodily Mg depletion is one of the most relevant [28]. Common loop (e.g. furosemide) and distal tubular (e.g. hydrochlorothiazide, chlorthalidone) diuretics have been found to cause hypermagnesiuresis in acute and chronic studies carried out in normal subjects and in patients suffering from various conditions [28, 30, 34]. The ensuing bodily Mg depletion is the principal determinant of diuretic-induced cardiac arrhythmias (hitherto ascribed to K depletion) [24, 28, 30], hampers the antihypertensive effect of diuretics [6], contributes to shifting the plasma lipid profile towards a pattern of high cardiovascular risk [28], and predisposes to sudden death and coronary and cerebrovascular spasms [1, 7, 16, 18].

The K-sparing diuretic amiloride exhibits weak dose-dependent, Mg-sparing actions when single doses are administered to healthy individuals [8, 17], whereas hydrochlorothiazide 50 mg (HCTZ) causes significant hypermagnesiuresis under similar experimental circumstances [15]. A combination of 50 mg hydrochlorothiazide and 5 mg amiloride (HCTZ + AMI) has not been found to induce a statistically significant increase in magnesiuresis when a single dose is given to normal subjects [10, 16]. In consequence, it could be postulated that HCTZ + AMI would be less likely to induce a decrease in bodily Mg than HCTZ, when given chronically to hypertensive patients.

The objective of this study was to comparatively assess the effects of HCTZ+AMI and of HCTZ on plasma Mg, during medium-term administration to patients with essential hypertension.

Patients and methods

Patients

Twenty-one males, clerical or manual workers, aged 35 to 56 years, were selected for the study after the objectives and implications of the trial were explained to them, using the vernacular where necessary to ensure that informed oral consent was obtained.

Twelve patients were Black and nine were Caucasoid. All had morning resting supine diastolic blood pressures of 95—110 mm Hg (inclusive), recorded after 2 and 4 weeks of treatment with placebo. Patients in whom comprehensive clinical — including ophthalmological —, laboratory electrical and radiological investigations revealed one or more of the following, were not included in the study: (i) secondary hypertension of any origin; (ii) malignant hypertension; (iii) haemodynamically significant valvular heart disease; (iv) congestive cardiac insufficiency; (v) left ventricular insufficiency; (vi) right ventricular insufficiency; (vii) sinus bradycardia of less than 54.min^{-1} after 3 minutes supine rest; (viii) second or third degree atrio-ventricular block; (ix) coronary insufficiency requiring pharmacological treatment; (x) myocardial infarction within the past six months; (xi) a history or clinical evidence of cerebrovascular impairment, including retinal hemorrhages; (xii) renal dysfunction evidenced by proteinuria, by a serum creatinine level higher than 1.5 mg.dL^{-1} or by a blood urea higher than 60 mg.dL^{-1}; (xiii) clinically relevant hyper- or hypokalaemia; (xiv) a history or clinical evidence of gout; (xv) diabetes mellitus requiring pharmacological treatment; (xvi) rheumatic conditions requiring drug therapy; (xvii) respiratory disorders requiring drug treatment other than with antibiotics; (xviii) anaemia or leucopenia; (xix) conditions requiring topical application of corticosteroids; (xx) any severe disease likely to interfere with the objectives of the study, either per se or because of the necessary medication; (xxi) psychotic disorders of any kind; (xxii) any specific contraindication for any of the study substances.

Measurements

Arterial pressures were measured in the working arm by the standard indirect technique, using the first appearance and final disappearance of Korotkoff's sounds to define systolic and diastolic pressures respectively. If sounds persisted to 0 mm Hg, the point at which muffling occurred was taken as the diastolic pressure reference. Patients rested supine for 10 minutes and erect for 3 minutes before measurement of the corresponding blood pressures; an average was obtained from three readings per patient in each bodily posture. The same mercury sphygmomanometer and stethoscope were used throughout the study and all clinical evaluations and measurements were made 2—4 h after the last intake of medication.

Serum Mg was measured by atomic absorption spectroscopy, using a Varian 275 instrument. Serum Na and K were measured by the ion-selective technique using a Nova 4 analyser. All other chemical analyses and haematological and urinary evaluations were done by standard laboratory methods. These determinations included plasma glucose, creatinine, uric acid, urea, SGOT alkaline phosphatase and bilirubin, hemoglobin, hematocrit, white blood cell count, platelet estimation and standard urinalysis. Blood samples for laboratory analyses were taken 2—4 h after the last intake of medication.

Operational procedures

At the first examination patients who were under antihypertensive treatment had current therapy withdrawn. Precise instructions were given that tablets had to be taken at h 08.00 a.m. and that magnesium-based antacids could not be used during the study. No medications other than the trial formulations were prescribed. One placebo tablet was indicated to be ingested daily for a 4-week baseline period. After the end of the second week of this period, another complete clinical evaluation was performed, and an electrocardiogram, a chest X-ray and complete laboratory analyses were carried out.

Patients with a supine diastolic blood pressure of 95—110 mm Hg at the end of the second and of the fourth week of the baseline period and who met none of the exclusion criteria proceeded to the active treatment phase of the study. Patients were randomly and double-blind allocated to monotherapy with a daily tablet of either HCTZ or HCTZ+AMI. Arterial blood pressure, pulse rate and body weight were measured every 2 weeks starting at the end of third week of treatment. Plasma Mg, K and Na were measured every 2 weeks between the end of the fifth and of the twenty-third week of active treatment. A complete clinical evaluation, an electrographic recording, a chest X-ray and complete laboratory measurements were carried out at the end of the twenty-third week of active treatment.

Compliance with treatment was assessed by counting the number of tablets returned at each clinical visit.

Statistics

Results are expressed as means and standard errors of means.

Normality of frequency distributions and homoscedasticity of variances were evaluated by the chi-square test for goodness of fit and the F ratio respectively. Minor departures from formal prerequisites for parametric tests were tolerated.

The t-test for dependent samples was used for assessing the significances of the differences between any two mean values within the same active treatment group, and a t-test for independent samples was used for evaluating the significances of the differences between any two mean values corresponding to different active treatment groups.

All statistical tests deployed were two-tailed and $p = 0.05$ was considered the limit of significance.

Results

Compliance with treatment was excellent in all cases.

Nine patients received HCTZ and twelve patients were treated with HCTZ+AMI.

The systolic and diastolic blood pressure mean values before and during treatment with HCTZ and HCTZ+AMI are depicted in Table I. The last measurement on placebo was taken as pretreatment (or untreated) blood pressure. Supine diastolic blood pressure showed mean values below 95 mm Hg after 3 weeks of treatment with HCTZ+AMI and after 5 weeks of therapy with HCTZ, and remained within the normal range under either formulation for the rest of the study.

Since plasma Mg, K and Na measurements were not carried out in all patients at all scheduled instances, values from two consecutive scheduled measurements corresponding to the same patient, if existent, were averaged. Mean results are shown in Table II, where values measured 15 days apart — one or two values per patient — have been ascribed to the corresponding median-point in time.

Mean plasma magnesium concentration fell significantly, with respect to pretreatment, after an average of 22 weeks of therapy with either HCTZ or HCTZ+AMI. Individual mean plasma magnesium concentrations were all above 0.75 mmol. L^{-1} after 8—10 weeks of treatment with either formulation. After a mean of 22 weeks of therapy, individual plasma magnesium concentrations were below 0.75 mmol. L^{-1} in eight out of the nine patients taking HCTZ and in six out of ten of the patients taking HCTZ+AMI.

Table I: Changes in blood pressure (mm Hg) induced by treatment of nine hypertensive patients with hydrochlorothiazide 50 mg (HCTZ) daily and of twelve hypertensive patients with a combination of hydrochlorothiazide 50 mg and amiloride 5 mg (HCTZ+AMI) daily. Values as means ±S.E.M.

Treatment	Blood pressure	Duration of treatment (weeks)											
		Pre-treatment	3	5	7	9	11	13	15	17	19	21	23
HCTZ	Supine systolic	156 ±4	147[b] ±5	140[a]*** ±4	144** ±4	143* ±6	140**** ±3	140* ±6	141*** ±5	144[a]*** ±5	136*** ±5	139**** ±4	146[c] ±8
	Supine diastolic	98 ±2	96[b] ±2	96[a] ±3	94* ±2	93** ±2	91*** ±2	92*** ±2	91*** ±2	94** ±2	90*** ±3	91** ±2	92[c] ±3
	Erect systolic	149 ±5	137[b]* ±4	137[a]* ±6	135*** ±3	136** ±5	132*** ±4	129*** ±5	132*** ±6	136[a]*** ±4	132*** ±6	133**** ±5	142[c] ±7
	Erect diastolic	100 ±4	92[b] ±2	94[a] ±3	92 ±2	92 ±1	89* ±3	90 ±2	90 ±2	93[a] ±2	89** ±3	90 ±2	94[c] ±3
HCTZ +AMI	Supine systolic	146 ±3	137[e] ±6	136 ±5	133** ±4	127*** ±3	129** ±4	129** ±4	125*** ±4	125*** ±4	126[d]*** ±3	132[d]* ±6	131[b]* ±6
	Supine diastolic	100 ±1	93[e]*** ±2	92**** ±2	93* ±3	90**** ±2	90*** ±3	89*** ±3	86**** ±2	87**** ±2	85[d]**** ±2	89[d]*** ±3	84[b]*** ±3
	Erect systolic	142 ±3	134[e] ±5	131* ±4	130** ±3	122**** ±2	132 ±5	121*** ±4	120*** ±3	120*** ±4	122[d]*** ±3	127[d]* ±5	126[b]*** ±6
	Erect diastolic	97 ±2	93[e] ±3	94 ±2	93 ±2	88*** ±1	89*** ±2	86*** ±3	85*** ±2	84*** ±2	84[d]*** ±2	88[d]*** ±3	84[b]*** ±3

[a] results from eight patients.
[b] results from seven patients.
[c] results from six patients.
[d] results from eleven patients.
[e] results from nine patients.
* $p < 0.05$ with respect to pretreatment mean value.
** $p < 0.02$ with respect to pretreatment mean value.
*** $p < 0.01$ with respect to pretreatment mean value.
**** $p < 0.001$ with respect to pretreatment mean value.

Table II: Changes in serum electrolyte concentrations (mmol. L^{-1}) induced by treatment of nine hypertensive patients with hydrochlorothiazide 50 mg (HCTZ) daily and of twelve other hypertensive patients with a combination of hydrochlorothiazide 50 mg and amiloride 5 mg (HCTZ + AMI) daily. Values as means ± S.E.M.

Treatment	Serum electrolyte concentration	Averaged duration of treatment (weeks)					
		Pre-treatment	6	10	14	18	22
HCTZ	Magnesium	0.77 ± 0.03 (8)	0.81 ± 0.03 (17)	0.80 ± 0.01 (16)	0.80[a] ± 0.03 (15)	0.76[b] ± 0.02 (18)	0.70* ± 0.01 (15)
	Potassium	3.8 ± 0.2 (8)	4.1 ± 0.2 (17)	3.7 ± 0.2 (16)	4.0 ± 0.1 (15)	3.9 ± 0.1 (17)	4.1 ± 0.1 (15)
	Sodium	146 ± 1 (7)	146 ± 1 (16)	146 ± 0.0 (16)	145 ± 1 (12)	146 ± 1 (15)	144 ± 1 (14)
HCTZ + AMI	Magnesium	0.82 ± 0.02 (12)	0.87** ± 0.02 (23)	0.83 ± 0.01 (24)	0.83[c] ± 0.01 (15)	0.80 ± 0.02 (22)	0.74[c]** ± 0.02 (15)
	Potassium	4.6 ± 0.2 (12)	4.3 ± 0.1 (23)	4.0** ± 0.1 (24)	4.0[c] ± 0.2 (15)	4.4 ± 0.1 (22)	4.2[c] ± 0.2 (15)
	Sodium	145 ± 1 (12)	147 ± 1 (23)	146 ± 0.0 (24)	145[c] ± 1 (15)	145 ± 1 (22)	144[c] ± 1 (15)

Figures between brackets depict the number of measurements from which the corresponding statistics were derived. Values from two consecutive measurements performed 15 days apart in the same patient were averaged whenever existent, and average values were used for evaluating the corresponding statistics.

[a] results from eight patients.
[b] results from seven patients.
[c] results from ten patients.
* $p < 0.05$ with respect to pretreatment mean value.
** $p < 0.01$ with respect to pretreatment mean value.

Plasma potassium and sodium, pulse rate and the clinical and instrumental variables studied did not reveal any important changes during treatment. Both formulations were equally well tolerated and no side-effects were reported or detected that merited withdrawal of any patient from the study.

Crossed comparisons between the plasma Mg, K and Na concentrations in the two groups did not yield any significant difference, either before the initiation of treatment or at any time during active therapy.

Discussion

The antihypertensive actions exerted by HCTZ and by HCTZ + AMI were consistent with the results of previous studies [26, 29].

Plasma Mg does not correlate linearly with tissue Mg. However, significant decreases — when existent — in plasma Mg are usually taken as tardy, albeit reliable, indicators of Mg depletion, especially within the context of studies on the effects of diuretics on bodily Mg status [13, 28, 30].

Hydrochlorothiazide provokes renal retention of Ca [4] and thus depresses parathyroid function [32] and decreases the parathormone-dependent reabsorption of Mg in the loop of *Henle* [19, 31], which is followed by hypermagnesiuresis [15]. In addition, hydrochlorothiazide hypercalcaemia per se might reduce Mg reabsorption in the loop of *Henle* [22], thus further contributing to the diuretic-induced hypermagnesiuresis.

Amiloride reduces the renal output of Mg, in a dose-dependent manner, when single doses of the drug are given to healthy volunteers [8, 17]. This Mg-sparing effect is however feeble and, when single standard-dose combinations of amiloride and common diuretics are administered to normal subjects, the hypermagnesiuretic effects of common diuretics are but partly counteracted by amiloride [9, 10, 16]. Apparently, amiloride-hydrochlorothiazide weight ratios higher than 1:10 would be necessary for amiloride to completely compensate for the hypermagnesiuretic effect of hydrochlorothiazide, though these higher weight ratios could be hazardous in terms of amiloride potential side-effects, especially acidosis and K intoxication.

Amiloride directly decreases the amount of Mg in the end portion of the distal convoluted tubule and in the early collecting duct, thus decreasing magnesiuresis [17, 28]. Whether this effect is due to decreased Mg secretion or to increased Mg reabsorption remains to be elucidated. The direct action of amiloride on Mg handling at the end-distal tubule and at the early-collecting duct is opposed by the Ca-mediated indirect effect of the drug on the handling of Mg in the early distale tubule, which parallels that of common distal tubular diuretics in so far as amiloride causes renal Ca retention [2, 3, 8, 12, 18, 20, 21, 23].

The addition of the Ca-retaining effects of amiloride and hydrochlorothiazide could explain that HCTZ + AMI increases magnesiuresis and lowers plasma Mg upon prolonged administration, since the positive feed-back mechanisms accounting for thiazide-induced hypermagnesiuresis [24, 28] could be set to a level unlikely to be counteracted by the direct effect of amiloride on the nephronal handling of Mg.

An alternative or complementary explanation for the deleterious effect of chronic treatment with HCTZ + AMI on magnesaemia, could lie on the fact both active principles in the formulation increase plasma aldosterone values. Hyperaldosteronaemia could increase magnesiuresis [25] to an extent that would not be overcome by the antagonistic action of amiloride.

Irrespective of the mechanisms accounting for the decrease in plasma Mg provoked by HCTZ + AMI, which matched that provoked by HCTZ, the present findings suggest that the deployment of HCTZ + AMI should be discouraged whenever a more innocuous alternative is available. HCTZ + AMI should be particularly avoided in patients under stress and in areas where the Mg content in drinking water is low, since these factors predispose to the development of Mg depletion [5, 11, 20]. Low doses of diuretics (e.g. hydrochlorothiazide 12.5 $mg.day^{-1}$ or chlorthalidone 25 $mg.day^{-1}$) are sufficient for achieving maximal antihypertensive effects and are not as likely as standard diuretic doses to cause Mg depletion [28, 33]. When higher doses of diuretics are needed for the treatment of cardiac insufficiency and/or oedema, HCTZ + AMI should be preferred to HCTZ because of its milder effect on bodily K, though plasma Mg should be measured frequently and Mg supplements given prophylactically to patients at high risk of developing Mg deficiency [28], or therapeutically if plasma Mg falls below 0.75 $mmol.L^{-1}$ [28].

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PAPER B9

Once-Daily Administration of Captopril and Hypotensive Effect

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Once-Daily Administration of Captopril and Hypotensive Effect

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Summary: The usual regimens of captopril—twice or thrice daily administration—are based on the duration of the decrease in plasma angiotensin II induced by captopril. In a study performed to evaluate the hypotensive effect of once daily captopril, 13 white patients with mild-to-severe uncomplicated hypertension were treated with one tablet of captopril 100 mg daily, taken 1–1.5 h before lunch, for 8 weeks. Arterial blood pressure was measured weekly, 22–23.5 h after medication. The patients' diet contained no more than 120 mmol/day of sodium. In the first week supine blood pressure fell from 210 ± 3/128 ± 4 (mean ± SEM) to 179 ± 5/116 ± 5 mm Hg ($p < 0.001$/ $p < 0.01$ compared with pretreatment). After the large decrease in the first week changes in systolic and diastolic pressures tailed off; they tended to fall towards stable values that would be maintained on prolonged treatment. At the end of the eighth week the supine values were 155 ± 3/104 ± 3 ($p < 0.001$/ $p < 0.001$). Changes in erect blood pressure paralleled those in the supine posture. No side effects were detected. These results confirm that captopril is efficacious when given alone to patients with essential hypertension who are taking a low sodium diet. Blood pressures were not, however, reduced to normotensive levels. Captopril's hypotensive effect in once daily administration appears to be independent of its effects on circulating angiotensin II. Captopril alone 100 mg/day is thus indicated in essential hypertension and should be prescribed once daily to obtain the best possible compliance. **Key Words:** Angiotensin I-converting enzyme inhibitors—Captopril—Essential hypertension—Once daily administration—Power antihypertensives.

The development of angiotensin I-converting enzyme (ACE) inhibitors, such as captopril, has been based on the assumption that these compounds decrease blood pressure by reducing the generation of circulating angiotensin II through ACE inhibition (1,2). In fact, captopril effectively lowers blood pressure in cases where augmented circulating angiotensin II plays a part in the pathogenesis of the disease, such as in malignant hypertension and hypertension secondary to renal artery stenosis. Moreover, captopril effectively reduced blood pressure when administered alone to patients with uncomplicated essential hypertension (3–9), irrespective of plasma renin concentrations (3–9), and with as few adverse reactions as hydrochlorothiazide (10).

Hitherto, captopril has been prescribed at least two or three times a day. The concept that captopril should be administered at frequent intervals has been derived from the results of short-term studies. When the drug is given to hypertensive patients with disorders of the circulating part of the renin-angiotensin-aldosterone system, the hypotensive effect of captopril parallels increases in plasma renin and angiotensin I and decreases in plasma angiotensin II and aldosterone (1,2). Nevertheless, recent investigations in such patients indicate that, after an initial response unequivocally mediated by the circulating components of the RAA system, blood pressure is reduced through other mechanisms (11), which operate long-term and possibly account for the therapeutic action of captopril in essential hypertension (12).

These putative mechanisms might originate from effects of captopril such as inhibition of local angiotensin II generation in different tissues, including the central nervous system (13–15), an increase in local renal and circulating (16) levels of kinins, an increase in the renal production of PGE_2 (17,18), and an increase in urinary sodium output (6,7,9,18,19), all of which might account for the decrease in total peripheral resistance caused by cap-

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topril in hypertension (20,21). All these processes may not require frequent dosing with captopril, just as the possible mechanisms underlying the chronic antihypertensive effects of diuretics or beta-adrenergic blockers may not depend upon actions determined by the duration of their specific diuretic or beta-adrenergic blocking effects.

Our object was to investigate the intensity and the medium-term duration of the antihypertensive effect of monotherapy with captopril prescribed once daily in essential hypertension.

PATIENTS AND METHODS

Patients

Thirteen white patients (two men) aged 22–77 (57.4 ± 4.0 years) were studied with their informed written consent. All had resting supine diastolic pressures of 100–140 mm Hg (Fig. 1) recorded on at least two occasions separated by 7 days before they were admitted to the study. Patients in whom comprehensive clinical (including ophthalmologic), laboratory, electrical, and radiologic investigations showed serious concomitant disease were excluded from the study.

Measurements

Arterial pressures were measured by the standard indirect technique, using the first appearance and final disappearance of Korotkoff's sounds to define systolic and diastolic pressures respectively. Patients rested supine or erect for 10 min before we measured the corresponding blood pressures; an average was obtained from three readings per patient and was adjusted to the nearest multiple of 10 mm Hg. The same mercury sphygmomanometer and stethoscope were used throughout the study and all measurements and clinical evaluations were made by the same cardiologist, 22–23.5 h after the last dose of captopril had been taken.

Plasma potassium, glucose, uric acid, and creatinine concentrations were measured immediately before the start of treatment and after 4 and 8 weeks' treatment. Blood cell counts were carried out at the same time.

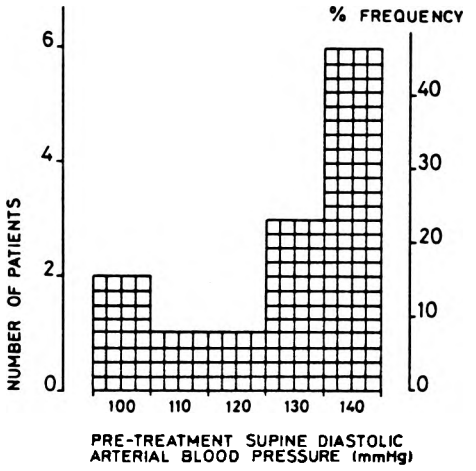

FIG. 1. Frequency distribution of pretreatment resting supine diastolic blood pressures corresponding to the 13 patients studied.

Urine samples were examined for microscopic and chemical abnormalities, including proteinuria, at the start of the trial and every 2 weeks thereafter. Standard laboratory techniques were used in all cases.

Operational procedures

Precise instructions were given to ensure that the diet contained 50–120 mmol of sodium daily from 1 week before the start of treatment until its completion.

All patients were given captopril 100 mg, as a tablet to be taken as the only treatment at 1100 daily for 8 weeks. It was emphasized to the patients that they should not take their lunch before 1230.

Clinical evaluations were carried out weekly, between 0900 and 1030 throughout the trial, starting 1 week before the initiation of treatment.

Compliance with treatment was assessed by counting the number of captopril tablets left at each clinic visit.

No other medications were prescribed during the study period and the use of topical corticosteroids was forbidden.

Statistics

Results are expressed as means and standard errors of means. The χ^2 test for goodness of fit and the F ratio were evaluated as appropriate for these purposes. Only minor departures from formal prerequisites were tolerated. A paired t test was used for assessing the significances of the differences between mean values of all variables except blood pressure. Arterial pressure values deviated significantly from normality (Fig. 1). Therefore, Wilcoxon's signed-rank test was used for evaluating differences between blood pressure mean values.

Standard linear correlation and regression techniques were applied to the descriptive analysis of linearized data (22).

All tests used were two tailed and $p = 0.05$ was considered the upper limit of significance.

RESULTS

Compliance with treatment was excellent in all cases and adherence to the prescribed diet was reported by all patients.

Blood pressure mean values from measurements carried out 1 week before entry to the study and immediately before the first dose of captopril (week 0, Table 1) did not differ significantly.

The weekly changes of supine and erect systolic and diastolic blood pressures during captopril treatment are depicted in Table 1. During the first week of treatment 100 mg/day induced statistically significant mean decreases of 30.4/11.5 mm Hg when supine and 36.2/6.9 mm Hg when erect. Thereafter, mean blood pressure values continued to decrease significantly—both statistically and clinically. By the end of the study the mean decreases from pretreatment levels were 55.0/23.8 mm Hg when supine and 54.3/23.1 mm Hg when erect (Fig. 2).

Patients reported no side effects and no abnormalities were detected on physical examination. No statistical changes were found at any time in any plasma urinary or electrocardiographic variables.

TABLE 1. *Blood pressure during treatment of 13 essential hypertensives with captopril 100 mg/day as a monotherapy (mean ± SEM)*

Blood pressure (mm Hg)	Weeks of treatment								
	0	1	2	3	4	5	6	7	8
Supine systolic	210 ± 3	179 ± 5*	169 ± 4*	168 ± 4*	163 ± 4*	163 ± 4*	161 ± 4*	156 ± 5*	155 ± 3*
Supine diastolic	128 ± 4	116 ± 5**	111 ± 5*	110 ± 4*	108 ± 4*	108 ± 4*	102 ± 3*	103 ± 4*	104 ± 3*
Erect systolic	205 ± 2	169 ± 4*	160 ± 3*	159 ± 3*	157 ± 4*	155 ± 3*	151 ± 3*	151 ± 4*	151 ± 4*
Erect diastolic	130 ± 4	123 ± 5***	118 ± 4*	111 ± 4*	112 ± 4*	115 ± 5*	108 ± 4*	108 ± 4*	107 ± 3*

Significances of the differences compared with pretreatment (week 0) means: *$p < 0.001$; **$p < 0.01$; ***$p < 0.05$.

Proteinuria did not occur in any patient. Plasma creatinine concentration rose from 0.62 mg/dl before the start of treatment to 1.42 mg/dl at the end of 8 weeks in one patient; the corresponding value at the end of the fourth week was 0.57 mg/dl.

DISCUSSION

In the present study captopril 100 mg was given once daily to a relatively small group of compliant, carefully supervised patients with mild-to-severe essential hypertension. Scrupulous attention was paid to the timing of clinical assessments relative to dosing with captopril and to dosing at the correct interval between meals, since it has been reported that the absorption of the drug is decreased by food (23,24).

We confirmed the findings of others (3–9) that captopril is effective and safe in essential hypertension.

Our results show that single daily doses of captopril 100 mg maintain their effect on blood pressure

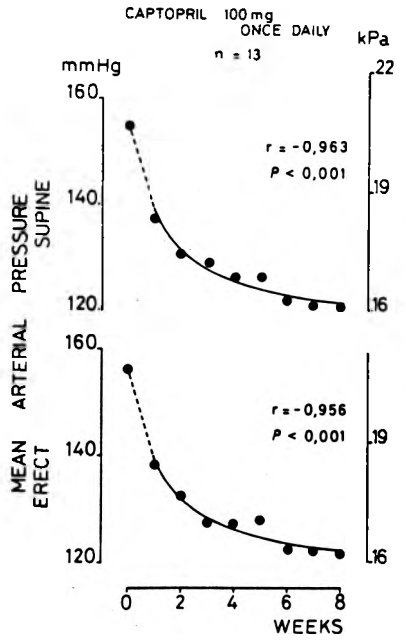

FIG. 2. Changes in resting supine and erect mean arterial pressures during treatment of 13 patients with essential hypertension with captopril 100 mg/day.

for 22 –23.5 h, suggesting that the plasma pharmacokinetics of captopril do not explain the duration of its antihypertensive action, since the plasma half life of the substance has consistently been found to be less than 2 h in normal subjects (25,26); the duration of the decrease in plasma angiotensin II provoked by captopril, which parallels that of the plasma concentration of the drug, may not account for the duration of the antihypertensive effect either. When captopril is used alone in essential hypertension, it would appear to be unnecessary to base the dosage scheme on the fact that the blood concentrations of the drug are low within 4 h of dosing (25,26). This issue is clinically relevant, inasmuch as patients' compliance with anithypertensive treatment is principally determined by the frequency of dosing. Similar considerations apply to antihypertensive treatment with some diuretics and beta-adrenergic blockers, which are taken once daily despite their rapid plasma turnover (27) and their characteristic pharmacodynamic actions, which last less than 24 h.

Since in all cases arterial pressure was reduced for a prolonged period after single daily doses of captopril 100 mg, the reduction in blood pressure cannot be accounted for solely by the decrease that captopril induces in plasma angiotensin II. Sodium intake was, however, curtailed to a maximum of 120 mmol daily in this study, and patients would therefore be expected to have high plasma renin activities. Possibly the effect of captopril on these high concentrations triggered off further long-lasting changes in the overall systemic blood pressure control and regulation system.

The course of the effect of captopril on blood pressure was fitted by a decreasing power function of time. This indicates that the compound reduces pressure very rapidly initially, the reduction being statistically significant and clinically relevant in the first week of treatment, after which pressure continues to decline gradually towards a clinically stable value that may be formally regarded as a final value. This gradual decline and the existence of a limit value indicate that resistance to treatment or exaggerated decreases in blood pressure should not be expected during prolonged monotherapy with captopril. Antihypertensive agents with these char-

acteristics ("power antihypertensives") (22) are particularly useful in hypertension uncomplicated by vascular insufficiency in the coronary or cerebral circulations. In patients with diminished blood flows in these areas linear antihypertensives such as the thiazide diuretics (22,27) are preferable, because they reduce blood pressure in a linear fashion, and therefore smoothly and slowly, before stabilization.

The results of this study require confirmation in carefully controlled double-blind trials, and single doses as low as 25–50 mg daily should also be assessed. If these results are confirmed, captopril could be considered a drug of first choice in the treatment of essential hypertension, since single therapy with it is efficacious and once-daily dosage is possible. Moreover, captopril provokes renal sodium excretion (6,7,9,18,19) rather than the sodium retention associated with most antihypertensive agents other than diuretics, and, unlike common diuretics, it has no deleterious effects on urinary potassium excretion (28).

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PAPER B10

Captopril Once Daily As Monotherapy in Patients with Hyperuricaemia and Essential Hypertension. Lancet 1985; 1: 1277.

This publication is only in the form of a letter but reports on significant findings and is therefore included in this presentation. Protocol, design, analysis and publication were joint efforts with Professor A.J. Reyes. Drs. Acosta-Barrios and Maharaj assisted in the collection and management of patients according to our instructions.

CAPTOPRIL ONCE DAILY AS MONOTHERAPY IN PATIENTS WITH HYPERURICAEMIA AND ESSENTIAL HYPERTENSION

SIR,—A multicentre comparative study in patients with essential hypertension showed that captopril 25 mg three times a day partly counteracted the hyperuricaemic response to hydrochlorothiazide 15 mg three times a day when these drugs were administered together.[1] Another study showed that prolonged therapy with enalapril alone (10–40 mg once daily) significantly reduced plasma urate levels in patients with essential hypertension.[2] In a placebo-controlled investigation[2] a single dose of enalapril 40 mg increased the renal clearance of uric acid, corrected for glomerular filtration rate, in fourteen hypertensive patients whereas there was no change in three. A further placebo-controlled study showed that a single dose of captopril 100 mg significantly increased the mean urinary output of uric acid in healthy volunteers, a single dose of hydrochlorothiazide 25 mg had an opposite effect, and 24 h urinary excretion of uric acid did not change when both drugs were given together.[3] Captopril 100 mg once daily in patients with moderate to severe essential hypertension significantly reduced blood pressure (BP) measured 22–23·5 h after dosing.[4]

We have studied twenty patients with hyperuricaemia and essential hypertension. After 4 weeks without active medication patients were treated openly, for ethical reasons, with captopril 100 mg daily for 4 weeks. The ten patients studied in Montevideo (all whites, five male) maintained a low-sodium (60–100 mmol daily), low-purine diet throughout the run-in and the captopril-treatment periods, whereas the ten male patients in Durban (nine white, one black) persisted with their usual high-sodium (200–300 mmol) and medium-purine diet. Serum uric acid and supine BP were measured at the end of the run-in period and after 4 weeks of active treatment. Post-captopril assessments took place 20–22 h (uric acid) and 22–23·5 h (BP) after the last intake of medication.

The serum urate fell from 0·53±0·07 (mean±SD) to 0·46±0·07 mmol/l ($p<0·05$) in Durban and from 0·46±0·05 to 0·35±0·07 mmol/l ($p<0·001$) in Montevideo. In Durban, BP varied from 168±14/114±8 to 159±18/112±11 mm Hg (not significant). In Montevideo BP fell from 192±15/119±11 to 144±8/95±10 mm Hg (both $p<0·001$); post-treatment diastolic values lower than 90 mm Hg were noted in six patients. The only recorded side-effect was pain in both legs in a patient with severe bilateral venous varicosities.

These findings support the use of captopril alone in patients with hyperuricaemia and essential hypertension. However, the efficacy of such monotherapy seems to depend on a reduction in salt intake, resulting in enhanced activity of the renin-angiotensin-aldosterone system which thus renders the BP contol very sensitive to angiotensin-I converting-enzyme inhibition.[5,6] Whether the diminution in serum urate level, which could be secondary to captopril-induced uricosuria, depends on angiotensin-I converting-enzyme inhibition to any extent remains to be elucidated.

The development of leg pain during treatment with captopril has been observed in patients with varicosities on three other occasions by one of us (T. N. A. B.). The mechanism is not known.

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SECTION C : PHARMACOLOGICAL EFFECTS OF DIURETICS

Diuretics are used at many centres as medications of first choice in the treatment of hypertension. They are relatively cheap and, in Africa at least, appear to be as effective as more expensive alternatives. Those who prefer to initiate treatment with a beta-receptor-antagonist or calcium channel blocker generally add an antihypertensive diuretic to the treatment regimen as a second step in managing patients who do not respond to monotherapy with the first-line drug used. Since diuretics occupy such an important place in the treatment of hypertension it is essential that their characteristics be described in detail and that possible adverse effects be identified.

The studies included in this section of the work deal with the effects of various diuretics upon the acute excretion of solutes. The possible consequences of these effects are discussed.

PAPER C1

Comparative Effects of Xipamide, Furosemide and Hydrochlorothiazide in Healthy Adults

I carried out all the clinical work presented here, including much of the analysis. Statistical analysis was carried out by Dr. J.J. Frey.

CURRENT
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Laboratory Study

COMPARATIVE EFFECTS OF XIPAMIDE, FUROSEMIDE AND HYDROCHLOROTHIAZIDE IN HEALTHY ADULTS

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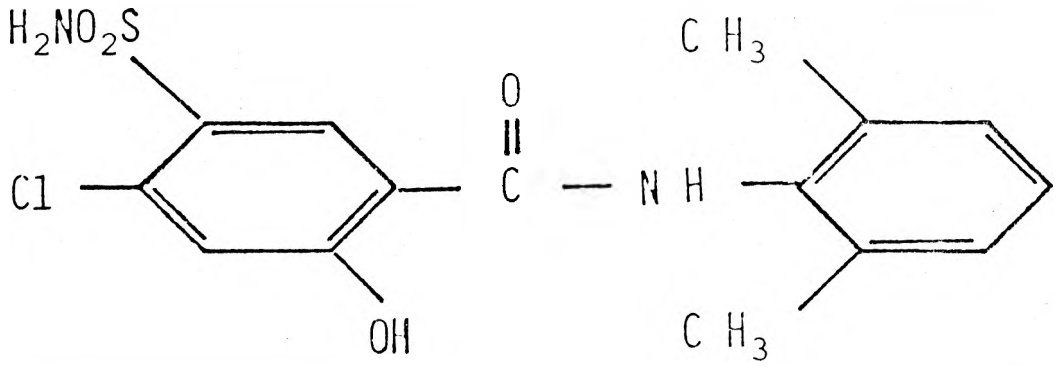
ABSTRACT

The diuretic, natriuretic and kaliuretic effects of single oral doses of xipamide 20, 40 and 60 mg, furosemide 40 mg and hydrochlorothiazide 50 and 100 mg were compared in 26 healthy adults who each received 2 treatments. Over 24 hours, furosemide 40 mg had less sodium and potassium excretion than xipamide 40 or 60 mg. In general, effects of these medicines were similar, although furosemide had a slightly shorter duration of activity than the others tested.

INTRODUCTION

Xipamide (Fig. 1) is a non-thiazide antihypertensive compound.[1,2] Preliminary investigation of healthy adult volunteers indicated that the effects of xipamide 5 or 10 mg resemble those of hydrochlorothiazide 50 mg when these diuretics are given orally in single daily doses.[3]

Figure 1 — *Structural formula of xipamide*



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The study reported here was initiated to compare the diuretic potency, natriuretic and kaliuretic effects following treatment of healthy volunteers with furosemide, hydrochlorothiazide, or high doses of xipamide.

EXPERIMENTAL METHODS

Twenty-six healthy, male, adult volunteers aged 21 to 45 years were selected for the study. Persons with any of the following characteristics were excluded:
(1) Receiving any medication within 3 weeks of the trial,
(2) History of cardiac, renal, or hepatic disorders,
(3) History of drug sensitivities,
(4) Abnormal values in pre-treatment laboratory tests, and
(5) Gross obesity.

A medical history was taken and physical examination carried out in each case. A 6-lead EKG was recorded and the following fasting laboratory tests were carried out before beginning the study: Haemoglobin concentration and white cell count, blood glucose, urea nitrogen, uric acid, creatinine, SGOT, and urinalysis.

All volunteers were placed on a controlled standard 10 mEq sodium chloride diet with a 24 hr fluid intake of 2000 ml. Subjects were randomly assigned to a treatment schedule so that each volunteer received 2 of the following 6 active medications:
(1) Xipamide 20 mg (Xip. 20).
(2) Xipamide 40 mg (Xip. 40).
(3) Xipamide 60 mg (Xip. 60).
(4) Hydrochlorothiazide 50 mg (HCT 50).
(5) Hydrochlorothiazide 100 mg (HCT 100).
(6) Furosemide 40 mg (Fur 40).

Medicines were given as single oral doses according to the following time schedule:
Period 1: 24-hr control period.
Period 2: 24-hr active medication.
Period 3: 72-hr washout.
Period 4: 24-hr active medication.

Medicines were administered at the doses appearing on the treatment schedule immediately after the last 24-hour urine and blood collections of the washout or control periods.

Weight and supine and standing blood pressure and pulse were recorded daily throughout the trial. The following "on trial" laboratory analyses were performed at defined intervals:
(1) Urine: Volume, pH, specific gravity and sodium, potassium and creatinine excretion.
(2) Blood: pH, CO_2 and sodium, potassium and chloride concentrations.

During periods 1 and 3, a total 24 hr urine collection and one blood sample were obtained for on trial laboratory analysis. This provided control data prior to therapy. During periods 2 and 4, on trial analyses were performed on blood collected after 6 and 24 hours and urine collected at 3-hr intervals from 0 to 24 hours.

On completion of the trial each volunteer was examined and EKG and laboratory determinations were carried out as previously described.

The study was randomized so that each volunteer received 2 of the 6 therapies being evaluated, following a partially balanced incomplete statistical design.

Data were treated by analysis of covariance with a model appropriate for a partially balanced incomplete block design. The covariate was the baseline measurement for the previous 24 hours for urinary data. The fifteen paired-treatment contrasts were evaluated by least significant difference contrasts.

RESULTS

Table I presents a summary of the urinary excretion data for each post-medication collection period and the total for 0 to 24 hours. The common baseline represents the result for the prior 24 hours. Results of statistical analyses are included in Table I by indicating which treatment contrasts were significant ($P < 0.05$) or by entering n.s. where none of the contrasts differed significantly.

There were no significant differences in urine volume at 3 to 6, 6 to 9, 9 to 12 hours or for the entire 24 hr collection period. However, furosemide 40 mg had an increased diuretic effect from 0 to 3 hours and xipamide 60 mg caused an increased urinary output from 12 to 18 hours by comparison with xipamide 20 mg or hydrochlorothiazide 50 and 100 mg.

There was an initial (0-3 hr) increase in sodium excretion followed by a sustained reduction for the remaining collections with furosemide 40 mg; 24-hour sodium excretion was lower than that with xipamide 40 and 60 mg. Hydrochlorothiazide 50 mg provoked less sodium excretion over 24 hours than xipamide 40 mg.

Urinary potassium excretion for the 24-hr period was lower after furosemide 40 mg than xipamide 40 or 60 mg and hydrochlorothiazide 100 mg. Furosemide 40 mg caused a significantly greater urinary chloride excretion for 0 to 3 hours than xipamide 20 mg or hydrochlorothiazide, but yielded a lower excretion thereafter with the 0 to 24-hour level significantly less than with all doses of xipamide or hydrochlorothiazide 100 mg. Chloride excretion was significantly reduced after hydrochlorothiazide 50 mg when compared to xipamide 40 and 60 mg or hydrochlorothiazide 100 mg. There were no significant differences between treatments for 24-hr creatinine excretion.

Urinary pH was significantly higher for the 24-hr treatment period after xipamide 40 and 60 mg than after hydrochlorothiazide 50 and 100 mg or furosemide 40 mg. There were no significant differences between the six treatments with regard to changes in urinary specific gravity.

Table II presents a summary of plasma and vital data and the results of statistical analysis. There were no differences between the six treatments in plasma sodium, potassium and chloride levels or in pulse

Table I (a) — *Summary of urine data from the incomplete block high dose xipamide electrolyte study in volunteers (N = 26) (Single-dose assay).*

Common Baseline (Prior 24 hours) By Variable	Treatment	Adjusted Mean for Hours Post-Dosing and Results of Statistical Analysis*						
		0 – 3	3 – 6	6 – 9	9 – 12	12 – 18	18 – 24	0 – 24
Urine volume	Xipamide 20 mg	426	514	281	372	329	356	2279
	Xipamide 40 mg	461	541	454	486	447	280	2669
	Xipamide 60 mg	506	384	471	351	565	238	2514
Common Baseline	HCT 50 mg	491	579	280	255	317	251	2173
	HCT 100 mg	429	484	431	338	385	241	2307
1239 ml	FUR 40 mg	899	523	259	348	480	159	2668
		*	n.s.	n.s.	n.s.	*	*	n.s.
	* HOURS	STATISTICALLY SIGNIFICANT CONTRASTS						
	0 – 3	FUR 40 XIP 20, XIP 40, XIP 60, HCT 50, HCT 100						
	12 – 18	XIP 60 XIP 20, HCT 50, HCT 100						
		FUR 40 HCT 50.						
	18 – 24	XIP 20 FUR 40						
Urine Specific Gravity	Xipamide 20 mg	1.012	1.009	1.005	1.004	1.010	1.015	1.010
	Xipamide 40 mg	1.010	1.011	1.009	1.008	1.012	1.013	1.010
	Xipamide 60 mg	1.005	1.009	1.011	1.016	1.015	1.011	1.012
Common Baseline:	HCT 50 mg	1.010	1.009	1.007	1.007	1.008	1.013	1.010
	HCT 100 mg	1.011	1.010	1.008	1.009	1.009	1.012	1.010
1.013	FUR 40 mg	1.006	1.010	1.004	1.009	1.011	1.013	1.010
		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Table I (b) — *Summary of urine data from the incomplete block high dose xipamide electrolyte study in volunteers (N = 26) (Single-dose assay).*

Common Baseline (Prior 24 hours) By Variable	Treatment	Adjusted Mean for Hours Post-Dosing and Results of Statistical Analysis* 0 – 3	3 – 6	6 – 9	9 – 12	12 – 18	18 – 24	0 – 24
Urinary Sodium	Xipamide 20 mg	50.9	72.7	50.2	51.1	61.6	51.1	337.5
	Xipamide 40 mg	65.7	85.8	61.5	65.2	75.1	38.9	392.0
	Xipamide 60 mg	59.9	66.3	69.8	65.3	67.0	33.5	361.8
Common Baseline:	HCT 50 mg	51.3	68.3	37.2	41.0	43.1	31.8	273.7
	HCT 100 mg	61.2	61.2	65.2	46.0	65.0	33.9	332.4
127.7 mmol	FUR 40 mg	102.5	72.5	27.7	13.5	32.8	6.2	255.1
Urinary Potassium	Xipamide 20 mg	15.0	10.7	9.1	9.2	5.1	12.6	61.8
	Xipamide 40 mg	13.4	12.6	12.6	10.9	14.5	12.6	76.7
	Xipamide 60 mg	17.5	13.1	11.7	11.3	20.7	7.4	81.7
Common Baseline:	HCT 50 mg	13.6	8.4	8.9	7.4	11.5	6.2	55.8
	HCT 100 mg	16.2	12.9	15.7	11.6	18.7	11.1	86.1
45.2 mmol	FUR 40 mg	13.1	9.7	3.0	2.7	8.0	1.6	38.1
		n.s.	n.s.	**	**	**	**	**

STATISTICALLY SIGNIFICANT CONTRASTS

* 0 – 3 hr: FUR 40 > XIP 20, HCT 50, HCT 100.
6 – 9 hr: FUR 40 < XIP 20, XIP 40, XIP 60, HCT 100.
HCT 50 < XIP 40, XIP 60, HCT 100.
9 – 12 hr: FUR 40 < XIP 20, XIP 40, XIP 60.
12 – 18 hr: FUR 40 < XIP 20, XIP 40, XIP 60, HCT 100.
HCT 50 < XIP 40.
18 – 24 hr: FUR 40 < XIP 20, XIP 40, XIP 60, HCT 100.
0 – 24 hr: HCT 50 < XIP 40.
FUR 40 < XIP 40, XIP 60.

** 6 – 9 hr: HCT 100 > XIP 20, HCT 50.
FUR 40 < XIP 40, XIP 60, HCT 50, HCT 100.
9 – 12 hr: FUR 40 < XIP 20, XIP 40, XIP 60, HCT 100.
12 – 18 hr: XIP 20 < HCT 100.
18 – 24 hr: FUR 40 < XIP 20, XIP 40, HCT 100.
0 – 24 hr: FUR 40 < XIP 40, XIP 60, HCT 100.

Table I (c) — *Summary of urine data from the incomplete block high dose xipamide electrolyte study in volunteers (N = 26) (Single-dose assay).*

Common Baseline (Prior 24 hours) By Variable	Treatment	Adjusted Mean for Hours Post-Dosing and Results of Statistical Analysis*						
		0 – 3	3 – 6	6 – 9	9 – 12	12 – 18	18 – 24	0 – 24
Urinary Chloride	Xipamide 20 mg	60.7	85.7	54.3	54.4	61.9	52.6	371-6
	Xipamide 40 mg	71.3	84.2	58.2	65.3	85.0	45.6	409.5
	Xipamide 60 mg	70.3	71.6	76.4	69.6	79.4	36.8	403.6
Common Baseline:	HCT 50 mg	65.3	73.3	43.5	44.6	47.5	33.5	307.7
	HCT 100 mg	68.8	69.6	82.5	47.4	77.8	43.7	389.7
132.5 mmol	FUR 40 mg	114.8 *	88.9 n.s.	24.6 *	9.4 *	25.9 *	2.5 *	266.3 *
Urinary Sodium/ Sodium/Potassium	Xipamide 20 mg	3.67	7.16	5.67	6.82	8.30	4.42	5.09
	Xipamide 40 mg	5.48	9.17	6.70	6.67	5.51	3.23	5.89
	Xipamide 60 mg	3.97	6.19	8.32	7.93	7.59	4.65	6.10
Common Baseline:	HCT 50 mg	3.83	11.00	6.82	7.70	5.33	5.53	5.83
	HCT 100 mg	5.46	7.59	5.82	5.66	4.27	5.08	5.17
3.09 mmol	FUR 40 mg	6.93 **	6.60 **	5.22 n.s.	4.83 n.s.	4.82 n.s.	2.64 n.s.	5.43 n.s.

STATISTICALLY SIGNIFICANT CONTRASTS

* 0 – 3 hr: FUR 40 > XIP 20, HCT 50, HCT 100
6 – 9 hr: FUR 40 < XIP 20, XIP 40, XIP 60, HCT 100; HCT 100 > XIP 20, HCT 50; XIP 60 > HCT 50
9 – 12 hr: FUR 40 < XIP 20, XIP 40, XIP 60, HCT 50, HCT 100
12 – 18 hr: FUR 40 < XIP 20, XIP 40, XIP 60, HCT 100; HCT 50 < XIP 40, XIP 60, HCT 100
18 – 24 hr: FUR 40 < XIP 20, XIP 40, XIP 60, HCT 50, HCT 100
0 – 24 hr: FUR 40 < XIP 20, XIP 40, XIP 60, HCT 100

** 0 – 3 hr: FUR 40 > XIP 20, HCT 50
3 – 6 hr: HCT 50 > XIP 20, XIP 60, HCT 100, FUR 40

Table I (d) — *Summary of urine data from the incomplete block high dose xipamide electrolyte study in volunteers (N = 26) (Single-dose assay).*

Common Baseline (Prior 24 hours) By Variable	Treatment	Adjusted Mean for Hours Post-Dosing and Results of Statistical Analysis*						
		0 – 3	3 – 6	6 – 9	9 – 12	12 – 18	18 - 24	0 – 24
Urine Ph	Xipamide 20 mg	6.01	6.01	5.78	5.79	6.34	5.37	5.88
	Xipamide 40 mg	6.34	6.81	6.30	6.01	5.62	5.62	6.12
	Xipamide 60 mg	6.94	7.53	6.88	6.20	5.41	6.00	6.49
Common Baseline:	HCT 50 mg	5.80	5.91	5.92	5.27	5.72	5.31	5.67
	HCT 100 mg	5.91	5.85	6.08	5.66	5.42	5.42	5.72
6.05	FUR 40 mg	6.13 **	5.36 *	5.75 n.s.	5.59 **	5.55 **	5.57 n.s.	5.66 **
Urinary Creatinine	Xipamide 20 mg	2.49	2.14	1.73	3.38	1.68	3.46	14.89
	Xipamide 40 mg	3.61	1.48	2.23	3.13	3.19	2.43	15.07
Common Baseline:	HCT 50 mg	2.50	2.57	1.34	1.93	2.75	2.50	13.59
	HCT 100 mg	2.18	2.05	1.94	0.77	3.17	2.86	12.98
13.30 mmol	FUR 40 mg	2.18	1.68	1.91	2.10	5.03	1.75	14.66

STATISTICALLY SIGNIFICANT CONTRASTS

* 6 – 9 hr	XIP 40 > XIP 20, HCT 50.	** 0 – 3 hr	XIP 60 > HCT 50.
	HCT 50 < XIP 40, HCT 100, FUR 40.	3 – 6 hr	XIP 60 > XIP 20, HCT 50, HCT 100, FUR 40.
12 – 18 hr	FUR 40 > XIP 20, XIP 40, XIP 60, HCT 50, HCT 100.		XIP 40 > HCT 50, HCT 100, FUR 40.
18 – 24 hr	FUR 40 < XIP 20.	9 – 12	HCT 50 < XIP 40, XIP 60.
		12 – 18 hr	XIP 20 > XIP 60, HCT 100, FUR 40.
		0 – 24 hr	XIP 40 > HCT 50, HCT 100, FUR 40.
			XIP 60 > HCT 50, HCT 100, FUR 40.

Table II (a)— *Summary of plasma and vital data from the incomplete block high dose xipamide electrolyte study in volunteers (N = 26) (Single-dose assay).*

Common Baseline By Variable	Treatment	Adjusted Mean For Hours Post-Dosing and Results of Statistical Analysis* 6 Hours	24 Hours
Plasma Sodium	Xipamide 20 mg	138.9	136.5
	Xipamide 40 mg	139.4	138.1
	Xipamide 60 mg	138.3	136.9
Common baseline:	HCT 50 mg	139.7	139.0
	HCT 100 mg	138.2	137.7
140.3 mmol/l	FUR 40 mg	138.1 n.s.	138.2 n.s
Plasma Potassium	Xipamide 20 mg	4.89	3.94
	Xipamide 40 mg	4.01	3.88
	Xipamide 60 mg	3.80	3.81
Common baseline:	HCT 50 mg	3.57	3.74
	HCT 100 mg	4.98	3.66
4.14 mmol/l	FUR 40 mg	4.26 n.s.	3.88 n.s.
Plasma Chloride	Xipamide 20 mg	100.92	99.43
	Xipamide 40 mg	99.67	96.74
	Xipamide 60 mg	97.83	96.21
Common baseline:	HCT 50 mg	100.45	99.11
	HCT 100 mg	100.96	98.49
103.9 mmol/l	FUR 40 mg	100.00 n.s.	100.72 n.s.

Table II (b) — *Summary of plasma and vital data from the incomplete block high dose xipamide electrolyte study in volunteers (N = 26) (Single-dose assay).*

Common Baseline By Variable	Treatment	Adjusted Mean For Hours Post-Dosing and Results of Statistical Analysis* 6 Hours	24 Hours
Plasma Bicarbonate	Xipamide 20 mg	24.45	25.28
	Xipamide 40 mg	23.15	28.62
	Xipamide 60 mg	26.44	25.99
Common baseline:	HCT 50 mg	22.96	24.21
	HCT 100 mg	24.88	23.90
24.68 mmol/l	FUR 40 mg	25.48 n.s.	24.97 *
Plasma Uric acid	Xipamide 20 mg	0.314	0.359
	Xipamide 40 mg	0.428	0.406
	Xipamide 60 mg	0.422	0.431
Common baseline:	HCT 50 mg	0.442	0.423
	HCT 100 mg	0.418	0.468
0.407 mmol	FUR 40 mg	0.404 **	0.410 **

STATISTICALLY SIGNIFICANT CONTRASTS

* 24 hr XIP 40 > XIP 20, XIP 60, HCT 50, HCT 100, FUR 40
** 6 hr XIP 20 < XIP 40, XIP 60, HCT 50, HCT 100, FUR 40
24 hr XIP 20 < HCT 100.

Table II (c) — *Summary of plasma and vital data from the incomplete block high dose xipamide electrolyte study in volunteers (N = 26) (Single-dose assay).*

Common Baseline By Variable	Treatment	Adjusted Mean For Hours Post-Dosing and Results of Statistical Analysis* 6 Hours	24 Hours
Diastolic Blood Pressure	Xipamide 20 mg	71.1	78.0
	Xipamide 40 mg	76.6	76.2
	Xipamide 60 mg	77.1	79.4
Common baseline: 73.7 mm Hg	HCT 50 mg	82.1	73.4
	HCT 100 mg	76.6	75.6
	FUR 40 mg	74.0 *	70.9 n.s.
Systolic Blood Pressure	Xipamide 20 mg	112.0	115.2
	Xipamide 40 mg	114.7	116.1
	Xipamide 60 mg	115.9	106.8
Common baseline: 115.4 mm Hg	HCT 50 mg*	114.9	108.4
	HCT 100 mg *	113.0	114.1
	FUR 40 mg	114.9 n.s.	109.1 n.s.

* STATISTICALLY SIGNIFICANT CONTRASTS

6 hr XIP 20 < HCT 50.
FUR 40 < HCT 50.

rate and systolic blood pressure. Plasma sodium, potassium and chloride levels fell slightly after each treatment.

For plasma bicarbonate xipamide 40 mg had a higher mean value than the other five treatments at 24 hours, but there were no statistically significant differences at 60 hours. All treatments caused a slight rise in plasma urate except xipamide 20 mg, the mean value being lower than the other treatments at 6 hours, and hydrochlorothiazide at 24 hours. Differences in diastolic pressure were slight and probably of no clinical importance. Negligible changes in pulse and body mass occurred and are not tabulated.

Safety laboratory data appear in Table III. The only significant change from pre- to post-study were increased mean values for haematocrit and red blood cell count and a decreased mean value for plasma urea.

SUMMARY AND CONCLUSIONS

This study was conducted on healthy male adult volunteers to compare the diuretic, natriuretic and kaliuretic effects of single oral doses of xipamide 20, 40 and 60 mg, hydrochlorothiazide 50 and 100 mg and furosemide 40 mg.

There were some differences between the six treatments in urine

Table III — *Summary of safety laboratory data for the incomplete block high dose xipamide electrolyte study in volunteers (N = 26)(Single-dose assay)*

Laboratory Test	Normal Values	No. Pts.	Mean ± s.e. Pre-Study	Mean ± s.e. Post-Study	Significance Level of Difference*
1. Hematology					
Hemoglobin	13.5 – 18.0 g	24	1.56 ± 0.02	1.57 ± 0.03	n.s.
Hematocrit	40.0 – 54.0 %	24	4.60 ± 0.07	4.76 ± 0.08	0.05
RBC	4.5 – 6.5	24	5.60 ± 0.07	5.74 ± 0.05	0.04
WBC	4.0 – 11.0	24	6.70 ± 0.27	7.10 ± 0.34	n.s.
2. Urinalysis					
Units					
Protein (0 to +3)	0	23	0.09 ± 0.09	0.13 ± 0.10	n.s.
Sugar (0 to +3)	0	23	0.13 ± 0.13	0.13 ± 0.13	n.s.
3. Blood Chemistry					
Plasma Urea	2.49 – 6.64 mmol/l	24	4.65 ± 0.31	3.95 ± 0.24	0.02
Serum Total Bilirubin	3.4 – 17.1 mmol/l	26	9.55 ± 1.03	10.37 ± 1.55	n.s.
SGOT	up to 12 u/l	26	18.30 ± 2.49	17.50 ± 1.81	n.s.
Uric Acid (Urate)	1.49 – 4.46 mmol/24 hrs	18	0.39 ± 0.02	0.41 ± 0.01	n.s.
S Serum Cholesterol	3.89 – 6.48 mmol/l	16	6.74 ± 0.25	6.74 ± 0.39	n.s.
Plasma Glucose	3.33 – 6.66 mmol/l	13	4.80 ± 0.14	5.22 ± 0.19	n.s.
Serum Triglycerides	.34 – 1.69 mmol/l	26	1.43 ± 0.15	1.51 ± 0.18	n.s.

* n.s. means not statistically significant ($P > .05$).

volume, urinary sodium and potassium excretion and urinary sodium/potassium ratio during the 24 hr collection period. Many of these results reflected the short duration of action of furosemide 40 mg. When results for the 0 to 24 hour period were compared furosemide 40 mg had significantly reduced sodium and potassium excretion compared to xipamide 40 or 60 mg. The 0 to 24 hr urinary sodium excretion for hydrochlorothiazide 50 mg was significantly lower than for xipamide 40 mg.

The results of the study indicate that the diuretic, saliuretic and kaliuretic effects of xipamide 20, 40 and 60 mg are similar in healthy adults and that they resemble those of hydrochlorothiazide 50 and 100 mg or furosemide 40 mg, although furosemide has a relatively short duration of action.

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PAPER C2

Xipamide: Diuretic Effects of Low Dosage in Healthy Adults

I carried out all the clinical work presented here, including much of the analysis. Statistical analysis was carried out by Dr. J.J. Frey.

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Laboratory Study

XIPAMIDE: DIURETIC EFFECTS OF LOW DOSAGE IN HEALTHY ADULTS

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ABSTRACT

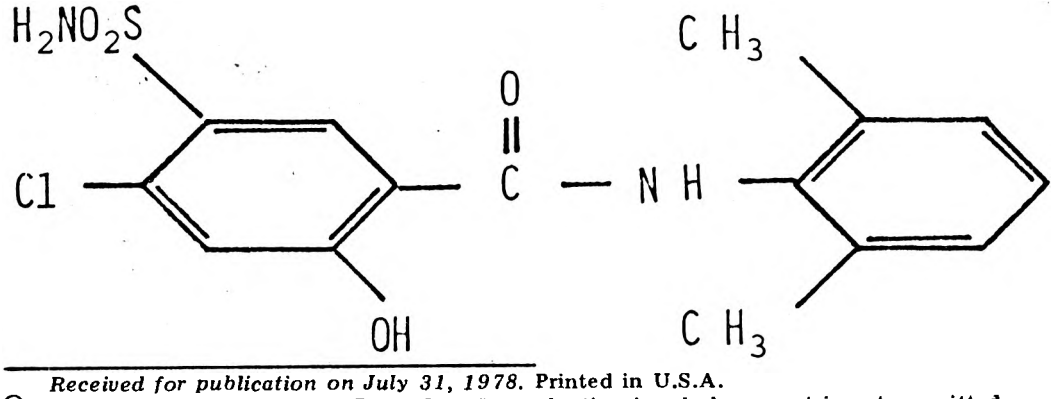
The diuretic, natriuretic and kaliuretic effects of xipamide 5 or 10 mg were compared to those of hydrochlorothiazide 50 mg in healthy male adult volunteers. Single doses were given in random order with suitable washout periods interposed.

The effects of xipamide resembled those of hydrochlorothiazide. No statistically significant differences among the 3 treatments for urine volume, urinary sodium or potassium excretion occurred. There were few differences for the other parameters measured.

INTRODUCTION

Xipamide, 5-(aminosulfonyl)-4-chloro-*N*-(2,6-dimethylphenyl) salicylamide, is a new antihypertensive non-thiazide compound. (Fig. 1). Preliminary studies in animals and man,[1-3] indicate that xipamide stimulates increased water and electrolyte excretion, with a more protracted effect than equipotent doses of frusemide (furosemide), but causes slightly less urinary potassium loss than the latter preparation.

Figure 1 — *Structural formula of xipamide.*



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This paper reports a further study in which the effects of relatively low doses of xipamide were compared to those of frusemide and hydrochlorothiazide in healthy adult volunteers.

EXPERIMENTAL METHODS

Twelve healthy male volunteers aged 21 to 45 years were selected for the study. In the course of selection, subjects with any of the following characteristics were excluded from the study:

(1) Receiving any medication within 3 weeks of the trial,
(2) History of cardiac, renal or hepatic disorders,
(3) History of drug sensitivities, and
(4) Abnormal values in pre-treatment laboratory tests.

After the medical history had been taken and a physical examination and EKG completed, a number of fasting laboratory tests were carried out as follows: haemoglobin concentration and white cell count, blood glucose, urea nitrogen, bilirubin, cholesterol, uric acid, creatinine, SGOT and urinalysis.

All volunteers were placed on a controlled standard 10 mEq sodium chloride diet with a 24 hr fluid intake of 2000 ml. Subjects were randomly assigned to a treatment schedule so that xipamide 5 and 10 mg or hydrochlorothiazide 50 mg tablets were taken in various sequences and each volunteer received 3 active medications according to the following schedule:

Period 1: 24-hr control period.
Period 2: 24-hr active medication (single dose).
Period 3: 72-hr washout.
Period 4: 24-hr active medication (single dose).
Period 5: 72-hr washout.
Period 6: 24-hr active medication (single dose).

Medication was administered in each case immediately after the last total 24-hr urine and blood collections of the control or washout periods.

During the trial, supine and standing blood pressure and pulse and weight were recorded daily. The following "on trial" laboratory analyses were performed at defined intervals:

(1) Urine: Volume, pH specific gravity, sodium, and potassium excretion.
(2) Venous: pH, CO_2, and sodium, potassium and chloride concentrations.

During periods 1, 3 and 5, a total 24-hr collection and one blood sample were obtained for on trial laboratory analysis. This served to provide control data prior to therapy. During periods 2, 4 and 6, on trial analyses were done on blood collected after 6 and 12 hours and urine collected at 0-3, 3-6, 6-9, 9-12, 12-18 and 18-24 hours after administration of active medication.

On completion of the study, each volunteer was examined and EKG and laboratory determinations were carried out as previously described.

Single oral doses of xipamide 5 and 10 mg and hydrochlorothiazide 50 mg were given utilising a randomized complete crossover Latin Square design in which all 6 possible orders of drug administration were used to balance for possible carry-over effects.

Data were treated by analysis of covariance with a model appropriate for a balanced Latin Square design where all possible orders are used. The covariate was the baseline measurement prior to treatment, or the measurement for the previous

24 hours for urinary data. The three paired-treatment contrasts were evaluated by least significant difference contrasts:
Xipamide 5 mg vs xipamide 10 mg.
Xipamide 5 mg vs hydrochlorothiazide 50 mg.
Xipamide 10 mg vs hydrochlorothiazide 50 mg.

RESULTS

Table I presents a summary of the urinary excretion data for each post-dosing collection period and the total for 0 to 24 hours. The common baseline represents the result for the prior 24 hours. The results of statistical analysis are included in Table I by indicating which treatment contrasts were statistically significant, ($P = < 0.05$), or by entering n.s. where none of the 3 contrasts was statistically significant.

There were no significant differences between the 3 treatments for urine volume or urinary sodium and potassium excretion which were all significantly increased by xipamide 5 or 10 mg and hydrochlorothiazide 50 mg. Xipamide 10 mg had a greater sodium/potassium ratio than hydrochlorothiazide for the 3- to 5-hr collection but there were no differences in the remaining collection period.

Mean urinary pH for hydrochlorothiazide 50 mg was higher than that for xipamide 10 mg at 0 to 3 hours and xipamide 5 mg was associated with a significantly higher pH than both xipamide 10 mg and hydrochlorothiazide at 18 to 24 hours. Average values over the 24-hr period changed very little. Urinary specific gravity was higher for hydrochlorothiazide as contrasted with xipamide 10 mg for the 12- to 18-hr collection.

Table II presents a summary of plasma and vital data collected during the investigation. There were no significant differences among the treatments of either 6- or 24-hr post-medication for plasma sodium, chloride or bicarbonate, although sodium and chloride levels fell slightly during all 3 treatments. Plasma potassium was reduced slightly by xipamide and hydrochlorothiazide, and the latter drug caused a greater fall at 6 hours than xipamide 5 mg. Urea was higher at 24 hours after hydrochlorothiazide 50 mg than xipamide 10 mg. Diastolic pressures were significantly reduced by xipamide 10 mg and 5 mg at 6 and 24 hours, respectively.

SUMMARY AND CONCLUSIONS

This study was conducted on healthy male adult volunteers to compare the diuretic, natriuretic and kaliuretic effects of single oral doses of xipamide 5 or 10 mg and hydrochlorothiazide 50 mg.

Analysis of the key urinary excretion parameters indicated there

Table I — *Summary of urine data from the Latin Square low dose xipamide electrolyte study in volunteers (N = 12) single dose assay.*

Common Baseline (Prior 24 hrs.) By Variable	Treatment	Adjusted Mean for Hours Post-Dosing and Results of Statistical Analysis*						
		0 – 3	3 – 6	6 – 9	9 – 12	12 – 18	18 – 24	0 – 24
Urine Volume	Xipamide 5 mg	289	427	444	355	399	308	2225
	Xipamide 10 mg	277	490	431	450	497	342	2490
Common Baseline: 1590 ml	HCT 50 mg	272	551	478	406	358	312	2372
	Pairwise Contrasts:	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Urinary Sodium	Xipamide 5 mg	34.1	45.8	31.1	24.7	30.6	15.1	181.7
	Xipamide 10 mg	32.6	50.4	41.8	32.4	29.6	22.0	209.1
Common Baseline: 86.9 mmol	HCT 50 mg	27.3	48.4	34.5	33.4	37.4	19.5	200.0
	Pairwise Contrasts:	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Urinary Potassium	Xipamide 5 mg	11.4	10.0	7.1	5.5	8.0	6.1	48.2
	Xipamide 10 mg	10.6	10.1	8.9	7.6	8.4	7.7	53.3
Common Baseline: 33.4 mmol	HCT 50 mg	9.4	10.2	6.9	6.4	6.1	7.0	45.9
	Pairwise Contrasts	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Urinary Sodium/Potassium Ratio	Xipamide 5 mg	3.10	5.43	4.77	5.84	4.89	3.03	4.21
	Xipamide 10 mg	3.40	6.03	5.21	6.17	5.96	3.66	4.56
Common Baseline: 2.69	HCT 50 mg	3.01	4.75	5.01	5.42	6.24	2.94	4.23
	Pairwise Contrast	n.s.	Xip 10 > HCT 50	n.s.	n.s.	n.s.	n.s.	n.s.
Urine pH	Xipamide 5 mg	5.89	6.83	5.98	5.84	5.66	5.91	5.85
	Xipamide 10 mg	5.73	6.06	5.75	5.59	5.59	5.61	5.72
Common Baseline: 5.89	HCT 50 mg	6.19	5.97	5.98	5.75	5.56	5.61	5.85
	Pairwise Contrasts:	HCT 50 > Xip 10	n.s.	n.s.	n.s.	n.s.	Xip 5 > Xip 10, HCT 50	n.s.
Urine Specific Gravity	Xipamide 5 mg	1.012	1.010	1.006	1.008	1.011	1.009	1.010
	Xipamide 10 mg	1.013	1.010	1.008	1.008	1.008	1.010	1.010
Common Baseline: 1.009	HCT 50 mg	1.010	1.008	1.007	1.008	1.013	1.012	1.010
	Pairwise Contrasts:	n.s.	n.s.	n.s.	n.s.	HCT 50 > Xip 10	n.s.	n.s.

* n.s. means no statistically significant ($P > .05$) results were obtained from the 3 pairwise treatment contrasts; where treatments are specified, statistically significant ($P < .05$) results are presented.

Table II — *Summary of plasma and vital data from the Latin Square low dose xipamide electrolyte study in volunteers (N = 12) single dose assay.*

Common Baseline By Variable	Treatment	Adjusted Mean for Hours Post-Dosing and Results of Statistical Analysis*	
		6 hours	24 hours
Plasma Bicarbonate	Xipamide 5 mg	24.7	25.3
	Xipamide 10 mg	25.1	25.4
Common Baseline: 24.8 mmol/l	HCT 50 mg	25.1	25.2
	Pairwise Contrasts:	n.s.	n.s.
Plasma Urea	Xipamide 5 mg	4.22	4.30
	Xipamide 10 mg	4.07	3.85
Common Baseline: 4.10 mmol/l	HCT 50 mg	4.36	4.37
	Pairwise Contrasts	n.s.	HCT 50> Xip 10
Body weight	Xipamide 5 mg	64.4	64.0
	Xipamide 10 mg	64.2	64.0
Common Baseline: 64.4 kg	HCT 50 mg	64.3	63.8
	Pairwise Contrasts:	n.s.	n.s.
Plasma Sodium	Xipamide 5 mg	139.6	140.8
	Xipamide 10 mg	139.0	140.8
Common Baseline: 142.0 mmol/l	HCT 50 mg	139.9	140.5
	Pairwise Contrasts:	n.s.	n.s.
Plasma Potassium	Xipamide 5 mg	4.18	4.10
	Xipamide 10 mg	3.95	4.10
Common Baseline: 4.34 mmol/l	HCT 50 mg	3.86	4.12
	Pairwise Contrasts:	Xip 5 > HCT 50	n.s.
Plasma Chloride	Xipamide 5 mg	100	99
	Xipamide 10 mg	99	98
Common Baseline: 103 mmol/l	HCT 50 mg	100	100
	Pairwise Contrasts:	n.s.	n.s.
Systolic Blood Pressure	Xipamide 5 mg	112.7	112.1
	Xipamide 10 mg	113.1	111.7
Common Baseline: 112.8 mm Hg	HCT 50 mg	114.5	111.2
	Pairwise Contrasts:	n.s.	n.s.
Diastolic Blood Pressure	Xipamide 5 mg	78.5	75.6
	Xipamide 10 mg	72.8	77.7
Common Baseline: 78.1 mm Hg	HCT 50 mg	80.4	80.1
	Pairwise Contrasts:	HCT 50 > Xip 10	HCT 50 > Xip 5

* n.s. means no statistically significant ($P > .05$) results were obtained from the 3 pairwise treatment contrasts; where treatments are specified, statistically significant ($P < .05$) results are presented.

were no significant differences between the 3 treatments with respect to urine volume, urinary sodium or potassium excretion and no important differences between observed responses in urinary pH, or specific gravity. A number of slight, though statistically significant, differences between the 3 treatments were noted on analysis of plasma and vital

data collected.

The results of this study indicate that single doses of xipamide 5 or 10 mg have similar diuretic, natriuretic and kaliuretic effects to hydrochlorothiazide 50 mg in healthy adults, as measured over a 24-hr period.

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PAPER C3

Urine Volumes and Flows After Oral Administration of Xipamide, Furosemide and Hydrochlorothiazide to Healthy Adults

The mathematical analyses reported in this paper were the responsibility of Professor A.J. Reyes who designed the models concerned. I carried out the clinical work and wrote much of the paper in its final form including the editing of the section dealing with mathematical methods.

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URINE VOLUMES AND FLOWS AFTER ORAL ADMINISTRATION OF XIPAMIDE, FUROSEMIDE AND HYDROCHLOROTHIAZIDE TO HEALTHY ADULTS

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ABSTRACT

Urine volumes and flows as functions of time after oral administration of xipamide 20 and 40 mg, furosemide 40 mg and hydrochlorothiazide 100 mg to healthy male individuals are described through mathematical models.

Urine volumes as functions of time, V, are accounted for by sigmoid functions that allow evaluating the percentage of the 24-hour urine volume that has been excreted at any time after intake. Fourteen hours after dosing, urine volume amounts to 74% for xipamide 20 and 40 mg, to 84% for furosemide and to 76% for hydrochlorothiazide, so that all the substances tried may be regarded as diurnal diuretics.

Urine flows as functions of time are defined and described as dV/dt. They show time to peak activity after dosing is about 4 to 4.5 hours for xipamide and hydrochlorothiazide and about 2 hours for furosemide.

INTRODUCTION

The comparative time-courses of the diuretic effects of xipamide 10 mg and hydrochlorothiazide 50 mg in healthy individuals have been described through mathematical techniques.[1]

The aim of the present article is to formally describe the corresponding effects of current therapeutic doses of xipamide, furosemide and hydrochlorothiazide. The experimental results backing the present analysis have been published.[2]

EXPERIMENTAL METHODS

Twenty-six healthy male volunteers aged 21 to 45 years were selected for the study. They were placed on a controlled standard 10 mEq sodium chloride diet with a

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24-hour fluid intake of 2000 ml. Subjects were randomly assigned to a treatment schedule so that each of the following active medications was received *per os* by eight volunteers:

(1) Xipamide 20 mg.
(2) Xipamide 40 mg.
(3) Furosemide 40 mg.
(4) Hydrochlorothiazide 100 mg

Medicines were given as single doses according to the following time schedule:

Period 1: 24-hr. control period.
Period 2: 24-hr. active medication.
Period 3: 72-hr. washout.
Period 4: 24-hr. active medication.

Medicines were administered at the doses appearing on the treatment schedule immediately after the 24-hour urine collections of the washout or control periods.

During periods 1 and 3, a 24-hour urine collection was obtained which provided control data prior to treatment. During periods 2 and 4, urine was collected at hours 3, 6, 9, 12, 18 and 24 after dosing.

The study was randomized so that each volunteer received 1 or 2 of the 4 therapies, following a partially balanced statistical design.

Data were treated by analysis of covariance with a model appropriate for a partially balanced incomplete block design. The covariate was the baseline measurement for the previous 24 hours for urine volume.

MATHEMATICAL METHODS

The urine volume, V, adjusted mean experimental values accumulated at the end of each fractioning collection period were plotted as functions of time, t. After inspection of the dispersion diagrams, a differential equation was postulated that upon integration yielded:

$$20\ V\ /\ \log\ (100\text{-}V)\ =\ 10^{[t/(a+bt)]}\ , \qquad (1)$$

where a (time) and b (dimensionless) are parameters of the function.

The fitnesses of (1) to the experimental data were evaluated through the standard linear correlation technique applied to:

$$t\ /\ [\log\ 20\ V\ -\ \log\ \log\ (100\text{-}V)]\ =\ a\ +\ bt\ ,$$

from where a and b were evaluated through linear regression.

Urine flow, U, as a function of time, was defined as:

$$U\ =\ dV/dt\ =\ a/\ (a+bt)^2\ \left[\left[(0.43/V)\ +\ [(0.43^2)/(100\text{-}V)\log\ (100\text{-}V)]\right]\right]$$

Standard statistical techniques (appliable t-tests) were used for assessing significance of differences between slopes (b) and intercepts (a).

RESULTS

Urine volumes after dosing as functions of time are shown in Figures 1 and 2. No significant differences were found between the values of any

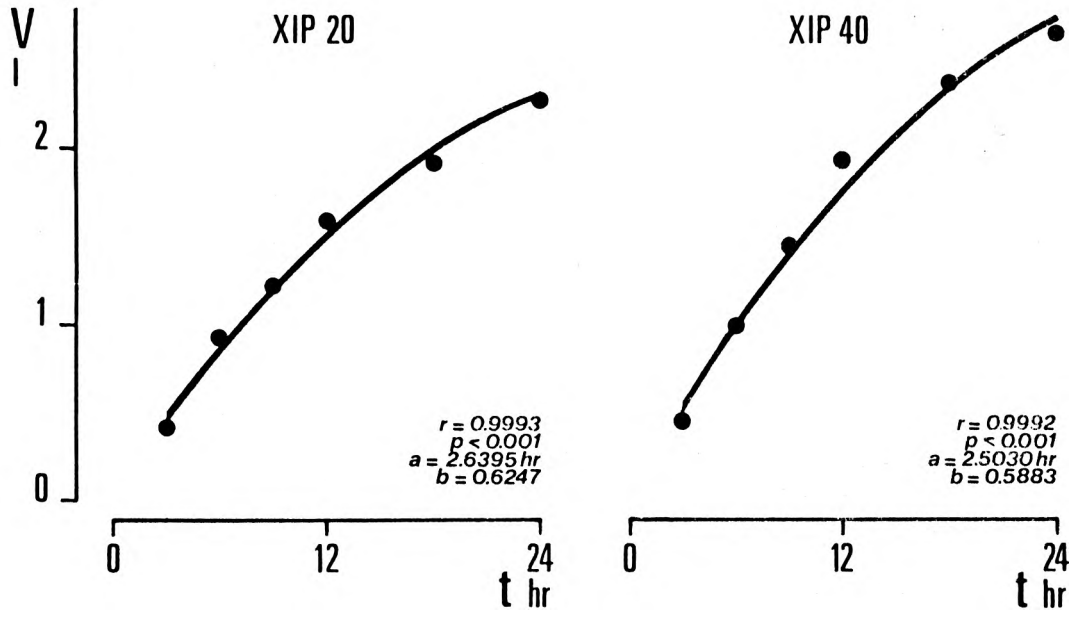

Figure 1 — *Experimental adjusted mean values of accumulated urine volumes, V, as functions of time, t, after xipamide 20 and 40 mg. The describing functions have been plotted.*

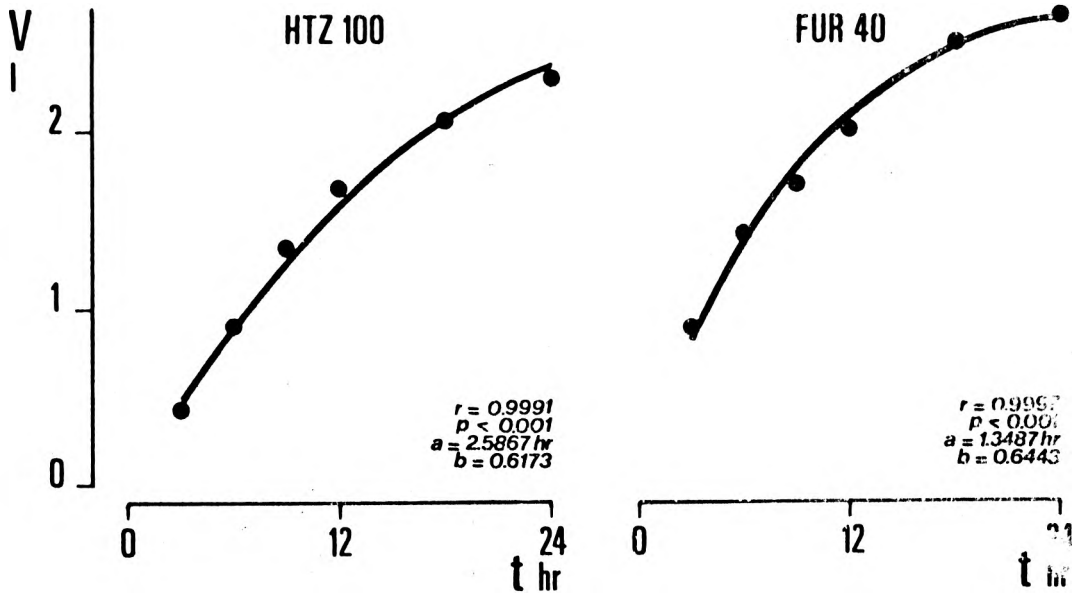

Figure 2 — *Experimental adjusted mean values of accumulated urine volumes, V, as functions of time, t, after hydrochlorothiazide 100 mg and furosemide 40 mg. The describing functions have been plotted.*

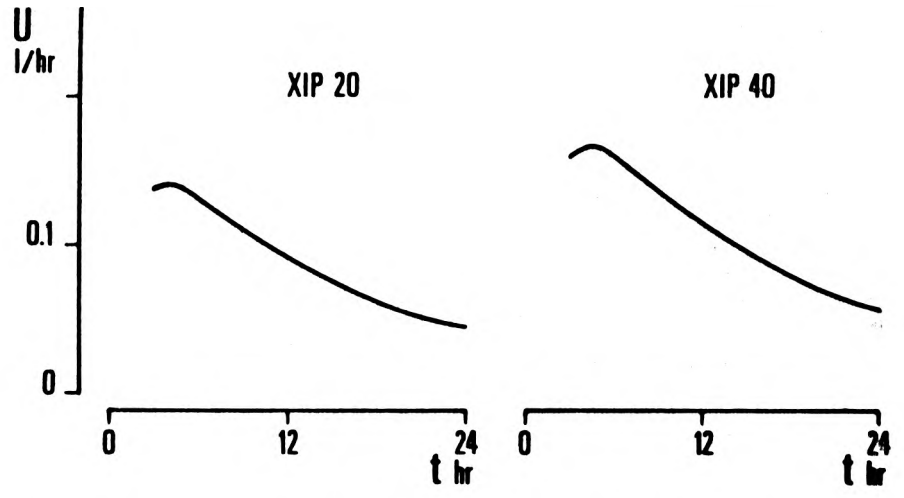

Figure 3 — *Urine flows, U, as functions of time, t, after xipamide 20 and 40 mg.*

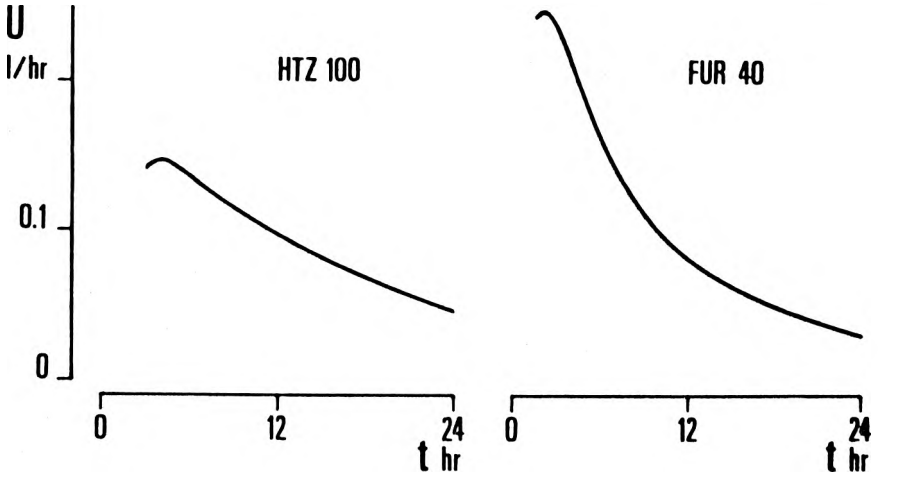

Figure 4 — *Urine flows, U, as functions of time, t, after hydrochlorothiazide 100 mg and furosemide 40 mg. The function for furosemide 40 mg has been extrapolated to the left of hr 3 after dosing with the objective of showing approximate peak diuretic activity.*

two slopes (b) of the linear transformations of V(t). The intercept value (a) for furosemide 40 mg was found to be significantly lower than for any other of the formulations tried ($p < 0.05$).

Fourteen hours after intake, 74% of the 24-hour urine volume had been excreted after xipamide 20 and 40 mg, 84% after furosemide 40 mg and 76% after hydrochlorothiazide 100 mg.

Urine flows after dosing as functions of time are shown in Figures 3 and 4. The time to peak diuresis after dosing was about 4 to 4.5 hours for xipamide and hydrochlorothiazide and about 2 hours for furo-

semide. The overall time-courses of the xipamide and hydrochlorothiazide induced diuresis were gentle whereas that of furosemide proceeded at a higher rate.

DISCUSSION

The time-courses of the diuretic effects of xipamide 20 mg and 40 mg and of hydrochlorothiazide 100 mg are similar and differ from that of furosemide. Time to peak diuresis after dosing is shorter for furosemide than for xipamide and hydrochlorothiazide. All three compounds tried may be regarded as diurnal diuretics because if given early in the morning they induce most of the forced urine volume within 14 hours after administration.

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PAPER C4

A Mathematical Model for the Clinical Pharmacology of Diuretics

This paper describes a method for dealing with data generated by diuretic studies. The mathematical model was designed by Professor A.J. Reyes. My major responsibility was to assist in expressing the model in terminology which could be understood by clinicians who needed to grasp the practical significance of the techniques involved.

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A MATHEMATICAL MODEL FOR THE CLINICAL PHARMACOLOGY OF DIURETICS

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ABSTRACT

Current research in the clinical pharmacology of diuretics uses an experimental design whereby, after voiding urine and single dosing with a diuretic, urine is collected at definite times, t, with the purpose of evaluating the time-courses of several renal excretory variables, Y, in normal or diseased subjects.

A mathematical model is presented that accounts for any Y as a continuous function of t: $20Y/\log (100 - Y) = \exp [2,3(t-t_1)/(a+bt)]$, where a (time), b (dimensionless) and t_1 (time) are parameters. This model describes the accumulated values after dosing and its derivative with respect to time, $y = dY/dt$, describes the corresponding flows as functions of time.

The mathematical model has been found to fit the experimental data very accurately (highly significant r values), even when the minimal number of urine collections and analyses compatible with the evaluations of correlation and regression (three) are considered. For similar t_1 and b values which frequently occur for a variable after different diuretics, higher a values mean slower overall rates of excretion, longer times to peak effect after dosing and less intensity and longer delay in onset of any rebounds which may exist.

The mathematical model has been found to hold after the administration of placebo and of all the diuretics that have been studied in this respect. It also applies to any renal excretory variable (volume, physiological solutes, drugs and their metabolites) so far studied. The model describes events in normal and diseased subjects equally well.

The repetibility of the parameter values of the mathematical model evaluated from different experiments is very high.

Applications of the mathematical model to the formal assessments of experimental designs have shown that it suffices to analyse urine collected at 3, 12 and 24 hours post-dosing for accurately describing the

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time-courses of renal excretory variables, after the administration of diuretics that complete their effects within 24 hours in normal subjects.

INTRODUCTION

The scope and sophistication of research techniques applied to clinical pharmacology are continuously increasing. Nonetheless, whereas evolution has been particularly rapid with regard to the generation of reliable data in certain fields, there has been a relatively slow development of mathematical models for the description and analysis of such data. The application of appropriate mathematical models to data greatly enhances their value in terms of the information that can reasonably be derived from them and may also prove useful in the evaluation and further improvement of experimental designs.

EXPERIMENTAL DESIGNS IN THE CLINICAL PHARMACOLOGY OF DIURETICS

With the purpose of assessing the effects of diuretics on urine and urinary solute outputs, an experimental design is currently followed in which, immediately after voiding urine, a single dose of the test substance is administered to normal subjects; urine is collected for analytical measurements at definite time intervals thereafter.[1-18] Such trials are carried out to directly assess the effects of new drugs,[12] to compare them with the effects of reference substances,[3,6,8,11,13,15] to study drug interactions[1,2,16] and to evaluate the urinary excretions of the diuretics and/or their metabolites.[4,7,17] Similar experimental designs are also applied to the study of patients.[19-25]

The total duration of experimental follow-up of the excretory effects of diuretics is usually 24 hours as was the case in 15 of 18 studies listed in Table I and in 5 of 7 listed in Table II. However, trials in normal subjects differ widely with respect to the timing of urine collections after medication (Table I). Analogue studies carried out in patients with syndromes likely to be treated with diuretics[19-25] show the same lack of uniformity in collection times (Table II). These differences are doubtless due to the fact that no consensus exists as to the experimental sensibility necessary for optimising the description of the time-courses of renal excretions. Such descriptions were invariably presented either as output values accumulated at urine collection times or as mean flow values corresponding to fractioning collection periods which are calculated by dividing the output value for the period by its duration until recently, when a mathematical model was designed to permit evaluation of post-dosing urinary excretions as continuous functions of time.[10,11,13-15]

Table I — *Summary of some recent studies carried out in normal subjects where urine was collected and analyzed after single doses of diuretics with the object of describing the time-courses of diuresis and/or with the object of describing the time-courses of diuresis and/or urinary solutes.*

Author(s)	Medication(s)	Urinary Variables Referred To Time	Hours Post-Dosing at Which Urine was Collected and Analysed
Brater[1]	furosemide, indomethacin	volume, Na	0.5, 1, 1.5, 2,3,4,5,6,8,24
Chennavasin et al[2]	furosemide, probenecid	volume, Na	0.5, 1, 1.5, 2,3,4,5,6,8,24
Cook et al[3]	furosemide, piretanide	volume, Na, K	2, 4, 6
Corrigan et al[4]	chlorothiazide	chlorothiazide	0.5, 1, 1.5, 2, 2.5, 3,4,5,7,24
González et al[5]	furosemide	volume, Na, furosemide	1,2,3,4,5,6
Irvin et al[6]	furosemide, indacrinone	volume, Na, K, uric acid	2,4,8,12,24
Knauf et al[7]	hydrochlorothiazide, triamterene	hydrochlorothiazide, triamterene, p-hydroxitriamterene	0.5, 1,2,3,4,5,6,8,12,24,48
Krück et al[8]	azosemide, furosemide	volume, Cl, Na, K	3,6,9,24
Krüger et al[9]	furosemide, xipamide	volume, Na, K	3,12,24
Leary et al[10]	furosemide, piretanide*	volume	3,6,9,12,24
Leary et al[11]	furosemide, tizolemide*	volume, Cl, Na, K, Ca, phosphate, creatinine, urea, urate	3,6,9,12,24
Piyasena et al[12]	xipamide	volume, Na, K	2,4,6,8,10,12,14,16,18,20,22,24
Reyes et al[13]	furosemide, hydrochlorothiazide, xipamide	volume	3,6,9,12,18,24
Reyes et al[14]	furosemide, piretanide*	volume, Cl, Na, K, Ca, phosphate, creatinine, urea, urate	3,6,9,12,24
Reyes et al[15]	furosemide, tizolemide*	volume	3,6,9,12,24
Smith et al[16]	furosemide, indomethacin	volume, Na	0.5, 1, 1.5, 2,3,4,5,6,8,24
Shah et al[17]	hydrochlorothiazide	hydrochlorothiazide	1,2,3,4,5,8,11,14,24
Stallings et al[18]	furosemide	volume, Cl, Na, K	6,12,24

* The effects after placebo were also studied.

Table II — *Summary of some recent studies carried out in patients where urine was collected and analyzed after single doses of diuretics with the object of describing the time-courses of diuresis and/or urinary variables.*

Authors	Syndrome	Medication(s)	Urinary Variables Referred to Time	Hours Post-Dosing at Which Urine was Collected and Analysed
Brater et al[19]	cardiac insufficiency	furosemide	furosemide	0.5, 1, 1.5, 2,3,4,5,6,8,24
Coodley et al[20]	cardiac insufficiency	furosemide, diapamide	volume	1,2,3,4,5,6,12,18,24
Gillies et al[21]	cardiac insufficiency	chlorothiazide, piretanide	volume, Na, K	6, 12
Kampf et al[22]	renal insufficiency	bumetanide, furosemide	volume, Cl, Na, K	3, 6, 12, 24
Pozet et al[23]	renal insufficiency	piretanide	wolume, Cl, Na, K, Ca, phosphate, uric acid	0.5, 1, 1.5, 2,3,4,5,6
Riva et al[24]	gestosis of pregnancy	furosemide	volume	2, 10, 24
Schmidt et al[25]	renal insufficiency	furosemide, muzolimine	volume, Na, K, creatinine, urea	6, 12, 24

DESCRIPTION OF MATHEMATICAL MODEL

Let Y be any renal excretory variable (urine volume or urinary chloride, sodium, potassium, etc.) accumulated at times t of urine collections after dosing. The function $20Y/\log (100-Y) = \exp [2.3 (t-t_1)/(a+bt)]$, where $t_1 = t$ for $Y = 0.1$ and a (time) and b (dimensionless) are other parameters, has been shown to fit the experimental data very accurately.[10,11,13-15]

With the object of evaluating the correlation coefficient and the a and b values through regression, the function is thus linearised:

$$(t-t_1)/[\log 20Y-\log \log (100-Y)] = a + bt.$$

For carrying out calculations, when experimental values are not within the range of from above zero to below ninety-nine, it is necessary to multiply them by ten or by a multiple or submultiple of ten in order to bring them within such a range (unit transformation). If more than one possibility of multiplication exists the coefficient yielding the highest values for analysis should be used, thus making the condition resulting from the $t = t_1$ for $Y = 0.1$ feature of the function itself more reliable if extrapolations to the left of its experimental range of validity are sought. Before proceeding to the statistical techniques, it is also necessary to evaluate t_1, which can be done either by iteratively finding the t_1 value yielding the highest r value, or, more easily and with sufficient accuracy, by linearly extrapolating t_1 from the experimental values as the t value for $Y = 0.1$ if urine is collected at hour 1, as the t value for $Y = 0.09$ if urine is collected for the first time at hours 2-4 and as the t value for $Y = 0.08$ if urine is collected for the first time at hours 4-7 post-dosing.

After having evaluated a and b, the renal excretory flow, y, as a function of time, is derived as:

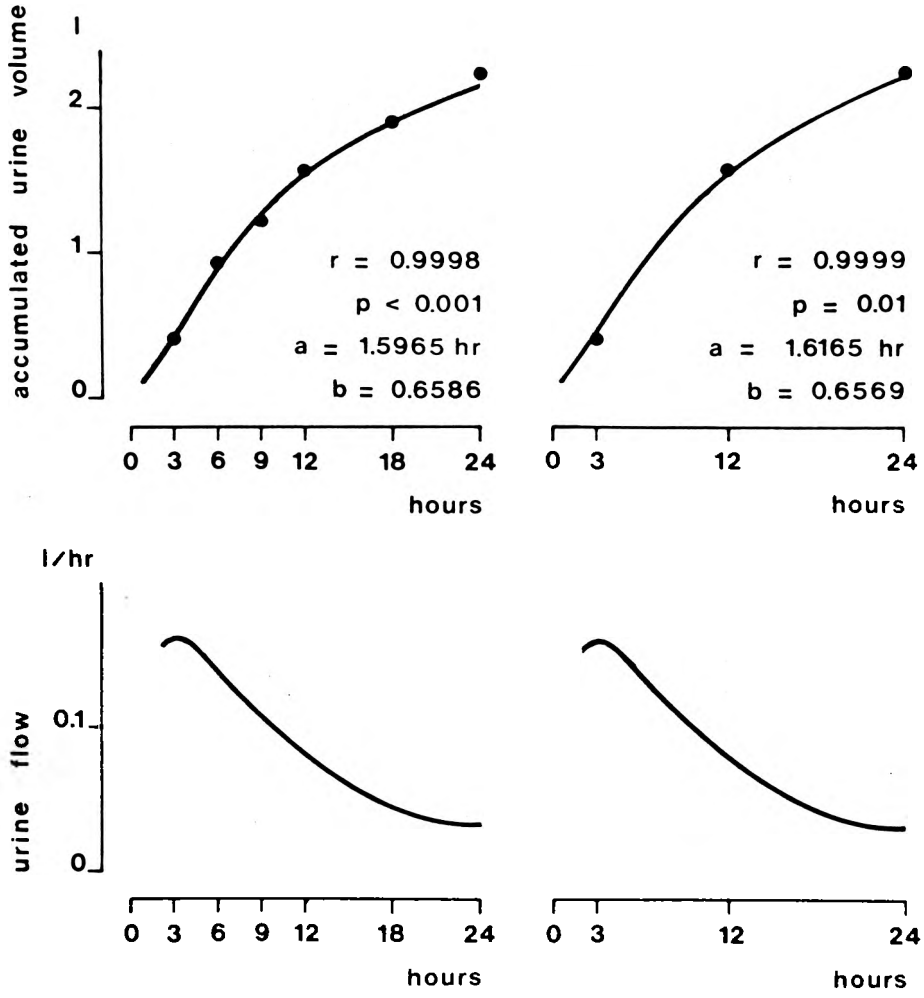
$$y = dY/dt = (a+bt_1)/(a+bt)^2 \| (0.43/Y) + [(0.43^2)/(100-Y) \log (100-Y)] \|$$

DISCUSSION OF THE MATHEMATICAL MODEL

No continuous description of the time-courses of renal excretory variables existed before the present mathematical model was used because no other satisfactory models were applied and in order to accomplish such descriptions experimentally it would be necessary to monitor the urine and urinary solute flows continuously.

The mathematical model significantly fits the experimental mean values of renal excretory variables such as urine volume,[10,13,15] urinary chloride, sodium, potassium, calcium, phosphate, creatinine and urate[11,14] measured after the administration of placebo[10,11,14,15] and various diuretics including furosemide,[10,11,13,15] hydrochloro-

Figure 1 — *The points in the upper left represent mean values of urine volume, Y, accumulated at hours 3, 6, 9, 12 and 24 post oral administration of xipamide 20 mg to eight healthy volunteers. The mathematical model Y (time — t —) was fitted to the entire set of experimental data and is shown as a continuous curve. The model was also fitted to mean values for t = 3, 12 and 24 hours only (upper right). t_1 = 0.7 hr in both cases. Urine flows (bottom) were derived as dY/dt. The functions have been extrapolated to the left of the first mean experimental value with the main purpose of showing their overall appearance.*



thiazide,[13] piretanide,[10,14] tizolemide[11,15] and xipamide.[13]

The functions Y (t) are sigmoid continuous functions and their derivatives with respect to time, y (t), describe the renal excretory variable flows with respect to time (Fig. 1) showing a post-dosing increasing

segment, a maximum ("peak effect") and a further monotonically decreasing segment. Times to onset of activity and peak excretions and the magnitude of any rebound (undershoot) with respect to placebo, can be very accurately evaluated through use of the mathematical model.

The model holds strictly for the time range from which the experimental data are derived, i.e., from the first until the last experimental points (urine collections and analyses). However, when there has been an interest in evaluating events such as maximal excretions which antecede the first urine collection, functions have been extrapolated to the left of their valid experimental ranges,[10,11,13,15] since that is the most accurate available method of describing the events. Such errors can be minimized by early urine collections after dosing, although, in practical terms, the extra experimental work involved is not worth the small gain in accuracy achieved. The mathematical model has been found to fit the experimental data satisfactorily even when only three experimental points (urine collections and analyses) are used.[26]

The a, b and t_1 parameter values summarise the principal features of the excretions studied. Commonly, t_1 and b values for a single renal excretory variable are similar for several diuretics;[10,11,13-15] then, higher a values indicate slower overall excretion time-courses, longer times to peak activity after dosing and decreased intensity and greater delay in onset of undershoots (rebounds) with respect to placebo, if they occur.

The mathematical model described covers physiological, post-placebo, excretions and all the pharmacologically-induced excretions that have been studied so far, thus allowing evaluation of alterations in normal or pathological physiology by diuretics. The model satisfactorily describes all the urinary excretory variables studied thus far including urinary volume, physiological solutes and drugs or their metabolites in urine. This has been evaluated (Reyes and Leary, unpublished) from published data,[4,7,17] and applies to results generated from both healthy and diseased subjects. The repetibility of the results of the evaluations of the mathematical model (parameter values) under different independent but similar experimental circumstances is very high.[26]

Similar parameter values are obtained from evaluating different sets of points from the same experiment, as shown in Figure 1, where the mathematical model has been fitted to mean experimental values for urine volume measured at 3, 6, 9, 12 and 24 hours after the oral administration of the diuretic xipamide to normal individuals. The model still holds when the mean experimental values corresponding to urine volume accumulated at 3, 12 and 24 hours post-dosing are the only points considered when carrying out calculations, and the a and b para-

meter values are practically the same as when all the points are entered into the calculations (Fig. 1). This coincidence has also been found (Reyes and Leary unpublished) for all drugs and variables studied[10,11,13-15] and means that, when diuretics that complete their effects within 24 hours are studied, most current experimental designs can be simplified in terms of the number of times at which urine should be collected for analysis after medication.

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PAPER C5

A Formal Method for the Therapeutic Classification of Anti-hypertensive Diuretics

This paper arose from analyses of data collected in several studies into the effects of various diuretics upon urinary excretion of various solutes.

The mathematical models used were those designed in collaboration with Professor A.J. Reyes. The paper was written by both authors working together.

CURRENT
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VOL. 30, NO. 6, SECTION 2, DECEMBER, 1981

A FORMAL METHOD FOR THE THERAPEUTIC CLASSIFICATION OF ANTIHYPERTENSIVE DIURETICS

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ABSTRACT

When high blood pressure, P, is treated with antihypertensive diuretics as a monotherapy, its evolution with time, t, has been satisfactorily described by an exponential function:

$$P = f(t) = P_f + (P_u - \Delta P - P_f) \exp(-t/T), \quad (I)$$

where P_f is a limit value to which P tends as t tends to infinity, P_u is untreated pressure, ΔP is the difference between P_u and P for t= O and T is a time-constant.

Alternatively, either a power or a linear function have been shown to fit the data to a statistically satisfactory degree similar to the one achieved by the exponential function in all cases:

$$P = g(t) = P_f + (P_u - P_s - P_f) \exp(-k . \ln t); \quad (II)$$

$$P = h(t) = P_u - P_d - bt, \quad (III)$$

where P_s is the difference between P_u and P for t = 1, k is a dimensionless constant, P_d is the difference between P_u and P for t = O and b is a pressure x time^{-1} dimensioned proportionality constant.

The mutually exclusive features of (II) and (III) allow classification of antihypertensive diuretics into "power" and "linear" types.

Comparisons between different drugs should be formally carried out through evaluations of the described functions. Power diuretics, such as xipamide, decrease blood pressure rapidly after the onset of treatment and should therefore be prescribed for most hypertensive patients. Linear diuretics, such as cyclothiazide, decrease blood pressure smoothly after the onset of treatment and are indicated in hypertensives with impaired peripheral perfusion in any vital organ.

INTRODUCTION

Antihypertensive diuretics are drugs of first choice in the treatment of hypertension. Changes in blood pressure induced by diuretics are usually

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expressed as differences between pre- and post-treatment values. Such expressions are, however, insufficient as they do not take into account time as a naturally occurring variable of the utmost importance in chronic diseases. Antihypertensive diuretics are not all identical and exhibit different characteristics with respect to the time-course of their therapeutic effect.[1-8] Thus, the application of a mathematical model to the time-course of the antihypertensive activity of diuretics has proved useful in formally characterising clinically relevant variables and parameters.

This communication describes the formal derivation and application of mathematical models to the anti-hypertensive effects of diuretics.

MATHEMATICAL MODELLING OF EXPERIMENTAL DATA

Blood pressure under treatment with a diuretic (P) as a function of time (t) can, in common with most medical variables, be described by different mathematical equations. The simplest approach to the mathematical description of a medical variable is the fitting of a linear function to the experimental data. This can usually be carried out in statistically acceptable form, provided some statistical pre-requisites are met and a sufficient number of points is analysed. However, if assumptions regarding well established clinical facts are taken into account, the derivation of a mathematical model must be based upon them and be worked out through a suitable differential equation.[1,4,5-7] Thus, for example, the application of a linear model to the evolution of treated blood pressure with time is not consistent with the assumption that blood pressure tends to a final limit value (P_f) as treatment is prolonged indefinitely[1,4-7]:

$$\lim_{t \to \infty} P = P_f . \qquad (1)$$

One of the simplest differential equations consistent with (1) is[1]:

$dP/dt = -K (P - P_f)$, where K is a $time^{-1}$ dimensional constant. (2)

Upon integration:

$$P = f(t) = P_f + (P_u - \Delta P - P_f) \exp(-t/T), \qquad (3)$$

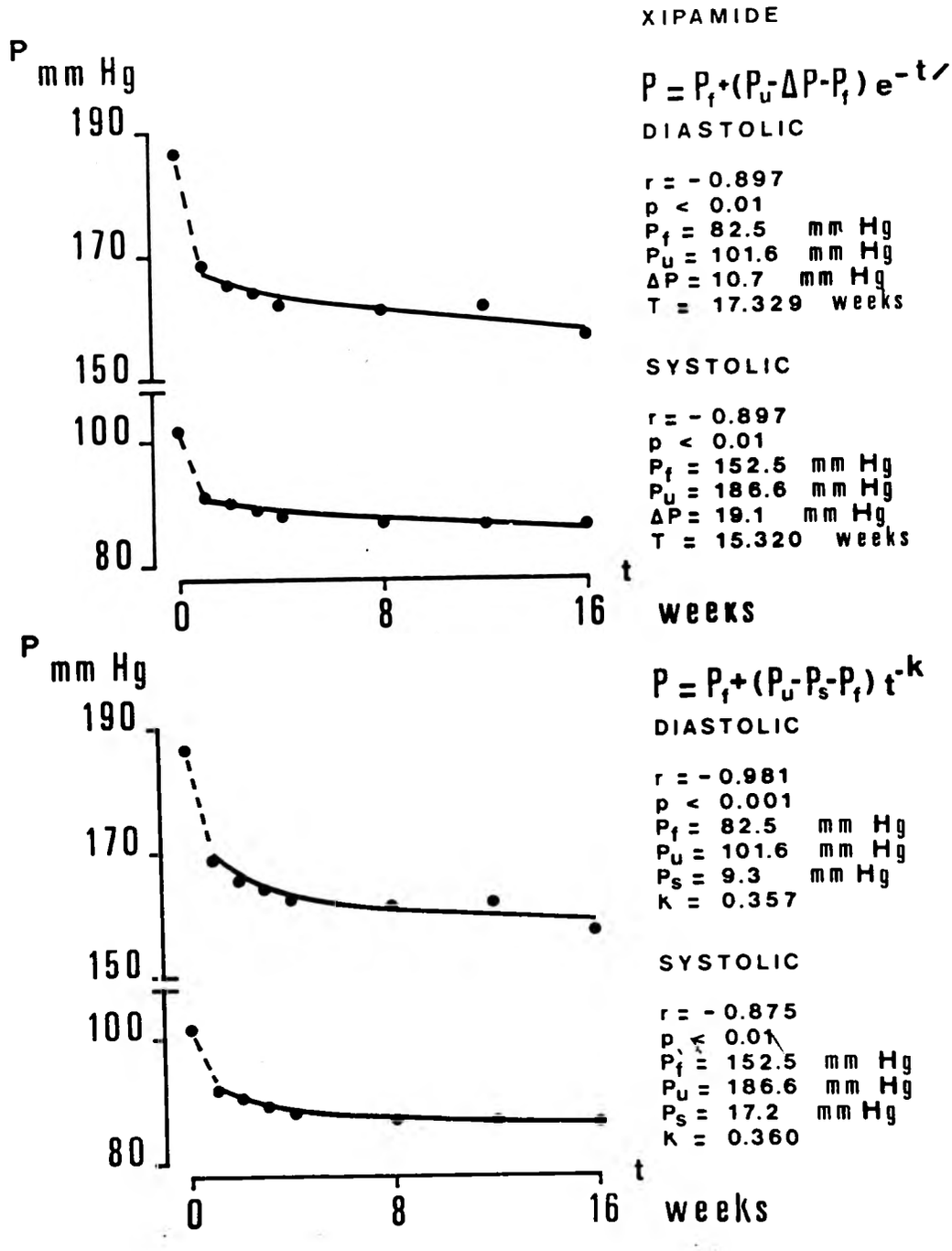
where P_u is untreated blood pressure, ΔP is the difference between P_u and the theoretical P value for t=0 and T is a time constant.

The fit of (3) to the experimental data can be easily assessed and the parameter values calculated through the correlation and regression techniques applied to:

$$\ln (P - P_f) = \ln (P_u - \Delta P - P_f) - (t/T). \qquad (4)$$

For evaluation purposes, a P_f value should be defined upon inspec-

Figure 1 — *Systolic and diastolic pressure (P) responses to xipamide in 25 hypertensive patients treated for 16 weeks (t) with an average dose of 23.6 mg/day. Exponential (top panel) and power (bottom panel) functions have been fitted to the data. Data from Castro and Reyes.*[7]



tion of the trend of the experimental data in a dispersion diagram.[1,4-7]

Equation (2) fits the experimental data expressed as the means of diastolic, systolic and mean arterial blood pressures under treatment with xipamide[1,4,7] (Fig. 1), cyclothiazide[2] (Fig. 2), a combination of hydrochlorothiazide and amiloride,[6] tizolemide[5] and hydrochlorothiazide.[8] As it is consistent with the postulate in (1), the validity of equation (2) still holds when very prolonged periods of treatment (2 years) are considered.[4,7]

An elementary alternative to equation (2) is provided by[7]:

$$dP/dt = -k\,(P - P_f)/t, \qquad (5)$$

where k is a dimensionless constant. Upon integration, (5) yields:

$$P = g(t) = P_f + (P_u - P_s - P_f)\exp(-k.\ln t), \qquad (6)$$

where P_s is the difference between P_u and the theoretical P value for t=1. This equation can be linearised for statistical purposes thus:

$$\ln(P - P_f) = \ln(P_u - P_s - P_f) - k.\ln t. \qquad (7)$$

Equation (7) has been found to fit the experimental data with as high a correlation coefficient value (r) as equation (4) in the case of some antihypertensive diuretics such as xipamide[7] (Fig. 1) and an association of hydrochlorothiazide and amiloride.[6] The validity of equation (7) also holds for extended periods of time as it, too, is consistent with the assumption in (1).[4,7]

In the case of other diuretics such as cyclothiazide, hydrochlorothiazide, and tizolemide, equation (7) does not hold to a similarly satisfactory statistical degree. In those circumstances a simple linear model (8) also fits the data to a statistically satisfactory degree similar to the one achieved by (4), when 3-4 months studies are considered:[2,5,8]

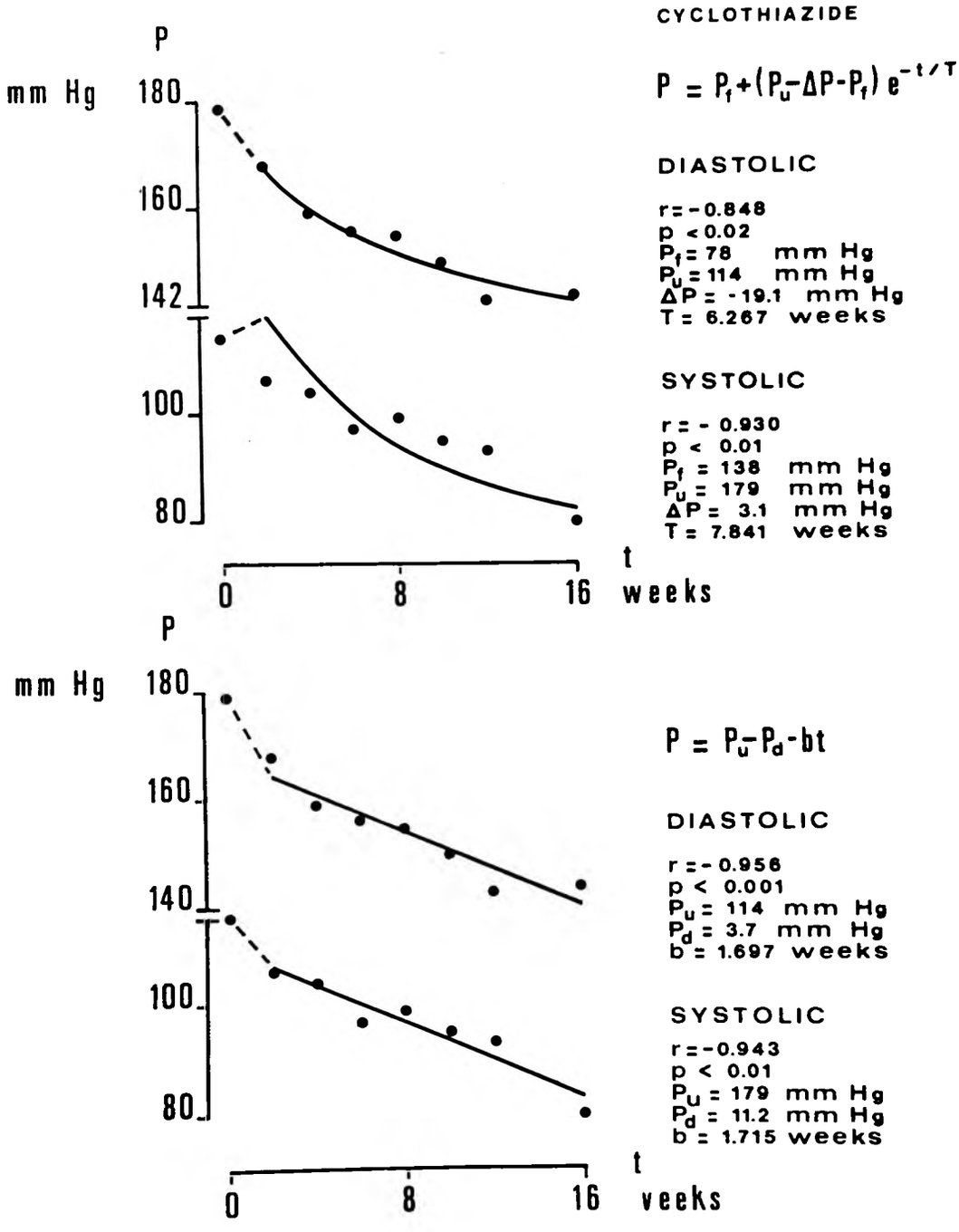
$$P = h(t) = P_u - P_d - bt, \qquad (8)$$

where P_d is the difference between P_u and the theoretical P value at t=0 and b is a pressure x time^{-1}-dimensioned proportionality constant.

Equation (8) is not consistent with (1) but indicates a fixed rate fall in pressure; for this reason it does not hold when long-lasting studies are considered.[5]

Equations (3), (6) and (8) are sufficient to describe all data analysed so far. Differences between them qualify two different responses of blood pressure to treatment with diuretics: Equation (3), (exponential model), is universally applicable, whereas equation (6), (power model) and equation (8), (linear model), apply to distinct blood pressure responses to treatment with different diuretics which may be classed as power or linear diuretics in accordance with the common mathematical denominations of the describing functions.

Figure 2 — *Systolic and diastolic pressure (P) responses to cyclothiazide in 15 patients treated for 16 weeks with an average dose of 5 mg/day. Exponential (top panel) and power (bottom panel) functions have been fitted to the data. Data from Isasi and Reyes.*[2]

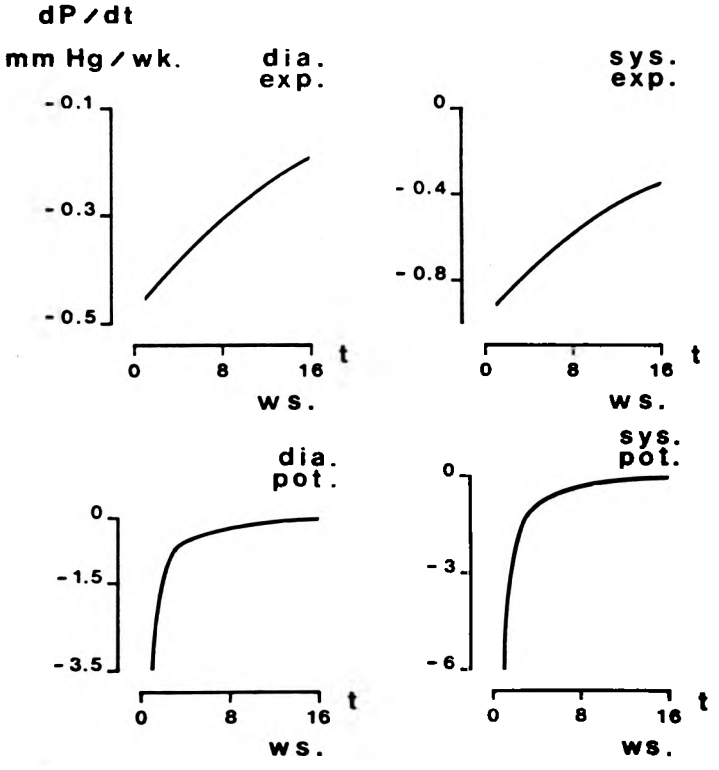


THE EXPONENTIAL MODEL

The finding that the exponential model provided by (3) describes the changes of blood pressure during treatment with any antihypertensive diuretic, formally confirms the established view that diuretics are not hypotensive drugs, that they do not reduce blood pressure unduly, and that tolerance to treatment with them does not develop.[9,10]

As equation (3) only applies to treated blood pressure, which represents the experimental range studied, the evolution of the independent variable from the onset of treatment until its first clinical evaluation is not covered by the model. The ΔP value is indicative of the parametric magnitude of the changes during this period, but ΔP only approximates to the actual experimental difference between untreated blood pressure and its first treated measurement. The use of a biexponential model fully covers blood pressure from the onset of treatment onwards; however, it further complicates the model. Therefore, the immediate evolution of blood pressure after the initiation of treatment is not described but arbitrarily represented by dotted lines in Figures 1 and 2.

Figure 3 — *Rates of change (dP/dt) in diastolic (dia.) and systolic (sys.) blood pressures in response to xipamide as functions of time (t) as derived from Fig. 1. Data corresponding to exponential (exp.) and power (pot.) models appear in upper and lower panels respectively. Abbreviations wk and ws signify week and weeks respectively.*



The rate of change of blood pressure as described by the exponential model is:

$$dP/dt = f'(t) = (-1/T)(P_u - \Delta P - P_f) \exp(-t/T). \quad (9)$$

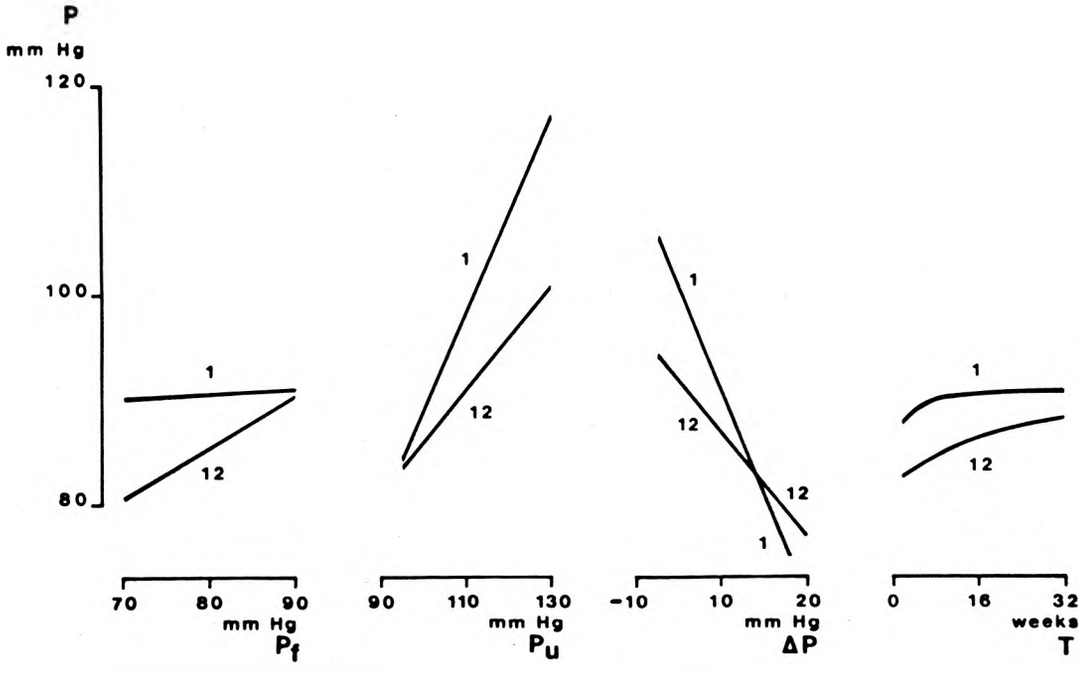
When expressed as a function of time, the rate of change increases exponentially and tends to zero (Fig. 3). The absolute rate of change is higher at the beginning of treatment and decreases thereafter, which means that diuretics exert a more intense antihypertensive effect immediately after treatment is initiated.

The universality of the exponential model allows comparisons between different diuretics or between responses of different groups of patients to the same diuretic. For such purposes, the influence of each parameter in (3) on the blood pressure value at any definite time must be taken into account. In the discussion that follows, it is assumed that all variable and parameter values, other than those two whose inter-relationship is analysed, remain constant.

Blood pressure (P) increases as a linear function of P_f (Fig. 4), that is, the higher P_f value, the higher the P value at any time during treatment. This relationship has a more marked slope, though lesser absolute values, earlier in treatment.

Blood pressure (P) increases as a linear function of P_u (Fig. 4), that is, the higher the P_u value the higher the P value at any time during

Figure 4 — *Diastolic blood pressure (P) during xipamide treatment as single functions of various parameters (P_f, P_u, ΔP, T) as derived from the exponential model in the upper panel of Fig. 1, at definite times (1= one week, 12= twelve weeks).*



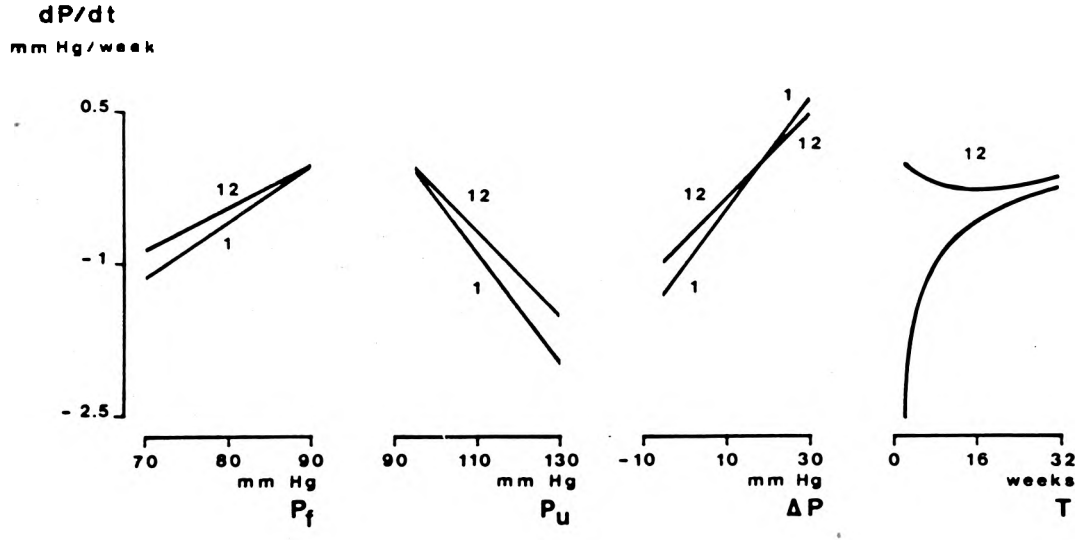
treatment. This relationship has a more marked slope at the early stages of treatment.

Blood pressure (P) decreases as a linear function of ΔP (Fig. 4), that is, the greater the ΔP value the lower the P value at any time during treatment. This is more marked early in treatment. The slope relationships between these functions early and late in treatment is such that the absolute P values early in treatment are higher than those at later stages, for relatively low P values, and vice versa.

Blood pressure P is an increasing exponential function of T (Fig. 4), that is, the higher the T value, the greater the P at any time during treatment. P tends to P_u - ΔP as T tends to infinity. The P, T relationship early in treatment lies within a higher range of P values than that which applies later.

The parameter values in (3) also affect the rate of change of treated blood pressure P when it is considered as a function of each of them. The single relationships between the rate of change, (dP/dt), and P_f and ΔP are increasing linear functions (Fig. 5). In both cases, the relative

Figure 5 — *Rate of change of diastolic blood pressure, dP/dt, during xipamide treatment as single functions of various parameters (P_f, P_u, ΔP, T) as derived from the exponential model in the upper panel of Fig. 1 at definite times (1= one week; 12= twelve weeks).*



positions of the functions at different stages of treatment are such that dP/dt values are higher at later than earlier phases, except when ΔP is greater than its experimentally derived value, when this relationship is reversed.

The rate of change of P is a decreasing linear function of P_u (Fig. 5), that is, higher untreated P values are assocaited with lower rates of blood pressure decrease at any time during treatment. This relationship has a more marked slope at the later stages of treatment.

Rate of blood pressure change with time, dP/dt, as a function of T (Fig. 5) is a non-monotonic function which early in treatment shows itself as rapidly increasing at low T values, its rate decreasing at higher T values. Thus, during the early stages of treatment the rate of change in P increases as T increases, this being true for T values higher than the t value considered. At later stages in treatment, dP/dt (T) is a clearly biphasic function with a minimum at T=t as derived when its first derivative is equated to 0:

$$(dP/dt)/dT = j(T) = [1-(t/T)]/T^2 . \quad (10)$$

In general, the rate of change of blood pressure as a function of T at any time is a decreasing relationship for the T value range below that T value equalling the time t considered; it is an increasing function thereafter.

When different treatments are compared, or the same treatment is evaluated in different groups of patients, the experimental data yield different parameter values. Comparisons between parameters arising from any two such experiments are limited to the P_u (t-test) and T values (t-test for the significance of differences between the slopes of linearised or linear functions). Therefore, between group comparisons of ΔP and P_f should be derived through their relationships with P_u and T as well as their intrinsic relationship. This necessity should be dealt with by an analysis of functions of multiple variables.

The analysis of the relationship between any two parameters, other parameters remaining constant, constitutes a simpler approach but is not as trustworthy. However, the technique will be briefly described, as an indication of the main directions of change in the relative relationships between variables.

When untreated blood pressure (P_u) is considered as a function of P_f, ΔP or T, it gives rise to linearly decreasing, linearly increasing and decreasing exponential relationships respectively, (Fig. 6):

$$P_u = P_f + \Delta P + (P - P_f) \exp (t/T). \quad (11)$$

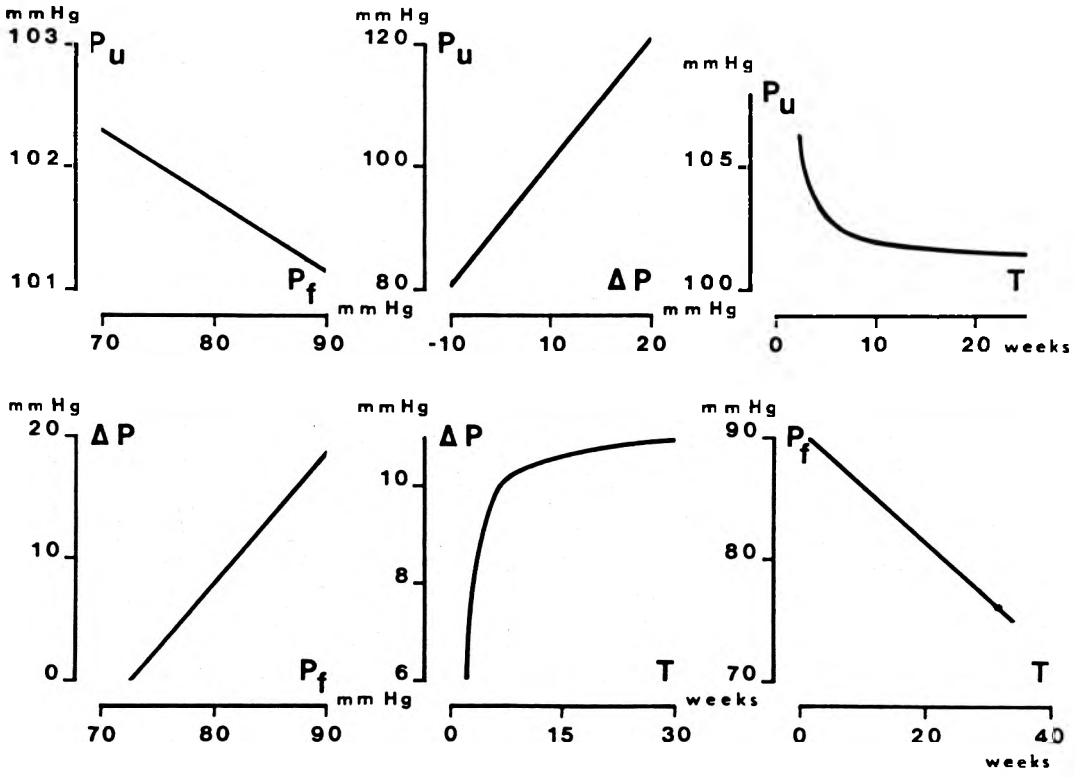
Higher P_f values mean lower P_u values and vice versa, all other variables and parameters being constant. The greater the ΔP value, the higher the P_u value and conversely. The higher the T value, the lower the P_u value, this relationship being more marked for low values of T or high values of P_u.

ΔP bears an increasing linear relationship with P_f and an increasing exponential relationship that tends to $P_u - P$ with T:

$$\Delta P = P_u - P_f - (P - P_f) \exp (t/T). \quad (12)$$

The higher the P_f value, the higher the ΔP and conversely. The higher the T value, the higher ΔP and vice versa, this relationship being steeper for small values of either variable. The P_f relationship with T is

Figure 6 — *Single inter-relationships between several parameters (P_u, ΔP, P_f and T) of the exponential model as derived from the diastolic blood pressure values, during xipamide treatment, in the upper panel of Figure 1.*



"an almost linear in shape" exponential function (Fig. 6):

$$P_f = [P-(P_u - \Delta P) \exp(-t/T)] \; / \; [1-\exp(-t/T)]. \qquad (13)$$

The higher the T value, the lower the P_f value, and conversely.

The time constant T as a function of all other parameters is expressed by:

$$T = -t/[\ln(P-P_f) - \ln(P_u - \Delta P - P_f)]. \qquad (14)$$

This equation clearly shows that solution of the multiple relationship $T(P_u, P_f, \Delta P)$ is necessary to allow an overall comparison between two different experiments through the T value. This relationship T (P_u, P_f, ΔP) constitute the "equivalent time constant" when evaluated at definite time, t, and could practically summarise the slopes of the P(t) functions. Thus, in order to determine the equivalent time-constant for purposes of comparisons between data, particularly when different diuretics are compared, it is necessary to multiply correct T in accordance with P_u, P_f and ΔP values for both sets of data. When the equivalent time-constant has a low value, that is, when P decreases markedly in the first weeks of treatment, the exponential model is matched by a power model. Power diuretics that exhibit a relatively high time-

constant value when compared to other diuretics whose effects upon blood pressure are not fitted by power functions, have in fact a low time constant value if it is corrected relative to the value of the other parameters (low equivalent time-constant value).

When the reductions in P that immediately follow the onset of treatment are of a mild degree, the exponential model also holds, and is not matched by a power but by an alternative linear model in terms of statistical correlation. The equivalent time-constant value is low in these circumstances. Thus, the great versatility of exponential functions makes them always applicable to the phenomena under study, whereas the value of the corrected time-constant allows differentiation between power and linear diuretics.

THE POWER MODEL

Equation (6) provides a power function which is a statistical equivalent alternative to equation (4), when the long-term evolution of blood pressure under treatment with diuretics such as xipamide is studied. The fact that the exponential term of the function varies with the logarithm of time makes this function evolve rapidly when the time values are small and continue with a smoother slope thereafter. For this reason, the power function satisfactorily describes events when they proceed rapidly after the onset of treatment, does not fit the data when blood pressure changes very gradually with time after the initiation of treatment and therefore formally characterises a group of antihypertensive diuretics.

Figure 3 shows how the slope of a power function varies with time. The initial evolution of the slope is such that small changes in time correspond to big changes in the slope, whereas after the first few weeks of treatment the rate of change of blood pressure slows down to a rate which tends asymptomatically to zero. Figure 3 clearly shows that even for the same data, fitted to analogous degrees of accuracy to power and exponential functions, different slopes arise from each model, thus proving that the justification for the denomination "power diuretics" lies in this mathematical uptake.

So far only xipamide and a combination of amiloride and hydrochlorothiazide have been shown to behave as power diuretics.[1,6,7,11] The data on xipamide to which power models fit have been derived from three independent studies covering periods which extend to two years of continuous treatments, whereas the only experiment dealing with the hydrochlorothiazide and amiloride combination was limited to 8 weeks and thus requires confirmation.

Comparisons between power diuretics can be summarised through

the analysis of the k values, provided the other parameter values are properly taken into account as for the exponential time constant:

$$P_u = P_f + P_s + (P - P_f) \exp (k. \ln t); \quad (15)$$

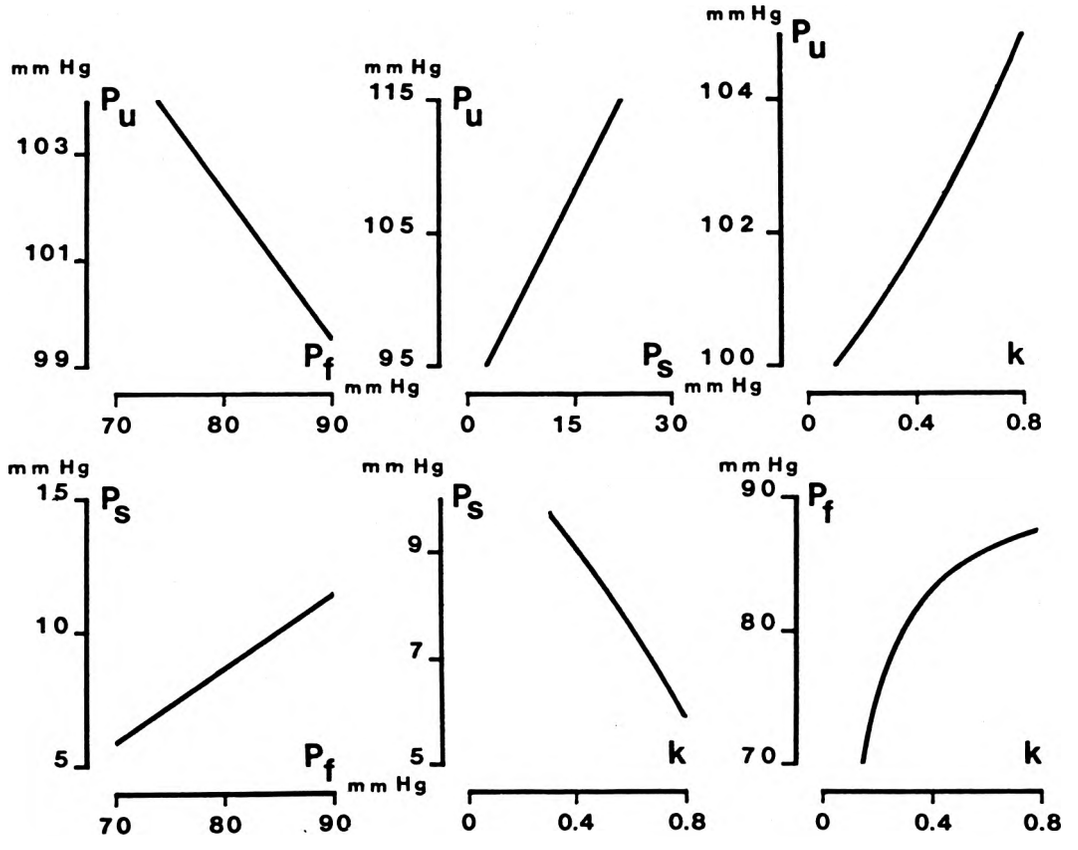
$$P_s = P_u - P_f - (P - P_f) \exp (k. \ln t); \quad (16)$$

$$P_f = [P - (P_u - P_s) \exp (-k. \ln t)] \; / \; [1-\exp(-k. \ln t)]; \quad (17)$$

$$k = -[\ln (P - P_f) - \ln (P - P_s - P_f)] \; / \ln t. \quad (18)$$

Figure 7 summarises the above expressions graphically.

Figure 7 — *Single inter-relationships between several parameters (P_u, P_s, P_f and k) of the power model, as derived from the diastolic blood pressure values, during xipamide treatment, in the lower panel of Figure 1.*



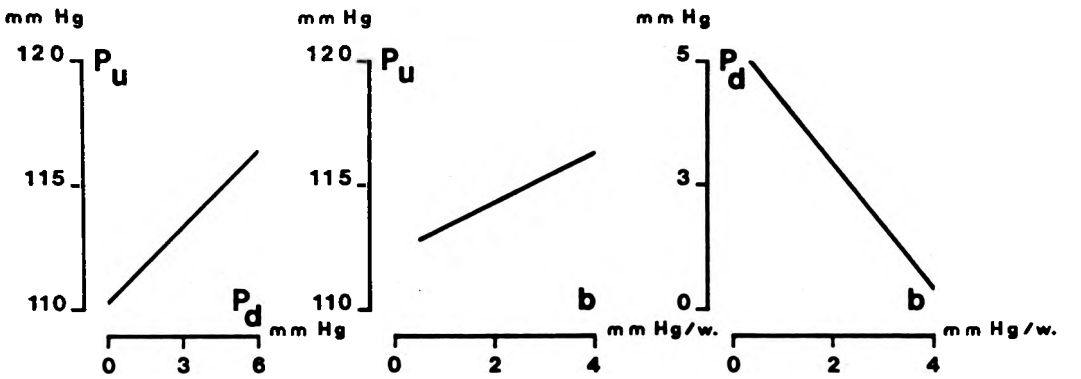
THE LINEAR MODEL

The antihypertensive action of some diuretics is very gradual throughout the first two or three months of treatment. Thus, upon inspection of the experimental data, it may be postulated that dP/dt = -b; equation

(8) is yielded upon integration. A linear model is not consistent with the assumption that blood pressure tends to a limit value when treatment is indefinitely prolonged;[1] however, it is the most frequently used mathematical descriptive instrument in the medical sciences and employment of the model is justified provided it fits the experimental data and no extrapolation outside its range is sought. The exponential model fits the same data and is consistent with the assumption in equation (1). Nevertheless, the fact that a linear model fits the data from experiments with diuretics such as cyclothiazide,[2] hydrochlorothiazide,[9] and tizolemide[5] implies a qualification of the slope of the change of blood pressure with time. This is not only constant but also has a very small equivalent time-constant value when the alternative exponential model is considered.

Figure 8 shows the relationship between any two parameters in the linear model (8), thus allowing comparisons between linear diuretics to be summarised in terms of corrected equivalent b values.

Figure 8 — *Single inter-relationships between several parameters (P_u, P_d, b) of the linear model as derived from the diastolic blood pressure values, during cyclothiazide treatment, in the lower panel of Figure 2.*



PATHOPHYSIOLOGICAL IMPLICATIONS

The investigations which provided the basis for the analysis carried out here were conducted in hypertensive patients, unselected with respect to certain functional characteristics which differ amongst hypertensives. These included differences in such variables as volaemia and peripheral renin activity. Therefore, the assumption has been made that the present descriptions apply to patients with essential hypertension in whom the percentile distribution of different functional pathophysiological characteristics is the same as in the general population of hyper-

tensives. This is justified by the fact that diuretics are first choice drugs in the management of essential hypertension and most patients are not subjected to the complicated laboratory studies necessary to classify them according to the functional features mentioned. Furthermore, proof that factors such as volaemia and renin level can explain the origins or degree of essential hypertension within any population has not been unequivocally provided. It is likely that other factors affecting the pathophysiology of essential hypertension will be considered in the future. Meanwhile, it remains valid to consider essential hypertension as a single disease, which justifies some broad pathophysiological speculations arising from the models described here.

The mechanisms whereby diuretics reduce high blood pressure remain largely obscure and therefore controversial.[10-12] An early depletion of volume followed by a chronic vasodilatory effect and, more recently, a functional basis for this vasodilation have been postulated as the most likely processes underlying the change in blood pressure.[13] The fact that blood pressure is rapidly reduced by some diuretics like xipamide, as expressed by the high ΔP or P_S values found, would be consistent with the theory that volume depletion intervenes as an early mechanism. In addition, the chronic gradual decreases seen at later stages do not contradict the view that chronic vasodilation plays an important role. However, the fact that loop diuretics such as furosemide[9,10,14] and piretanide[15] are not effective antihypertensive drugs, even early during treatment, suggests that early volume depletion alone cannot account for the changes in blood pressure noted initially as responses to antihypertensive diuretics. Similarly, chronic vasodilation is not the most likely explanation for the long lasting fall in blood pressure provoked by diuretics because some loop diuretics show vasodilatory effects in acute animal experiments[10] but are relatively ineffective as antihypertensive agents in man even when long-acting formulations of the compounds are used.[9,10,14,15]

Linear, exponential and power functions are all derived from ordinary first-order differential equations, whereas the control and regulatory mechanisms of blood pressure are known to be based upon non-linear differential equations. Not only the direct molecular and secondary functional mechanisms of the antihypertensive effect of diuretics should be taken into account but also their interplay with physiologically acting controls and homeostatic regulations. Therefore, the models described here constitute an oversimplification in terms of pathophysiological and pharmacological description in as much as they are based upon linear differential equations and their value lies in the possibility they can afford for formal comparisons between the clinical effects of these drugs.

CLINICAL IMPLICATIONS

Diuretics with antihypertensive effects may be divided into two clearly defined groups on the basis of the mathematical models described here. Those exhibiting a relatively rapid onset of action followed by a more gradual effect upon blood pressure are best described by power functions whereas diuretics which reduce pressure relatively gradually throughout treatment are best described by linear functions.

The risks associated with hypertension are such that the pressure should be lowered rapidly and to the maximum degree compatible with effective organ perfusion. In consequence, a well-established power diuretic like xipamide should be used as the first choice antihypertensive in most cases.

In patients with coexistent impairment in perfusion of vascular beds, such as the cerebral, renal or coronary circulations, it is likely that high rate decreases in blood pressure may cause further deterioration in organic function by the operation of the critical closing pressure mechanism. In these patients, a well-established linear diuretic such as hydrochlorothiazide should be tried as the first choice antihypertensive.

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PAPER C6

Mathematical Evaluation of the Effects of Tizolemide, Furosemide and Placebo in Healthy Adults

This paper describes the diuretic effects of tizolemide (at the time a new diuretic) and compares it to furosemide and placebo. The study was designed by both authors in consultation, carried out by me and the paper written jointly.

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Mathematical evaluation of the effects of tizolemide, furosemide and placebo in healthy adults

W. P. LEARY, A. J. REYES

Summary

The effects of single oral doses of tizolemide 100 mg, furosemide 80 mg and placebo on outputs of various electrolytes and metabolites in urine, on their corresponding urinary flows and on several blood solute concentrations were compared in 16 healthy volunteers.

Tizolemide and furosemide exhibited similar quantitative effects on urinary and blood variables for 24 hours after administration but had different time-courses of renal excretion. Furosemide acted abruptly as loop diuretics do; tizolemide had more physiological time-courses, as shown by most diuretics whose main site of renal action lies at the cortical diluting segment.

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Tizolemide and furosemide are clinically effective diuretics with slightly dissimilar properties, presumably related to different sites of action within the nephron. Tizolemide, a relatively new sulphonamide diuretic, acts mainly at the cortical diluting segment of the distal convoluted tubule, whereas furosemide exerts its main effects within the ascending limb of Henle's loop.[1]

The object of this study was to describe the effects of tizolemide and furosemide on the concentrations of various electrolytes and metabolites in urine and blood of healthy volunteers and to compare them with concentrations measured after treatment of the same subjects with placebo.

Subjects and methods

Sixteen healthy male medical students or technologists volunteered to participate in the experiments after a full explanation of their aims and implications had been given. All the subjects were aged 20-35 years and none had any history of renal, cardiovascular, endocrine or metabolic disorders.

Tizolemide 100 mg, furosemide 80 mg and placebo, labelled A, B or C, were administered orally with 100 ml water on different treatment days. Volunteers carried out their normal daily work and received a standardized diet containing approximately 150 mEq sodium and 2 000 ml fluid on treatment days and during the preceding 24 hours. Heavy muscular work and ingestion of alcohol or any medicine other than the trial preparations were forbidden on treatment days. A specified schedule was followed so that volunteers received medicines in random order, at least 7 days separating each treatment. All laboratory analyses were carried out by experienced technologists unaware of the protocol or the medicines being tested.

All urine passed on pretreatment and treatment days was collected. Urine collected during the 24 hours preceding medication was pooled, whereas urine excreted after dosing on each treatment day was divided into samples collected 0-3, 3-6, 6-9, 9-12 and 12-23 hours after medication. The volume of the urine and the urinary sodium, potassium, chloride, calcium, phosphate, creatinine, urea and urate levels were measured in each specimen. On treatment days a venous blood sample was drawn just before medication and 6 and 24 hours later for measurement of blood osmolality and sodium, potassium, chloride, calcium, phosphate, creatinine, urea and urate levels. Plasma cholesterol, triglyceride, bilirubin, alkaline phosphatase and SGOT values were also measured before and 24 hours after medication.

The mean experimental values of the urinary outputs, Y, accumulated by the end of each collection period were plotted as functions of time, t. After inspection of the dispersion diagrams the following equation relating Y and t was postulated:[2]

$$20Y/\log(100-Y) = \exp[2{,}3\,(t - t_1)/(a + b)] \quad \text{------ (1)},$$

where t_1 is the time at which $Y = 0{,}1$ and a (time) and b (dimensionless) are parameters of the function. The fit of (1) to the experimental data was evaluated through the standard linear correlation and regression techniques applied to:

$$(t - t_1)/[\log 20Y - \log\log(100 - Y)] = a + bt.$$

The t_1 values were calculated as the t values yielding $Y = 0{,}09$ upon extrapolation from the mean 3-hour accumulated output experimental values, as the t values corresponding to $Y = 0{,}1$ would constitute an overestimation because of the final shape of the functions.

Flows of urinary variables, Y, were defined as:

$$y = dY/dt = (a + bt_1)/(a + bt)^2\,((0{,}43/Y) + [(0{,}43)^2/(100 - Y)\log(100 - Y)]).$$

Standard statistical techniques (applicable *t* tests) were used for evaluating the significances of differences between slopes and between mean values. All tests used were two-tailed and the significance level was $P \leq 0{,}05$.

Normality of frequency distributions and homoscedasticity of samples variances were evaluated prior to the use of the statistical methods through the chi-square test for goodness of fit and the *F* test respectively.

Results

Any one 24-hour pretreatment urinary variable mean value did not differ significantly from any other or from the 24-hour urinary variable mean value after placebo.

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Date received: 27 May 1981.

Mean experimental values of urinary variables after dosing are shown in Tables I and II. Twenty-four-hour mean urine volumes after both diuretics were alike and significantly higher than the 24-hour urine volume after placebo ($P < 0,001$). Twenty-four-hour mean urinary sodium, potassium, chloride and calcium outputs were significantly higher after tizolemide ($P < 0,001$, $P < 0,02$, $P < 0,01$ and $P < 0,05$ respectively) and furosemide administration ($P < 0,01$, $P < 0,001$, $P < 0,001$ and $P < 0,001$ respectively) than after placebo. Phosphate, creatinine, urea and urate excretion after tizolemide, furosemide and placebo did not differ significantly.

Mean urinary variable outputs accumulated after dosing as functions of time are parametrically described in Tables III and IV and the Y (t) and y (t) functions for potassium plotted in Figs 1 and 2. The time-courses of the urinary flow of urea, urate, potassium and creatinine did not differ significantly between tizolemide and placebo (similar a values); sodium, chloride and calcium flows after tizolemide proceeded at higher and lower rates than after placebo or furosemide respectively. All electrolyte and metabolite urinary flows induced by furosemide, except phosphate, proceeded at higher rates than the corresponding rates after tizolemide or placebo (lower a values). All urinary phosphate flows proceeded at the same rate.

No significant differences were found between any two b values corresponding to the same variable.

Mean values of blood variables before and after treatments are shown in Table V. Significant increases in serum urate concentration were recorded 24 hours after dosing with furosemide or tizolemide ($P < 0,05$), and there was a significant fall in serum potassium 6 hours after furosemide administration ($P < 0,05$).

TABLE I. SUMMARY OF URINE DATA FROM THE COMPARATIVE TIZOLEMIDE, FUROSEMIDE AND PLACEBO STUDY IN 16 HEALTHY ADULTS

		Mean values accumulated at hours after dosing				
Variable	Treatment	3 h	6 h	9 h	12 h	24 h
Urine volume (l)	TIZ 100 mg	0,368	0,734	1,171	1,508	2,283
	FUR 80 mg	1,113	1,681	1,932	2,114	2,457
	PLA	0,162	0,469	0,648	0,858	1,369
Urinary sodium (mmol)	TIZ 100 mg	45,77	95,22	152,61	199,18	313,99
	FUR 80 mg	110,84	116,82	187,16	200,05	223,62
	PLA	162,30	458,20	781,80	100,41	148,38
Urinary potassium (mmol)	TIZ 100 mg	8,49	16,32	24,91	32,30	50,62
	FUR 80 mg	15,53	26,25	34,13	40,44	53,84
	PLA	5,03	13,70	21,29	26,68	39,05
Urinary chloride (mmol)	TIZ 100 mg	58,96	116,86	172,18	216,41	331,90
	FUR 80 mg	149,36	219,07	241,52	255,55	287,71
	PLA	19,84	63,77	84,77	106,53	152,54
Urinary calcium (mg)	TIZ 100 mg	0,57	1,10	1,60	1,84	2,71
	FUR 80 mg	1,35	2,28	2,61	2,87	3,27
	PLA	0,31	0,69	1,03	1,27	1,93

TIZ = tizolemide; FUR = furosemide; PLA = placebo.

TABLE II. SUMMARY OF URINE DATA FROM THE COMPARATIVE TIZOLEMIDE, FUROSEMIDE AND PLACEBO STUDY IN 16 HEALTHY ADULTS

		Mean values accumulated at hours after dosing				
Variable	Treatment	3 h	6 h	9 h	12 h	24 h
Urinary phosphate (mg)	TIZ 100 mg	0,06	0,13	0,40	0,55	1,03
	FUR 80 mg	0,06	0,13	0,32	0,44	0,89
	PLA	0,04	0,12	0,25	0,35	0,74
Urinary creatinine (mg)	TIZ 100 mg	190,22	320,48	531,04	783,26	1 555,89
	FUR 80 mg	353,72	518,78	693,54	843,15	1 437,43
	PLA	97,74	283,94	479,70	633,69	1 187,15
Urinary urea (mg)	TIZ 100 mg	2,62	6,24	9,12	12,17	20,71
	FUR 80 mg	4,25	7,95	9,93	12,01	17,26
	PLA	1,75	6,48	9,03	11,49	18,18
Urinary urate (mg)	TIZ 100 mg	64,28	109,07	167,47	217,75	408,02
	FUR 80 mg	64,75	107,28	138,08	158,69	247,44
	PLA	46,86	102,83	154,92	206,93	330,43

TIZ = tizolemide; FUR = furosemide; PLA = placebo.

TABLE III. STATISTICAL EVALUATIONS AND PARAMETERS OF THE LINEARIZED TRANSFORMATIONS OF THE FUNCTIONS Y (t)

Variable	Treatment	*r*	*P*	t_1 (h)	a (h)	b
Urine volume (l)	TIZ 100 mg	0,999	<0,001	0,80	2,097	0,622
	FUR 80 mg	0,999	<0,001	0,25	0,584	0,687
	PLA	0,998	<0,001	2,00	2,205	0,711
Urinary sodium (mmol 10^{-1})	TIZ 100 mg	0,999	<0,001	0,06	0,759	0,363
	FUR 80 mg	0,999	<0,001	0	0,216	0,412
	PLA	0,999	<0,001	0,16	1,040	0,409
Urinary potassium (mmol 10^{-1})	TIZ 100 mg	0,999	<0,001	0	0,642	0,335
	FUR 80 mg	0,999	<0,001	0	0,395	0,340
	PLA	0,999	<0,001	0	0,696	0,349
Urinary chloride (mmol 10^{-1})	TIZ 100 mg	0,999	<0,001	0,50	0,408	0,367
	FUR 80 mg	0,999	<0,001	0	0,189	0,394
	PLA	0,999	<0,001	0,15	0,839	0,416
Urinary calcium (mg)	TIZ 100 mg	0,999	<0,001	0,50	1,510	0,621
	FUR 80 mg	0,999	<0,001	0,20	0,528	0,631
	PLA	0,999	<0,001	0,90	2,194	0,656

TIZ = tizolemide; FUR = furosemide; PLA = placebo.

TABLE IV. STATISTICAL EVALUATIONS AND PARAMETERS OF THE LINEARIZED TRANSFORMATIONS OF THE FUNCTIONS Y (t)

Variable	Treatment	*r*	*P*	t_1 (h)	a (h)	b
Urinary phosphate (mg 10)	TIZ 100 mg	0,995	<0,001	0,45	2,151	0,390
	FUR 80 mg	0,998	<0,001	0,45	2,176	0,409
	PLA	0,999	<0,001	0,70	2,408	0,413
Urinary creatinine (mg 10^{-1})	TIZ 100 mg	0,998	<0,001	0,15	1,339	0,399
	FUR 80 mg	0,998	<0,001	0,08	0,827	0,429
	PLA	0,999	<0,001	0,30	1,473	0,412
Urinary urea (mg)	TIZ 100 mg	0,999	<0,001	0,11	0,939	0,388
	FUR 80 mg	0,999	<0,001	0,07	0,613	0,418
	PLA	0,999	<0,001	0,16	0,975	0,394
Urinary urate (mg 10^{-1})	TIZ 100 mg	0,999	<0,001	0,04	0,782	0,346
	FUR 80 mg	0,999	<0,001	0,04	0,571	0,392
	PLA	0,999	<0,001	0,06	0,756	0,360

TIZ = tizolemide; FUR = furosemide; PLA = placebo.

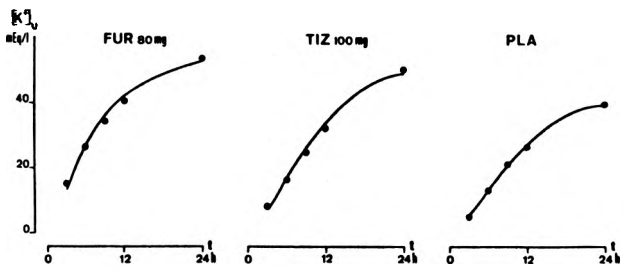

Fig. 1. Experimental mean values of accumulated urinary potassium as functions of time, after furosemide 80 mg, tizolemide 100 mg and placebo. The describing functions have been plotted.

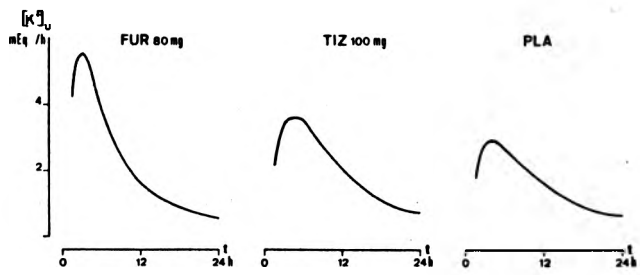

Fig. 2. Urinary potassium flows as functions of time, t, after furosemide 80 mg, tizolemide 100 mg and placebo. The functions have been derived from those in Fig. 1.

TABLE V. BLOOD VARIABLES IN 16 SUBJECTS BEFORE AND 6 AND/OR 24 HOURS AFTER TREATMENT WITH TIZOLEMIDE 100 mg, FUROSEMIDE 80 mg AND PLACEBO (MEAN ± SEM)

	Pretreatment			Post-treatment					
				6 h			24 h		
Variable	TIZ	FUR	PLA	TIZ	FUR	PLA	TIZ	FUR	PLA
Sodium (mEq/l)	140,5 ± 0,6	139,5 ± 0,7	14,1 ± 0,9	138,3 ± 0,8	139,4 ± 1,1	139,6 ± 0,8	138,3 ± 1,3	139,6 ± 1,1	139,7 ± 1,3
Potassium (mEq/l)	4,2 ± 0,08	4,11 ± 0,05	4,09 ± 0,09	3,96 ± 0,08	3,8 ± 0,08	4,16 ± 0,1	4,07 ± 0,11	4,06 ± 0,06	4,24 ± 0,1
Chloride (mEq/l)	102,2 ± 0,8	100,5 ± 0,8	101,2 ± 0,9	100,1 ± 0,9	99,0 ± 1,1	101,2 ± 0,6	97,7 ± 1,0	100,7 ± 1,0	100,8 ± 0,8
Calcium (mmol/l)	2,35 ± 0,02	2,33 ± 0,04	2,37 ± 0,03	2,41 ± 0,04	2,39 ± 0,05	2,37 ± 0,03	2,37 ± 0,03	2,36 ± 0,04	2,33 ± 0,03
Phosphate (mg/dl)	4,6 ± 0,23	4,43 ± 0,27	4,7 ± 0,33	4,83 ± 0,17	5,1 ± 0,19	4,88 ± 0,13	4,37 ± 0,2	4,14 ± 0,18	3,62 ± 0,17
Osmolality (mOsm/l)	288,9 ± 1,9	283,2 ± 2,4	289,0 ± 1,3	285,7 ± 2,0	287,8 ± 2,8	291,8 ± 2,0	281,3 ± 4,9	291,0 ± 2,3	288,8 ± 4,8
Creatinine (mg/dl)	1,04 ± 0,04	1,04 ± 0,03	1,01 ± 0,03	1,12 ± 0,03	1,17 ± 0,04	1,1 ± 0,04	1,14 ± 0,05	1,09 ± 0,03	1,05 ± 0,04
Urea (mg/dl)	25,2 ± 1,9	25,5 ± 1,6	26,9 ± 1,8	22,7 ± 2,0	23,6 ± 1,7	25,6 ± 1,7	26,0 ± 2,0	29,2 ± 2,3	25,3 ± 1,5
Urate (mg/dl)	5,24 ± 0,17	5,48 ± 0,16	5,41 ± 0,12	5,94 ± 0,16	6,5 ± 0,21	5,99 ± 0,25	6,07 ± 0,21	6,11 ± 0,3	5,36 ± 0,18
Cholesterol (mg/dl)	148,7 ± 8,3	153,1 ± 7,9	157,0 ± 6,5				164,9 ± 8,2	158,7 ± 7,4	155,7 ± 7,0
Triglycerides (mg/dl)	136,3 ± 29,3	11,8 ± 14,6	146,5 ± 25,0				111,5 ± 1,5	109,5 ± 16,1	136,7 ± 19,5
Bilirubin (mg/dl)	0,48 ± 0,05	0,49 ± 0,06	0,48 ± 0,05				0,44 ± 0,04	0,43 ± 0,04	0,45 ± 0,04
Alkaline phosphatase (U/l)	26,5 ± 1,7	28,2 ± 1,7	27,8 ± 1,6				29,4 ± 1,4	27,7 ± 1,9	26,7 ± 1,9
SGOT (U/l)	8,44 ± 1,13	7,81 ± 0,7	7,63 ± 0,65				7,88 ± 0,8	7,13 ± 0,88	6,63 ± 0,86

TIZ = tizolemide; FUR = furosemide; PLA = placebo.

Discussion and conclusions

Despite different sites of action within the nephron, tizolemide 100 mg and furosemide 80 mg had similar effects on the 24-hour urine volume and urinary outputs of potassium, chloride, calcium, phosphate, creatinine, urea and urate. Tizolemide had a greater natriuretic effect than furosemide.

The time-courses of several important functions were continuously described through a derivation from the mathematical model accounting for the post-dosing accumulated outputs. The Y (t) values are not statistically independent. In addition, their variances increase as functions of time because they are accumulated values which include all previous values. Therefore, two of the basic assumptions underlying standard correlation are not met by the experimental data and there is actual covariation. However, the present evaluation has the principal objective of deriving the flow functions which, upon detailed fractioning collections, have been shown to follow a pattern that is very well described by the fitted function. In so far as the evaluations of the a and b parameter values are satisfactorily discriminant when different medications are tried, the standard correlation and regression techniques have been used.

Lower parameter a values mean quicker time-courses of excretory activities, i.e. lower times to peak excretions after dosing, higher maximal flow values and lower after-effect flow values, sometimes with undershoots, all other parameters being equal.

Tizolemide resembles hydrochlorothiazide and xipamide rather than furosemide in time-courses of excretory activities.[3,4]

The fact that the serum potassium level fell significantly 6 hours after furosemide was administered could mean that the rate at which potassium was eliminated from plasma into urine after furosemide exceeded the rate at which it was correspondingly shifted from within the cells into the interstitial fluids.

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PIONEER

PAPER C7

Urinary Magnesium and Zinc Excretions After Monodosing Healthy Volunteers with Chlorthalidone

This provides new data on the effects of a well established diuretic. This was a collaborative project.

CURRENT
THERAPEUTIC
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URINARY MAGNESIUM AND ZINC EXCRETIONS AFTER MONODOSING HEALTHY VOLUNTEERS WITH CHLORTHALIDONE

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ABSTRACT

Accumulated excretions and flows of urinary volume, sodium, potassium, chloride, magnesium, and zinc are described as sigmoid functions of time and their time-derivatives respectively, after oral administration of 100 mg chlorthalidone to ten healthy volunteers. The effects of the medication on several blood variables are also evaluated.

Chlorthalidone provoked significant increases in mean volume, sodium, potassium, chloride, magnesium and zinc 24-hour and 36-hour urinary outputs with respect to mean 24-hour and 36-hour renal excretions without medication. The time-courses of the chlorthalidone-induced excretions of volume, potassium, sodium and chloride coincided (peak-flows around 4.5 hours post-dosing) whereas the urinary magnesium and zinc excretions after dosing proceeded at significantly slower rates (peak-flows around 10 and 8.5 hours post-dosing, respectively).

The only significant changes in 24-hour post-dosing blood variables with respect to the corresponding pre-dosing mean values were decreases in serum chloride and magnesium concentrations; mean serum sodium, potassium, glucose, uric acid and total bicarbonate values remained statistically unchanged.

The fact that urinary magnesium and zinc flows after chlorthalidone exhibited delayed time-courses with respect to those of other electrolytes suggests that endocrine mechanisms intervene in the determination of the hypermagnesiuric and hyperzinciuric responses to the distal tubule diuretic chlorthalidone.

The important magnesium losses provoked by chlorthalidone are of profound pathophysiological significance in the development of perilous cardiac arrhythmias until now ascribed to depletion of bodily potassium. The ultimate significance of the zinc losses provoked by chlorthalidone could only be speculated upon.

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INTRODUCTION

The time-courses of urinary volume and solute excretions after monodosing healthy volunteers with various diuretic formulations have been described recently through the fitting of a mathematical model to the experimental results.[1-8,12-19]

The objectives of this study are to evaluate the effect of the distal tubule diuretic chlorthalidone on 24-hour renal magnesium output and to describe the time-course of the post-dosing urinary magnesium excretion, in normal adults.

It is important to know the magnitude of the magnesiuric effect of chlorthalidone because magnesium losses induced by diuretics are presently considered to be the principal factor underlying perilous complications of diuretic therapy such as ventricular arrhythmias.[9-11,20] The time-course of urinary magnesium excretion after the administration of chlorthalidone is worth detailed investigation because magnesium flows after the administration of other diuretics do not follow the common pattern of other urinary solutes,[16-20] thus suggesting clues to the elucidation of the mechanisms underlying the hypermagnesiuric effects of these drugs.[16-20]

The effects of chlorthalidone on urinary zinc excretion may also be important given that other distal tubule diuretics, such as thiazides, provoke hyperzinciuria[21,22] and that symptoms of zinc deficiency such as sexual impotence, hypogeusia and hiposmia[23] are sometimes reported by patients undergoing chronic treatment with some diuretics (Reyes, unpublished).

SUBJECTS AND METHODS

Subjects and Experimental Design

Ten healthy caucasoid medical technologists or secretaries (three males and six women) volunteered to participate in this study after a full explanation of its implications had been given. All were aged between 21 and 46 years and had not taken any medication, supposedly acting in any way at the time of the experiments, within the previous two months. No volunteers were obese or had any history of renal, cardiovascular, endocrine or metabolic disorders.

A standardized diet was prescribed on control and trial days which contained around 100-125 mEq of sodium and 2000 ml of liquid. The ten subjects received chlorthalidone 100 mg *per os* on the treatment day. The medication was given at hour 08.00 with 100 ml tap water. Volunteers carried out their normal daily work but heavy muscular exercise and ingestion of alcohol or any medicine other than the trial formulation was forbidden on treatment and control days. Control days anteceded treatment days by 96 hours.

The laboratory analyses were carried out by technologists who were unaware of the protocol being used. All urines passed on treatment and control days were collected. Urines collected for 24 and 36 hours on control and following days were

pooled, whereas separate samples were collected at 3, 6, 12, 24 and 36 hours on treatment and following days. Urinary volume and contents of sodium, chloride, magnesium and zinc were measured in each plasma specimen by standard laboratory techniques (atomic absorption was used for magnesium and zinc evaluations). On treatment days venous blood was drawn just before medication and 6 and 24 hours later, for measurement of plasma concentrations of sodium, potassium, chloride, magnesium and total bicarbonate and of serum glucose and uric acid at hours 0 and 6.

Mathematical Methods

The mean experimental values of the urinary volume and solutes, Y, accumulated by the end of each post-dosing period, as functions of time, t, were fitted by a mathematical model[8]:

$$20Y / \log(100-Y) = \exp[[2.30(t-t_1)] / (a+bt)],$$

where t_1 is the time at which Y = 0.1 and a (time) and b (dimensionless) are parameters of the function. The fitting procedure has already been described.[8]

Flows of urinary variables were defined as:

$$dY/dt = (a+bt_1) / [(a+bt)^2 [(0.43/Y)+(0.43^2)/(100-Y)\log(100-Y)]].$$

Standard statistical techniques (paired t-test, linear correlation and regression) were used. Normality of frequency distributions and homoscedasticity of sample variances were evaluated through the chi square and the F tests, respectively.

RESULTS

Mean experimental values of urinary variables after dosing with chlorthalidone and with placebo are shown in Table I. Twenty-four hour

Table I — *Urinary excretory variables measured before (control period) and after dosing with 100 mg chlorthalidone per os in ten healthy volunteers. Values as means ± S.E.M..*

		Control Period		Post-Dosing Period	
Variable	Unit	0-24 hr	0-36 hr	0-24 hr	0-36 hr
Volume	l	1.368 ± 0.171	2.018 ± 0.184	2.308**** ± 0.257	2.997**** ± 0.325
Sodium	mmol	106.9 ± 14.3	155.1 ± 17.2	283.7***** ± 23.9	342.8***** ± 24.0
Chloride	mmol	126.6 ± 14.7	195.2 ± 21.1	317.0***** ± 21.0	404.4***** ± 22.5
Potassium	mmol	39.08 ± 2.93	60.70 ± 5.45	66.29**** ± 6.47	94.47**** ± 10.48
Magnesium(1)	mmol	2.69 ± 0.55	4.86 ± 0.91	5.03**** ± 0.62	7.34***** ± 0.72
Zinc	mcg	383.0 ± 62.6	686.73 ± 124.5	589.3* ± 108.4	909.5** ± 169.0

(1) Data from nine probands.
Significances of the differences with respect to the corresponding control period mean values:
* $p < 0.05$; ** $p < 0.02$; **** $p < 0.005$; ***** $p < 0.001$.

Figure 1 — *Mean accumulated excretions of several urinary variables after administration of 100 mg chlorthalidone per os to ten healthy probands. The Y(t) function has been fitted in all cases.*

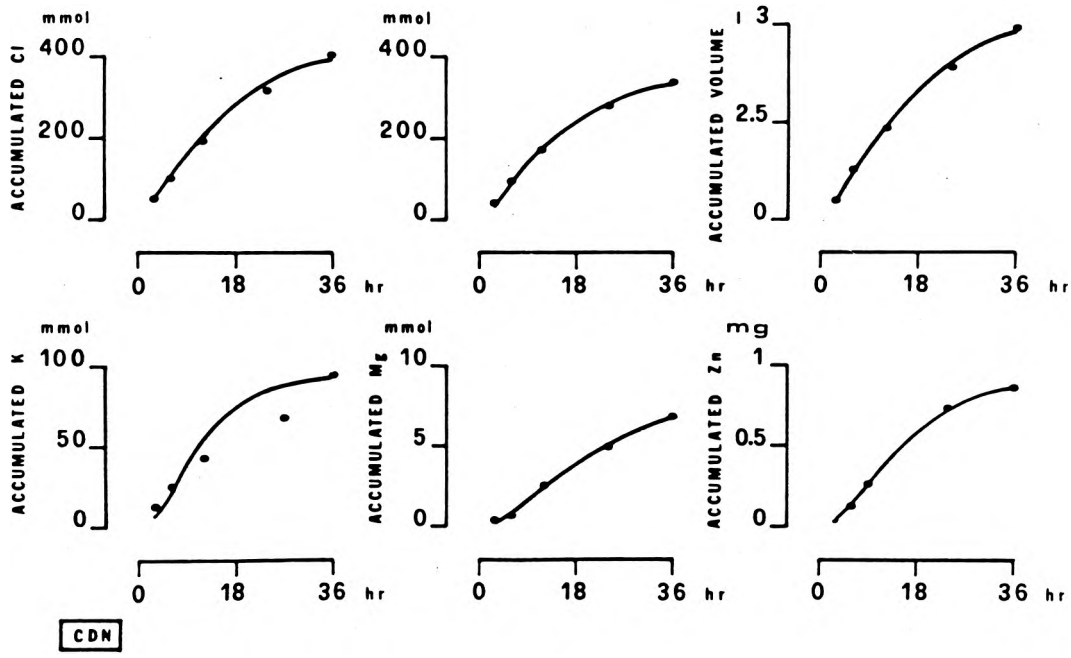


Table II — *Statistical and parametrical features of the linear transformations of the functions Y(t).*

Variable	Unit	r	p	t_1 (hr)	a (hr)	b
Volume	$l \times 10^{-1}$	0.9996	<0.001	0.08	0.9211	0.3747
Sodium	mmol x 10	0.9998	<0.001	0.06	0.8571	0.3651
Chloride	mmol x 10	0.9997	<0.001	0.05	0.7719	0.3569
Potassium	mmol	0.9948	<0.001	0	0.8695	0.2807
Magnesium	mmol $\times 10^{-1}$	0.9981	<0.001	0.06	1.2532	0.3013
Zinc	mg $\times 10^{-2}$	0.9953	<0.001	0	1.1188	0.2842

mean urine volume and urinary outputs of sodium chloride, potassium, magnesium and zinc after dosing with chlorthalidone were significantly higher than the control 24-hour and 36-hour excretions.

Mean urinary variable values accumulated after dosing as functions of time are shown in Figure 1, where the Y function has been fitted to all variables, after having evaluated the a and b parameters (Table II).

The time-courses of chlorthalidone-induced diuresis and flows of sodium, chloride and potassium resembled each other (peak-flows around 4.5 hours post-dosing), whereas the magnesium and the zinc flows after chlorthalidone proceeded at significantly slower rates (peak-flows around 10 and 8.5 hours post-dosing, respectively), as evaluated from the dY/dt functions. Figure 2 shows the time-courses of all

Figure 2 — *Mean flows of several urinary variables after administration of 100 mg chlorthalidone per os to ten healthy probands. The describing functions are the time-derivatives of those portrayed in Figure 1.*

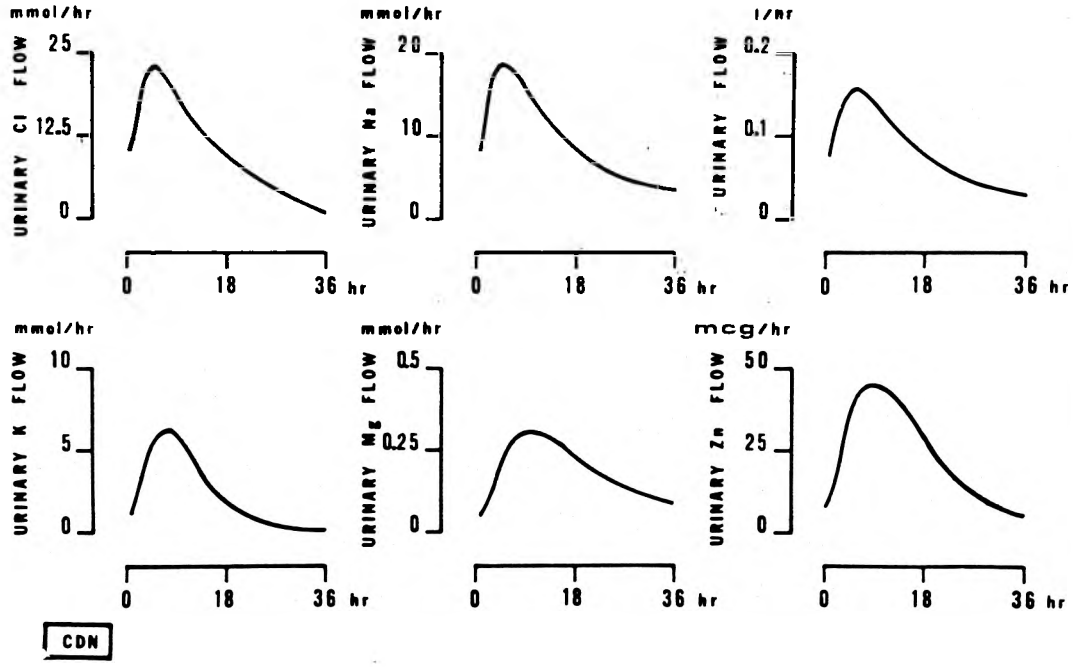


Table III — *Values of several serum variables after dosing with 100 mg chlorthalidone and with placebo in ten healthy volunteers. Values in mmol/l as means ± S.E.M.*

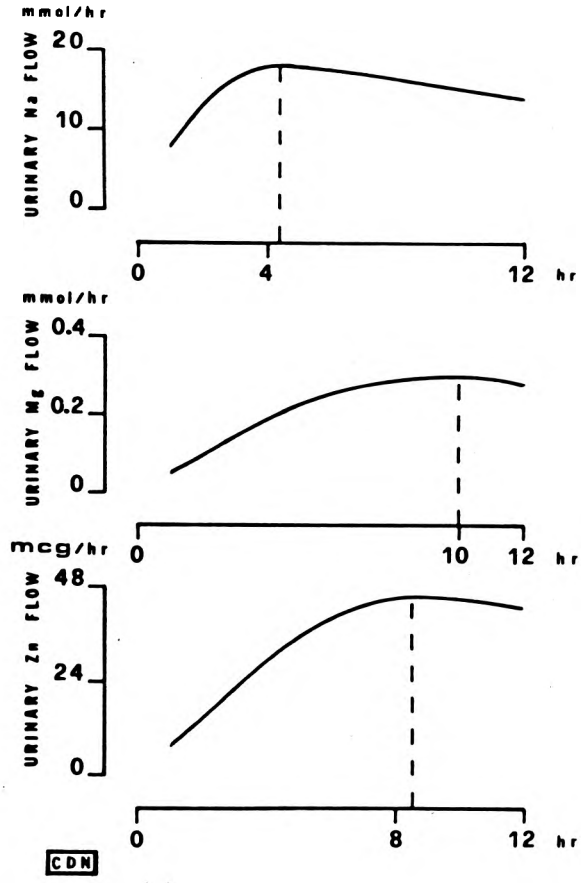
Variable	Hours Post-Dosing 0	12		24	
Sodium	144.0 ± 0.5	142.7 ± 0.6	($p < 0.05$)	143.3 ± 0.6	(N.S.)
Potassium	4.19 ±				
Sodium	144.0 ± 0.5	142.7 ± 0.6	($p < 0.05$)	143.3 ± 0.6	(N.S.)
Potassium	4.19 ± 0.11	3.75 ± 0.14	($p < 0.001$)	4.05 ± 0.13	(N.S.)
Chloride	108.9 ± 0.5	106.9 ± 0.6	($p < 0.01$)	107.0 ± 0.7	($p < 0.005$)
Magnesium	0.79 ± 0.01	0.80 ± 0.02	(N.S.)	0.75 ± 0.01	($p < 0.02$)
Glucose	4.95 ± 0.15			5.01 ± 0.15	(N.S.)
Uric acid	0.31 ± 0.03			0.34 ± 0.03	(N.S.)
Total CO_2	0.31 ±	28.1 ± 0.6	(N.S.)	27.7 ± 0.6	(N.S.)

N.S. : non-significant

urinary variables after chlorthalidone and Figure 3 describes the post-dosing flows of sodium, magnesium and zinc comparatively.

Mean values of blood variables before and after treatment are shown in Table III. Significant changes included decreases in serum chloride and potassium concentrations at hour 6 post-dosing and decreases in serum chloride and magnesium concentrations at hour 24 post-dosing.

Figure 3 — *Mean flows of urinary sodium, magnesium and zinc after administration of 100 mg chlorthalidone to ten healthy probands.*



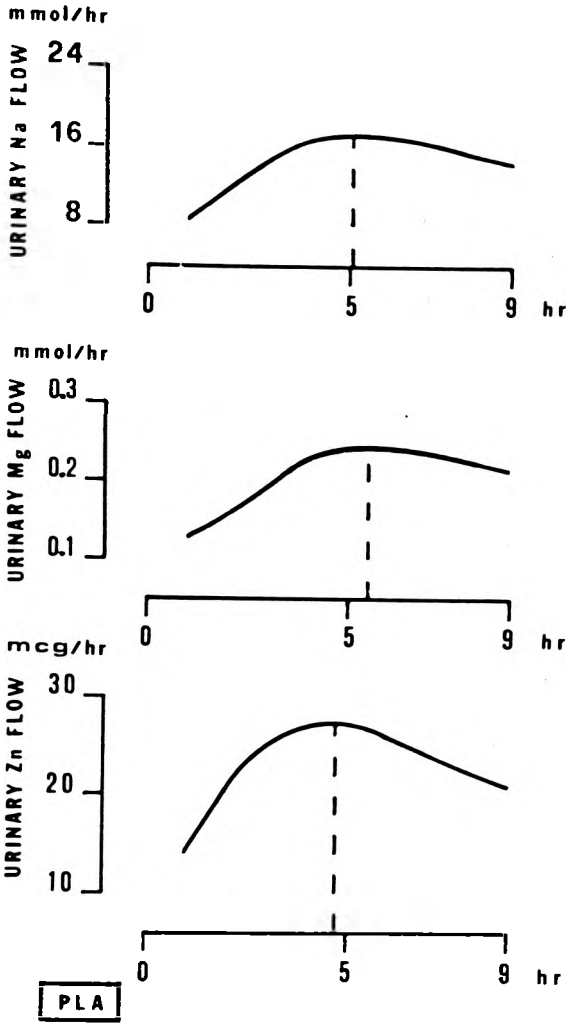
DISCUSSION

Critical descriptions of the mathematical methods used have been published.[8,12]

Chlorthalidone is a diuretic whose main site of renal activity is an acceptor located at the first portion of the distal convoluted tubule;[9-11] it therefore provokes increased urinary excretions of volume, sodium, chloride, potassium, magnesium and zinc that proceed non-abruptly. The rates of volume, sodium, chloride and potassium excretions are lower than those encountered in response to the distal tubule diuretics hydrochlorothiazide[7] and xipamide[3,7,17] lower than those after the loop diuretics furosemide[4,5,6,12,16] and piretanide[5,6] and higher than those after placebo.[4,7,12,19]

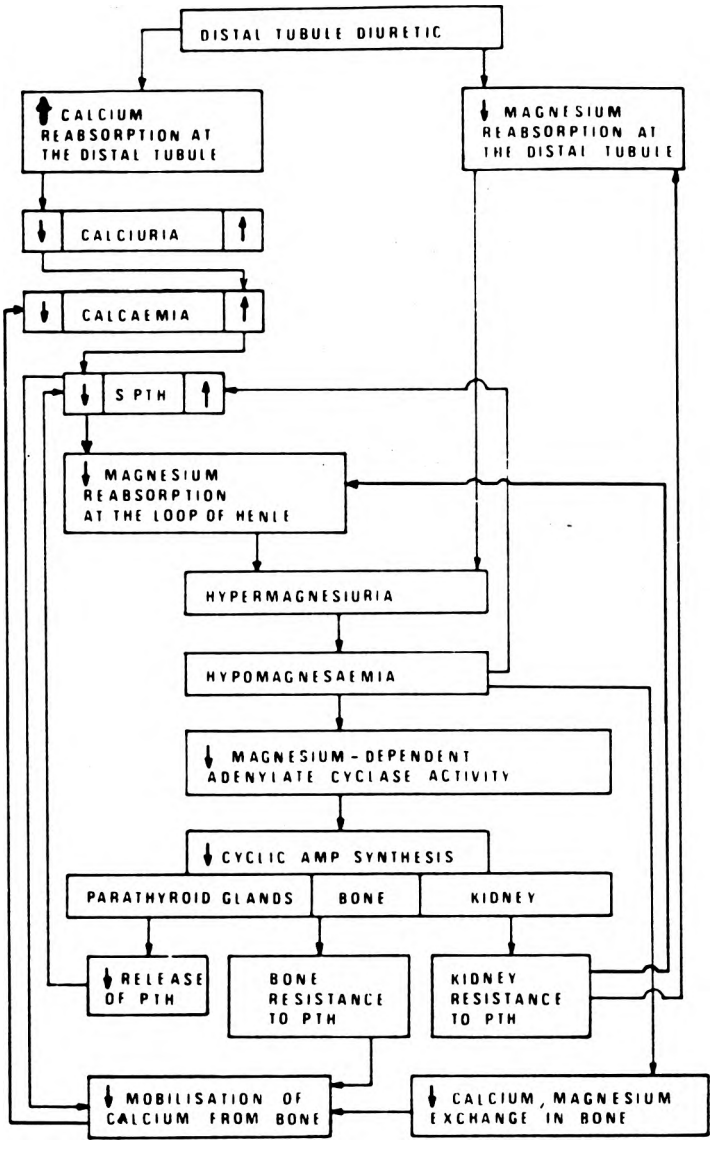
The fact that urinary magnesium excretion after chlorthalidone is significantly delayed with respect to volume, sodium, chloride and potassium excretions is consistent with the findings after all other distal

Figure 4 — *Mean flows of urinary sodium, magnesium and zinc after administration of placebo to ten healthy probands. From Reyes and Leary.*[18]



tubule diuretics studied in this respect[13,18-19] and differs from what has been found after the administration of placebo in similar experiments,[18] where the time-courses of all urinary solute excretions coincide at about hour 5 post-dosing, as illustrated in Figure 3. This may be due to the intervention of a relatively slow endocrine mechanism in the hypermagnesiuria that follows the administration of distal tubule diuretics.[19] Since reabsorption of magnesium in the distal tubule does not surpass 5 to 8% of the filtered ion, the direct blockade of this process by chlorthalidone would not suffice to account for the marked increase in magnesiuria induced by the drug. As a distal tubule diuretic, chlorthalidone induces a decrease in urinary calcium, an increase in serum calcium and a corresponding increase in serum parathormone

Figure 5 — *Mechanisms whereby loop diuretics provoke significant hypermagnesiuria.*



(sPTH) activity. Thus, since the reabsorption of magnesium at the loop of Henle, which accounts for 70% of the filtered ion, is under positive sPTH control, the delayed hypermagnesiuric effect of chlorthalidone could be a tardy consequence of its primary hypocalciuric action. Other concurrent mechanisms, such as an end-organ resistance to sPTH secondary to the negative rate of change in magnesaemia, could also account for the hypermagnesuria provoked by chlorthalidone[20] (Fig. 5).

The renal magnesium losses induced by chlorthalidone could be a most important factor in predisposing to the occurrence of perilous

cardiac arrhythmias since, during chronic treatment, these losses determine a decrease in intracellular magnesium concentration with a corresponding increase in intracellular free calcium concentration in the myocardium.[20]

Significant urinary zinc losses after the administration of chlorothiazide,[22] bendroflumethiazide, hydrochlorothiazide and chlorthalidone[21] have been described. The present finding of hyperzinciuria after the single doses of chlorthalidone is thus confirmatory. The physiological renal handling of zinc remains largely unknown so no mechanisms for the post-chlorthalidone hyperzinciuria could reasonably be postulated. However, no hyperzinciuria has been found after administrations of triamterene or the loop diuretics furosemide and bumetanide;[22] therefore, post-diuretic hyperzinciuria would only occur after medication with diuretics whose principal renal acceptors lie at the cortical diluting segment of the nephron.

The clinical significance of renal zinc losses induced by chronic zinc-losing diuretic medication is not apparent from available information. However, some early symptoms of bodily zinc depletion such as hyposmia, hypogeusia and impotence[23] have been found amongst patients treated with thiazides for hypertension (Reyes, unpublished).

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PAPER C8

The Magnesiuric Effect of a Single Dose of Furosemide in Healthy Adults

This was the first detailed analysis of the magnesiuric effect of furosemide published. It was a joint project with Professor A.J. Reyes and the findings have relevance to the chronic effects of this drug in the treatment of hypertension. Since the appearance of this publication reports have been published of cardiac arrhythmias developing in furosemide-treated patients and of their reversal by magnesium infusions.

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THE MAGNESIURIC EFFECT OF A SINGLE DOSE OF FUROSEMIDE IN HEALTHY ADULTS

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ABSTRACT

Accumulated excretions and flows of urinary volume, sodium, chloride and magnesium are described as sigmoid functions of time and their time-derivatives, respectively, after oral administration of 40 mg furosemide to nine healthy volunteers. The effects of the medication on several blood variables are also evaluated.

Furosemide provoked significant increases in mean volume, sodium, chloride and magnesium 24-hour urinary outputs compared to mean 24-hour control renal excretions without medication. The time-courses of the furosemide-induced excretions of volume, sodium and chloride coincided (peak-flows around 1 hour post-dosing), whereas the urinary magnesium excretion after dosing proceeded at a significantly slower rate (peak-flow around 3 hours post-dosing).

The only significant change in 24-hour post-dosing blood variables with respect to the corresponding pre-dosing mean value was an increase in serum uric acid; serum sodium, chloride, potassium, magnesium, total bicarbonate and glucose concentration values remained statistically equal.

The fact that urinary magnesium flow after furosemide exhibited a delayed time-course with respect to those of other electrolytes suggests that an endocrine mechanism intervenes in the determination of the hypermagnesiuric response to furosemide, in addition to whatever direct blockade of nephronal magnesium reabsorption this drug might provoke.

INTRODUCTION

The time-courses of the urinary excretions of volume and several solutes after monodosing healthy volunteers with various diuretic formulations have recently been described through the fitting of a mathematical model to the experimental results.[1-8,12-19]

Requests for reprints to Prof. Dr. A.J. Reyes, Holanda 1724, Montevideo, Uruguay.
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The objectives of this study are to evaluate the effect of the loop diuretic furosemide on 24-hour renal magnesium output and to describe the time-course of the post-dosing urinary magnesium excretion in normal adults. It is important to know the magnitude of the magnesiuric effect of furosemide because magnesium losses induced by diuretics are presently deemed to be the principal factor causing complications of diuretic therapy, such as muscular cramps and cardiac arrhythmias, that have been classically ascribed to hypokalaemia.[9-11,20]

The time-course of urinary magnesium excretion after dosing with furosemide is worth investigating because urinary magnesium flows after the administration of other diuretics have been found not to follow the common pattern of other urinary solutes, thus affording a clue to the elucidation of the mechanisms underlying the hypermagnesiuric effect of the drugs in question.[16-20]

SUBJECTS AND METHODS

Subjects and Experimental Design

Nine healthy Caucasoid medical technologists or secretaries (three male and six female) volunteered to participate in this study after a full explanation of its implications had been given. All were aged between 21 and 46 years and had not taken any medication, which would be active in any way at the time of the experiments, within the previous two months. No volunteers were obese or had any history of renal, cardiovascular, endocrine of metabolic disorders.

A standardized diet was prescribed on control and trial days containing approximately 140 mEq of sodium and 2000 ml of liquid. The nine subjects received furosemide 40 mg *per os* on treatment days. The medication was given with 100 ml tap water at 08.00 hr. Volunteers carried out their normal daily work but heavy muscular exercise and ingestion of caffeine, alcohol or any other medicine than the trial formulation were forbidden on treatment and control days. The control day preceded the treatment day by 24 hours.

The laboratory analyses were carried out by technologists who were unaware of the protocol being used. All urines passed on treatment and control days were collected. Urines collected during control days were pooled, whereas separate samples were collected at 3, 6, 12 and 24 hours on treatment days. Urinary volume and contents of sodium, chloride and magnesium were measured in each specimen. On treatment days venous blood was drawn just before medication and 6 and 24 hours later, for measurement of plasma concentrations of sodium, potassium, chloride, magnesium and total bicarbonate. Plasma glucose and uric acid concentrations were measured in blood samples withdrawn at hours 0 and 24. All variables were measured by standard Laboratory techniques using a Nova 4 analyser for sodium, chloride, potassium and bicarbonate determinations and a Varian 275 atomic absorption spectrophotometer to measure magnesium levels.

Mathematical Methods

The mean experimental values of the urinary volume and solutes, Y, accumulated by the end of each post-dosing period, as functions of time, t, fitted a mathematical

model[8]:

$$20Y / \log(100\text{-}Y) = \exp \left[[2.30(t\text{-}t_1)] / (a+bt) \right],$$

where t_1 is the time at which Y = 0.1 and a (time) and b (dimensionless) are parameters of the function. The fitting procedure has been described.[8]

Flows of urinary variables were defined as:

$$dY/dt = (a+bt_1) / \left\| (a+bt)^2 [(0.43/Y)+(0.43^2)/(100\text{-}Y)\log(100\text{-}Y)] \right\|.$$

Standard statistical techniques (paired t-test, linear correlation and regression) were used. Normality of frequency distributions and homoscedasticity of sample variances were evaluated through the chi square and the F tests, respectively.

Table I — *Urinary excretory variables measured during the collection periods after dosing with 40 mg furosemide in nine healthy volunteers. Values as means ± S.E.M..*

Variable	Unit	Post-dosing Period 0-3 hr	3-6 hr	6-12 hr	12-24 hr
Volume	litre	1.067 ± 0.140	0.800 ± 0.152	0.250 ± 0.023	0.504 ± 0.057
Sodium	mmol	100.2 ± 11.6	59.3 ± 13.5	10.7 ± 1.4	33.7 ± 7.2
Chloride	mmol	130.5 ± 15.5	78.5 ± 16.8	10.7 ± 1.1	22.4 ± 3.1
Magnesium	mmol	1.18 ± 0.09	0.86 ± 0.12	0.60 ± 0.09	1.72 ± 0.22

Table II — *24-hour urinary volume and solute excretions after dosing with 40 mg furosemide per os and during the previous 24-hours control. Values as means ± S.E.M..*

Renal Excretory Variable	Pre-treatment (24-hr Control)	Treatment (24-hr Post-Dosing)	Significances of the Differences Between Mean Values
Urinary volume (litres)	1.750 ± 0.195	2.621 ± 0.161	$p < 0.005$
Urinary sodium (mmol)	138.2 ± 16.5	203.9 ± 15.8	$p < 0.005$
Urinary chloride (mmol)	162.2 ± 23.0	242.1 ± 8.07	$p < 0.02$
Urinary magnesium (mmol)	2.90 ± 0.55	4.37 ± 0.25	$p < 0.05$

RESULTS

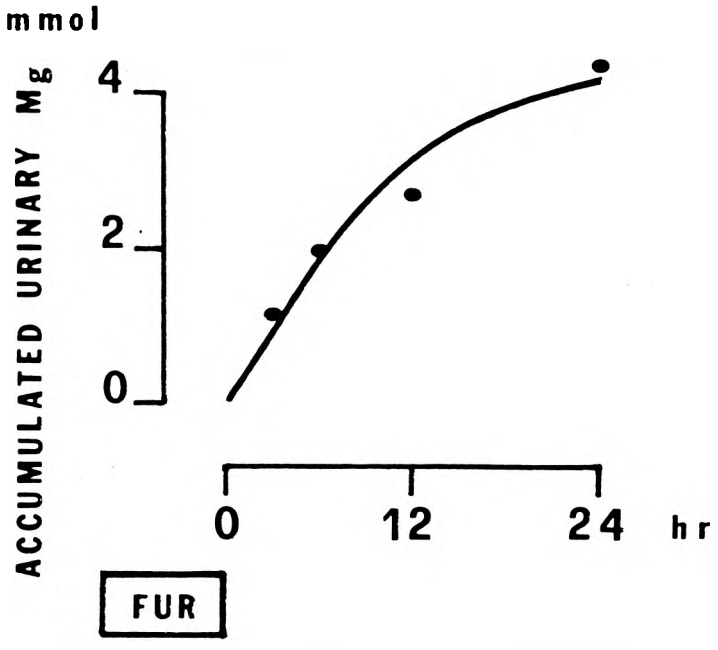
Mean experimental values of urinary variables after dosing are shown in Table I. Twenty-four-hour mean urine volume and urinary outputs of sodium, chloride and magnesium after dosing with furosemide were significantly higher than the control 24-hour excretions (Table II).

Mean urinary variable values accumulated after dosing as functions of time are parametrically described in Table III and the Y function for magnesium is shown in Figure 1. The time-courses of the hydrochloro-

Table III — *Statistical and parametrical features of the linear transformations of the functions Y (t).*

Variable	Unit	r	p	t_1 (hr)	a(hr)	b
Volume	$l \times 10^{-1}$	0.9999	<0.001	0	0.2736	0.3979
Sodium	mmol x 10	0.9999	<0.001	0	0.2278	0.4203
Chloride	mmol x 10	0.9999	<0.001	0	0.1555	0.4083
Magnesium	mmol $\times 10^{-1}$	0.9993	<0.001	0	0.4630	0.3545

Figure 1 — *Mean urinary excretions of magnesium in nine normal probands accumulated after medication with 40 mg furosemide per os at hour 0. The Y(t) function has been fitted to the experimental data.*



thiazide-induced diuresis and flows of sodium and chloride were alike (peak-flows around 1 hour post-dosing), whereas the magnesium flow after furosemide proceeded at a significantly slower rate (peak-flow around 3 hours post-dosing), as evaluated from the dY/dt functions.

Figure 2 — *Mean urinary sodium and magnesium flows in nine healthy volunteers after medication with 40 mg furosemide per os at hour 0. The describing functions are the time-derivatives of the corresponding Y(t) functions.*

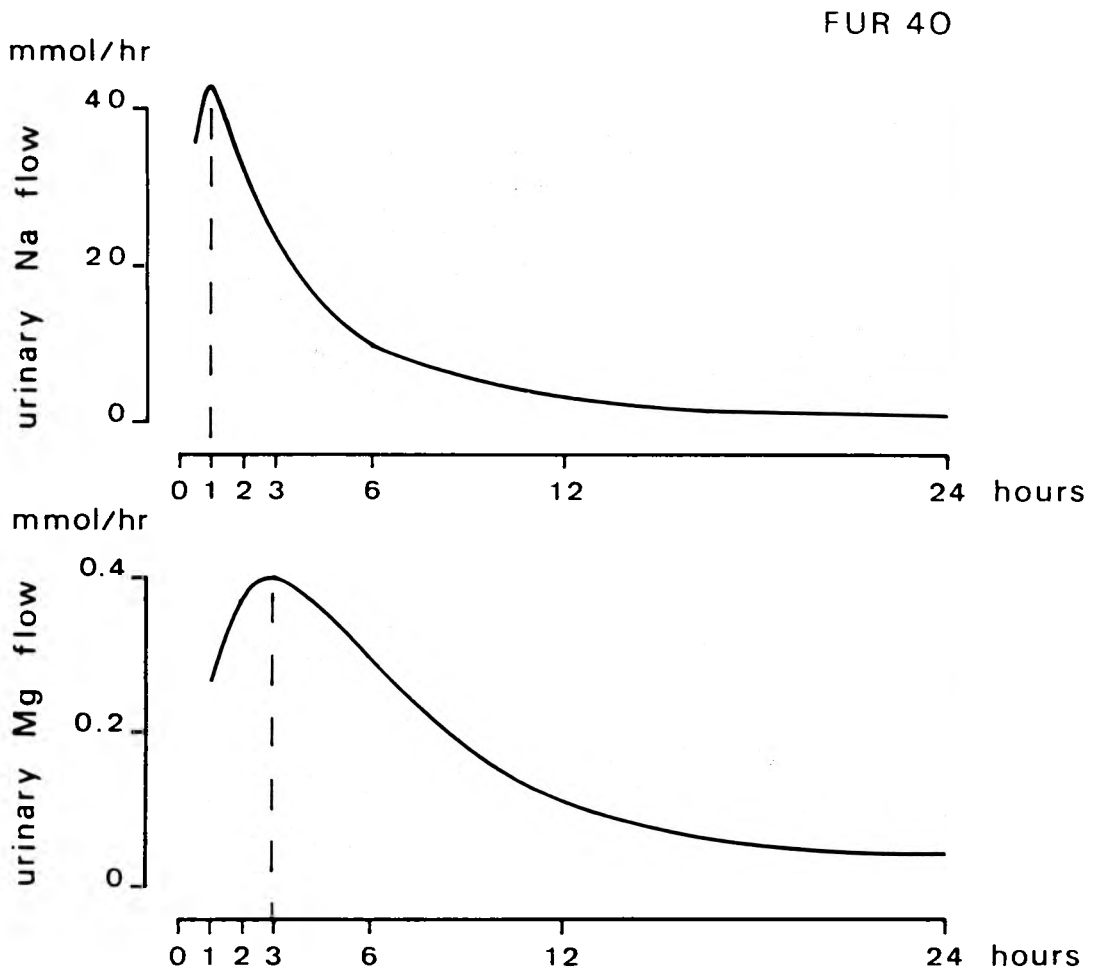


Figure 2 shows the time-courses of urinary sodium and magnesium excretions after dosing with furosemide.

Mean values of blood variables before and after treatment are shown in Table IV. Significant changes included increases in serum sodium and total bicarbonate concentrations and decreases in serum potassium and chloride and total bicarbonate concentrations at hour 6 after dosing and an increase in plasma uric acid concentration 24 hours after medication.

Table IV — *Values of several serum variables after dosing with 40 mg furosemide and with placebo in nine healthy volunteers. Values in mmol/l as means ± S.E.M..*

Variable	Hours Post-Dosing 0	6	24
Sodium	138.4 ± 1.1	141.5 ± 0.7 [2] ($p < 0.05$)	139.3 ± 0.9 [2] (N.S.)
Potassium	4.22 ± 0.10	3.93 ± 0.15[2] ($p < 0.05$)	4.08 ± 0.18[2] (N.S.)
Chloride	103.5 ± 1.2	99.8 ± 0.6 [2] ($p < 0.01$)	100.1 ± 0.5 [2] (N.S.)
Magnesium	0.80 ± 0.04	0.80 ± 0.02[2] (N.S.)	0.79 ± 0.02[2] (N.S.)
Glucose	5.09 ± 0.25		5.16 ± 0.14[2] (N.S.)
Uric acid	0.25 ± 0.02		0.30 ± 0.03[2] ($p < 0.005$)
Total CO_2	23.4 ± 0.6	26.0 ± 0.6 [2] ($p < 0.005$)	24.4 ± 0.7 [2] (N.S.)

[2] Data from eight volunteers

The p values indicate the significance of the differences with respect to hr 0 — mean values.
N.S. = non significant.

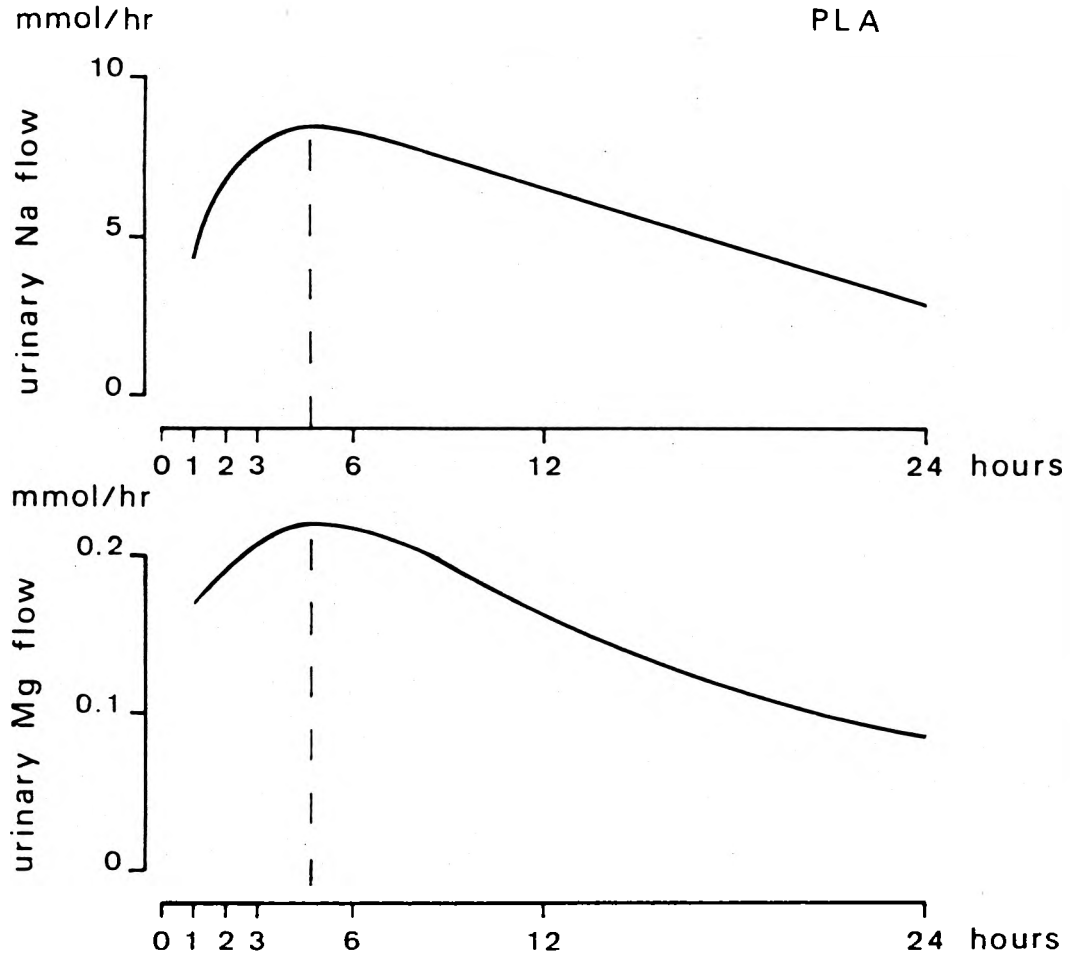
DISCUSSION

Critical descriptions of the mathematical methods used have been published.[8,12]

Furosemide is a diuretic whose main site of renal activity is an acceptor located at the thick portion of the ascending limb of the loop of Henle;[9-11] it therefore provoked increased urinary excretions of volume, sodium and chloride that proceeded abruptly. The rates of volume, sodium and chloride excretions resemble those encountered after the loop diuretics furosemide 80 mg[4,5,6,12,16] and piretanide[5,6] and are higher than those after the distal tubule diuretics hydrochlorothiazide,[7] xipamide,[3,7,17] chlorexolone[18] and chlorthalidone[19] or after the administration of placebo.[4,7,12,19]

The fact that magnesium excretion after furosemide is significantly delayed with respect to the volume, sodium and chloride excretions differs from what is found after the administration of placebo in similar experiments,[18] when the time-courses of all urinary solute excretions coincide, as illustrated by Figure 3. This is possibly due to the intervention of a relatively slow endocrine mechanism in the hypermagnesiuria that follows the administration of distal tubule diuretics.[19,20] The hypercalciuria elicited by furosemide induces an increase in serum parathyroid hormone (sPTH) activity, which in turn tends to reduce the effect of furosemide on urinary magnesium reabsorption because magnesium reabsorption at the loop of Henle is under positive sPTH con-

Figure 3 — *Mean urinary sodium and magnesium flows from nine healthy volunteers after administration of placebo per os at hour 0. The describing functions are the time-derivatives of the Y(t) functions fitted to the experimental data in Reyes and Leary.*[17]



trol. However, the primary negative rate of change of serum magnesium concentration caused by the hypermagnesuria induced by furosemide gives place to a series of events, including an end-organ resistance to sPTH, that reinforce the renal magnesium losses determined by the direct blockade of magnesium reabsorption provoked by furosemide[20] (Fig. 4).

The renal magnesium losses induced by furosemide may well be a most important factor in the development of potentially lethal arrhythmias since, during chronic treatment, such losses determine a decrease in

the intracellular magnesium concentration with a corresponding increase in the intracellular free calcium concentration in the myocardium,[20] as shown in Figure 5.

The changes encountered in blood solute concentrations after the administration of furosemide correspond to what could be expected upon the basis of established knowledge.

Figure 4 — *Orientated graph showing the mechanisms whereby there is an increased urinary magnesium excretion after a loop diuretic such as furosemide.*

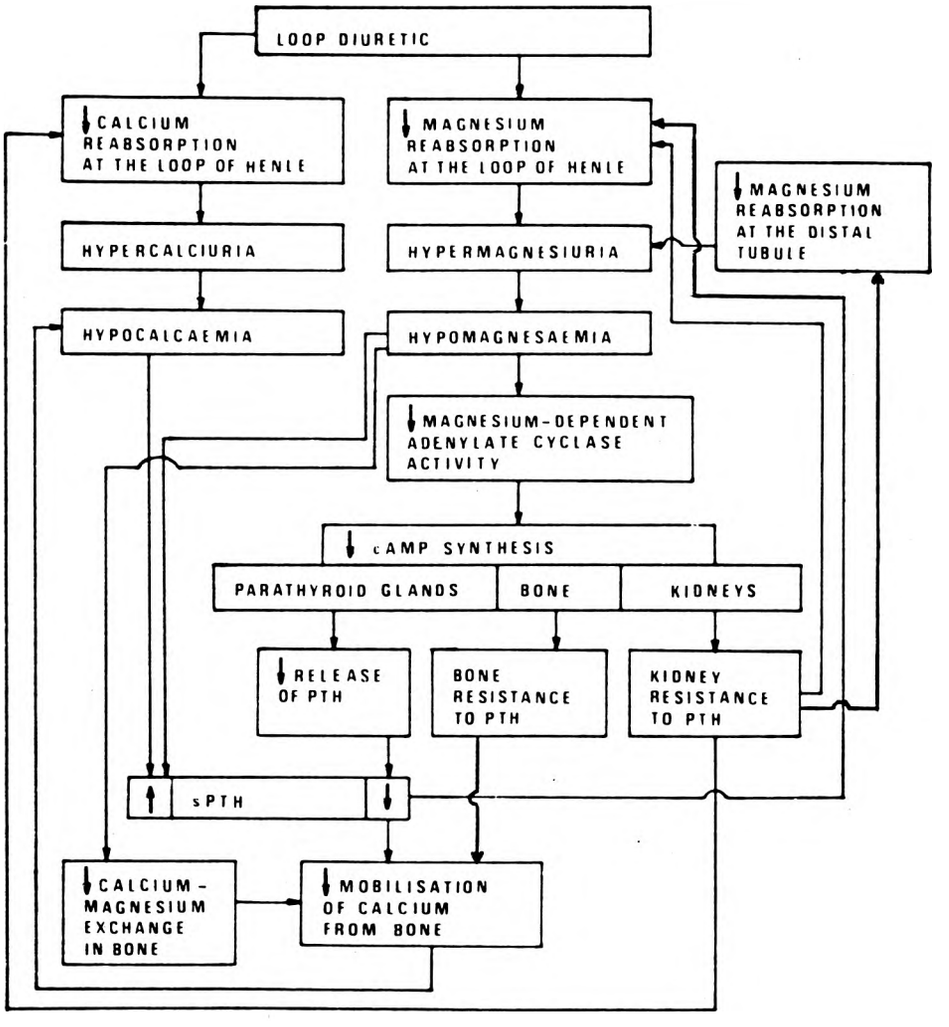
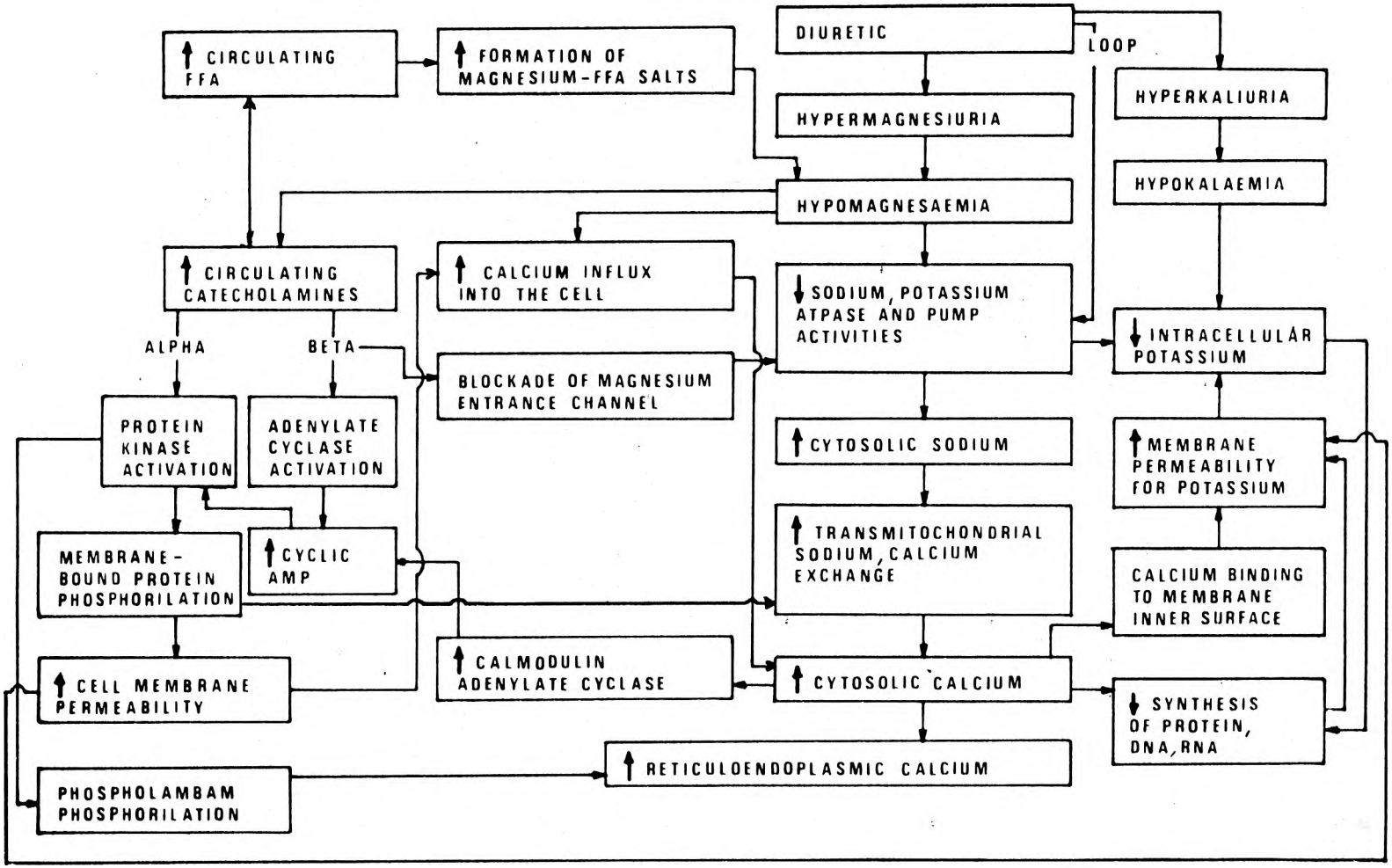


Figure 5 — *Orientated graph showing the mechanisms whereby diuretic-induced hypermagnesiuria conduces to a decrease in intracellular magnesium and to an increase in free intracellular calcium.*



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PAPER C9

The Magnesiuric Effect of a Single Dose of Hydrochlorothiazide in Healthy Adults

This was the first detailed analysis of the magnesiuric effect of hydrochlorothiazide. It was another joint project with Professor A.J. Reyes and the findings have relevance to the chronic effects of this drug in the treatment of hypertension.

CURRENT
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THE MAGNESIURIC EFFECT OF A SINGLE DOSE OF HYDROCHLOROTHIAZIDE IN HEALTHY ADULTS

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ABSTRACT

Accumulated excretions and flows of urinary volume, sodium, chloride and magnesium are described as sigmoid functions of time and their time-derivatives, respectively, after oral administration of 50 mg hydrochlorothiazide to nine healthy volunteers. The effects of the medication on several blood variables are also evaluated.

Hydrochlorothiazide provoked significant increases in volume, sodium, chloride, and magnesium mean 24-hour urinary mean outputs with respect to 24-hour renal excretions without medication. The time-courses of the hydrochlorothiazide-induced excretions of volume, sodium and chloride coincided (peak flows around 3.5 hours post-dosing), whereas the urinary magnesium excretion after dosing proceeded at a significantly slower rate (peak flow around 6 hours post-dosing).

The only significant changes in 24-hour post-dosing blood variables with respect to the corresponding pre-dosing mean values were a decrease in serum chloride and an increase in total plasma bicarbonate. Serum sodium, potassium and magnesium values remained statistically unchanged.

The fact that urinary magnesium flow after hydrochlorothiazide exhibited a delayed time-course with respect to those of other electrolytes suggests that an endocrine mechanism intervenes in the determination of the hypermagnesiuric response to the distal tubule diuretic hydrochlorothiazide.

INTRODUCTION

The time-courses of the urinary excretions of volume and several solutes after monodosing healthy volunteers with various diuretic formulations have recently been described through the fitting of a mathematical

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model to the experimental results.[1-8,12-19]

The objectives of this study are to evaluate the effect of the distal tubule diuretic hydrochlorothiazide on 24-hour renal magnesium output and to describe the time-course of the post-dosing urinary magnesium excretion, in normal adults.

SUBJECTS AND METHODS

Subjects and Experimental Design

Nine healthy caucasoid medical technologists or secretaries (three males and six females) volunteered to participate in this study after a full explanation of its implications had been given. All were aged between 21 and 46 years and had not taken any medication, which might act in any way at the time of the experiments, within the previous two months. No volunteers were obese or had any history of renal, cardiovascular, endocrine or metabolic disorders.

A standard diet was prescribed on control and trial days containing approximately 140 mEq of sodium and 2000 ml of fluid. The nine subjects received hydrochlorothiazide 50 mg *per os* given with 100 ml tap water at 08.00 hour on the treatment days. Volunteers carried out their normal daily work but heavy muscular exercise, tobacco and the ingestion of alcohol, caffeine or any medicine other than the trial formulation were forbidden on treatment and control days. The control day anteceded the treatment day by 24 hours.

The laboratory analyses were carried out by technologists who were unaware of the protocol being used. All urines passed on treatment and control days were collected. Urines collected during control days were pooled, whereas separate samples were collected at 3, 6, 12 and 24 hours on treatment days. Urinary volume and contents of sodium, chloride and magnesium were measured in each urine specimen. On treatment days venous blood was drawn just before medication and 6 and 24 hours later for measurement of plasma concentrations of sodium, potassium, chloride, magnesium and total bicarbonate and of serum glucose and uric acid at hours 0 and 6. All variables were measured by standard laboratory techniques using a Nova 4 analyser for determinations other than magnesium levels, which were measured on a Varian 275 atomic absorption spectrophotometer.

Mathematical Methods

The mean experimental values of the urinary volume and solutes, Y, accumulated by the end of each post-dosing period, as functions of time, t, were fitted a mathematical model[8]:

$$20Y / \log(100-Y) = \exp [[2.30 (t-t_1)] / (a+bt)],$$

where t_1 is the time at which $Y = 0.1$ and a (time) and b (dimensionless) are parameters of the function. The fitting procedure has been already described.[8]

Flows of urinary variables were defined as:

$$dY/dt = (a+bt_1) / [(a+bt)^2 [(0.43/Y)+(0.43^2)/(100-Y)\log(100-Y)]].$$

Standard statistical techniques (paired, t-test, linear correlation and regression) were used. Normality of frequency distributions and homoscedasticity of sample variances were evaluated through the chi square and the F tests, respectively.

Table I — *Urinary excretory variables measured during the collection periods after dosing with 50 mg hydrochlorothiazide in nine healthy volunteers. Values as means ± S.E.M..*

Variable	Unit	Post-dosing Periods 0-3 hr	3-6 hr	6-12 hr	12-24 hr
Volume	litres	0.455 ± 0.086	0.392 ± 0.050	0.547 ± 0.066	0.968 ± 0.124
Sodium	mmol	42.3 ± 2.3	36.2 ± 6.0	62.1 ± 9.3	85.4 ± 6.5
Chloride	mmol	53.0 ± 8.1	41.6 ± 7.1	77.6 ± 10.0	85.8 ± 6.6
Magnesium	mmol	0.50 ± 0.09	0.47 ± 0.09	1.02 ± 0.05	2.18 ± 0.27

RESULTS

Mean experimental values of urinary variables after dosing are shown in Table I. Twenty-four-hour mean urine volume and urinary outputs of sodium, chloride and magnesium after dosing with hydrochlorothiazide were significantly higher than the control 24-hour excretions (Table II).

Mean urinary variable values accumulated after dosing as functions of time are parametrically described in Table III and the Y function for magnesium is shown in Figure 1. The time-courses of the hydrochloro-

Table II — *24-hour urinary volume and solute excretions after dosing with 50 mg hydrochlorothiazide per os and during 24-hour previous control. Values as means ± S.E.M..*

Renal Excretory Variable	Pre-treatment (24-hr Control)	Treatment (24-hr Post-dosing)	Significances of the Differences Between Mean Values
Urinary volume (litres)	1.750 ± 0.195	2.320 ± 0.279	$p < 0.05$
Urinary sodium (mmol)	138.2 ± 16.5	222.0 ± 20.0	$p < 0.005$
Urinary chloride (mmol)	162.2 ± 23.0	258.0 ± 21.3	$p < 0.05$
Urinary magnesium (mmol)	2.90 ± 0.55	4.17 ± 0.40	$p < 0.05$

Table III — *Statistical and parametrical features of the linear transformations of the functions Y (t).*

Variable	Unit	r	p	t_1 (hr)	a(hr)	b
Volume	$l \times 10^{-1}$	0.9995	<0.001	0.06	0.7095	0.3890
Sodium	mmol x 10	0.9997	<0.001	0.06	0.7283	0.3910
Chloride	mmol x 10	0.9998	<0.001	0.05	0.6375	0.3837
Potassium	mmol	0.9997	<0.001	0	0.5001	0.3411
Magnesium	mmol x 10^{-1}	0.9988	<0.01	0	0.9021	0.3401

Figure 1 — *Mean values of the accumulated urinary excretions of magnesium after dosing with 50 mg hydrochlorothiazide in nine healthy volunteers. The Y(t) functions have been fitted in the experimental data.*

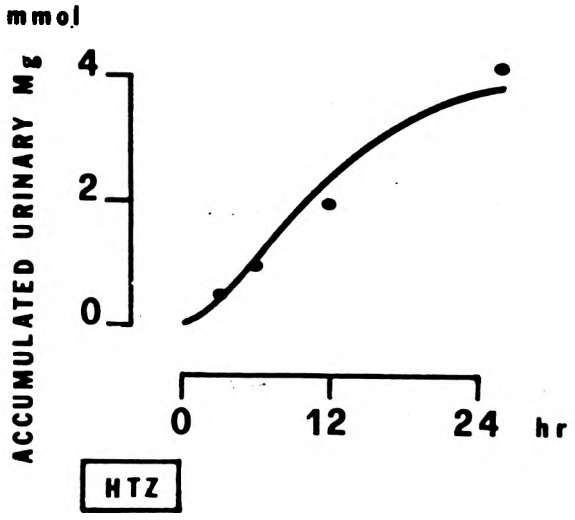
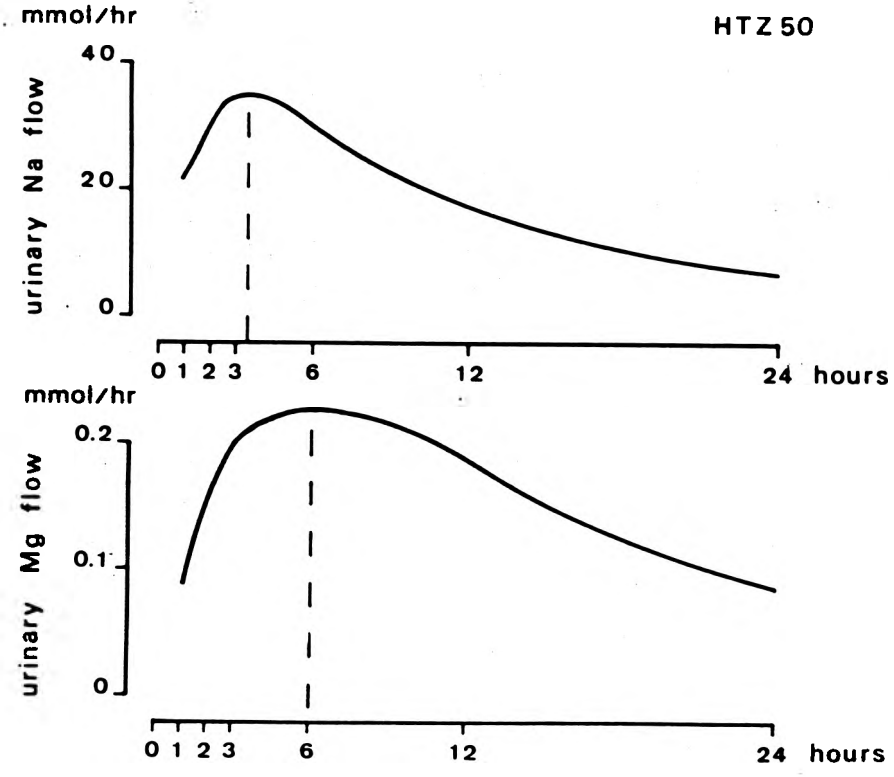


Figure 2 — *Urinary flows of sodium and magnesium after dosing with 10 mg furosemide in nine healthy volunteers. The plotted functions are the time-derivatives of the corresponding Y(t) functions.*



thiazide-induced diuresis and flows of sodium and chloride were alike (peak flows around 3.5 hours post-dosing), whereas the magnesium flow after hydrochlorothiazide proceeded at a significantly slower rate (peak flow around 6 hours post-dosing), as evaluated from the dY/t functions. Figure 2 shows the time-courses of urinary sodium and magnesium excretions after dosing with hydrochlorothiazide.

Mean values of blood variables before and after treatment are shown in Table IV. Significant changes included decreases in serum

Table IV — *Values of several serum variables after dosing with 50 mg hydrochlorothiazide and with placebo in nine healthy volunteers. Values in mmol/l as means ± S.E.M.*

Variable	Hours Post-dosing 0	6	24
Sodium	143.8 ± 2.0	136.5 ± 0.9 ($p < 0.02$)	138.7 ± 1.5 (N.S.)
Potassium	4.31 ± 0.13	3.94 ± 0.08 ($p < 0.02$)	4.22 ± 0.14 (N.S.)
Chloride	108.8 ± 0.6	103.7 ± 0.9 ($p < 0.001$)	103.7 ± 0.7 ($p < 0.001$)
Magnesium	0.80 ± 0.02	0.80 ± 0.02 (N.S.)	0.80 ± 0.02 (N.S.)
Glucose	5.49 ± 0.21		5.33 ± 0.26 (N.S.)
Uric acid	0.25 ± 0.02		0.28 ± 0.02 (N.S.)
Total CO_2	24.7 ± 0.7	26.8 ± 0.5 ($p < 0.005$)	26.3 ± 0.8 ($p < 0.01$)

* Data from ten volunteers.
The p values indicate the significance of the differences with respect to hr 0 — mean values.
N.S. = non significant.

sodium and potassium concentrations at hour 6 post-dosing, increases in serum bicarbonate concentration at hours 6 and 24 post-dosing and decreases in serum chloride concentration at 6 and 24 hours after medication.

DISCUSSION

Critical descriptions of the mathematical methods have been published.[8,12]

Hydrochlorothiazide is a diuretic whose main site of renal activity is an acceptor located at the first portion of the distal convoluted tubule;[9-11] it therefore provokes increased urinary excretions of volume, sodium, chloride, and magnesium that proceed non-abruptly. The rates of volume, sodium, and chloride excretions are similar to those that have been encountered after hydrochlorothiazide 100 mg[7] and xipamide,[3,7,17] lower than those after the loop diuretics furosemide[4,5,6,12,16] and piretanide[5,6] and higher than those after placebo[4,7,12,19] and the distal tubule diuretics chloreoxolone[18] and chlorthalidone.[19]

The fact that urinary magnesium excretion after hydrochlorothiazide was significantly delayed with respect to the volume, sodium and chloride excretions resembles what has been found after all the other distal tubule diuretics studied in this respect.[13,17-19] This fact is possibly due to the intervention of a relatively slow endocrine mechanism in the genesis of the hypermagnesiuria that follows the administration of distal tubule diuretics.[19] In so far as distal tubule reabsorption of magnesium only affects 5-8% of the filtered ion, the direct blockade of this process by hydrochlorothiazide cannot account for the marked increase in magnesiuria induced by the drug. Hydrochlorothiazide reduces urinary calcium excretion causing an increase in serum calcium and a corresponding decrease in serum parathormone (sPTH) activity. As the reabsorption of magnesium at the loop of Henle, which accounts for 70% of the filtered ion, is under positive sPTH control, the hypermagnesiuric effect of hydrochlorothiazide could follow its primary hypocalciuric action.

The changes in blood variables after medication with hydrochlorothiazide were those that could be expected on the basis of established knowledge.

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PAPER C10

Urinary Magnesium and Zinc Excretions After Two Different Single Doses of Amiloride in Healthy Adults

This study was of particular interest since it investigated the claim that Amiloride is a magnesium-sparing diuretic. The findings stimulated a number of subsequent research projects in various laboratories. Mr. K. van der Byl was included as an author in recognition of his assistance with computer facilities.

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URINARY MAGNESIUM AND ZINC EXCRETIONS AFTER TWO DIFFERENT SINGLE DOSES OF AMILORIDE IN HEALTHY ADULTS

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ABSTRACT

Accumulated excretions and flows of urinary volume, sodium, potassium, chloride, magnesium and zinc are described as functions of time and their time-derivatives, respectively, after oral administration of placebo, amiloride 5 mg and amiloride 10 mg to thirteen healthy volunteers. The effects of the treatments on several blood variables are also evaluated.

Both doses of amiloride provoked significant increases in mean 24-hour urinary volume and sodium outputs compared to the corresponding excretions after placebo. The 24-hour urinary excretion of potassium was significantly reduced by both doses of amiloride and that of zinc was significantly decreased by the higher dose. Magnesium output was unaffected by amiloride. The time-courses of urinary volume, sodium and potassium flows after amiloride were accelerated by comparison with the corresponding events after placebo, whereas the time-courses of chloride, magnesium and zinc urinary excretions were not significantly altered by amiloride. The fact urinary magnesium flow was unaltered by amiloride would indicate the drug did not affect the tubular handling of the cation.

The effects of amiloride on the blood variables measured were unremarkable.

INTRODUCTION

Urinary potassium losses caused by loop and distal tubule diuretics such as furosemide and the thiazides, respectively, have been of concern to clinicians largely because subsequent depletion of intracellular potassium may cause electrical destabilisation of the sarcolemma and serious cardiac arrhythmias.[1-5] The coprescription of potassium-sparing diuretics such as

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amiloride, triamterene, and spironolactone with common diuretics is used as a therapeutic manoeuvre for limiting urinary potassium losses; however, it does not influence the hypermagnesiuria and consequent depletion of bodily magnesium which also results from the use of diuretics.[1-3,6-11] Since magnesium is a cofactor for the myocardial Na^+, K^+ -ATPase responsible for the active incorporation of potassium into cells, intramyocardial potassium concentration may remain abnormally low when magnesium is depleted despite normal serum potassium levels.[6] In addition, other changes encouraging arrhythmias such as increased intramyocardial sodium and free cytosolic calcium levels develop in association with magnesium depletion.[10] Thus, simple measures which conserve or replace extracellular potassium may not be adequate to prevent cardiac arrhythmias complicating treatment with diuretics.[12]

Common distal tubule diuretics increase zinc excretion and a syndrome of zinc depletion with clinical manifestations such as impotence, impaired senses of taste and smell and retarded wound-healing may develop during treatment with these preparations.[13]

This study evaluates the effects of separate single doses of amiloride (5, 10 mg) on the urinary excretions of magnesium and zinc in healthy volunteers.

SUBJECTS AND METHODS

Subjects and experimental design

Thirteen healthy male Caucasoid medical technologists or students volunteered to participate in this study after a full explanation of its implications had been given. All were aged between 20 and 45 years and had taken no other medication within the previous two months. None were obese or had any history of renal, cardiovascular, endocrine or metabolic disorders. Smokers were not studied.

A standardized diet containing 130 to 150 mEq of sodium and 2000 ml of liquid was prescribed on "treatment" days and on the previous 24 hours "control" days. Subjects received placebo, 5 mg amiloride, and 10 mg amiloride separately and in random order on three different "treatment days", separated by at least 96 hours. Medications were given at 0800 hr with 100 ml tap water. Volunteers carried out their normal daily work but heavy muscular exercise and ingestion of alcohol, caffeine or any medicine other than the trial formulation was forbidden on treatment days.

All laboratory analyses were carried out by technologists who were kept unaware of the protocol being used. All urines passed on treatment and control days were collected. Urine samples were collected at 3, 6, 12, and 24 hours on treatment days. Urinary volume and contents of sodium, potassium, chloride, magnesium and zinc were measured in each urine specimen by standard laboratory techniques (flame photometry was used for sodium, potassium and chloride evaluations and atomic absorption for magnesium and zinc evaluations). On treatment days venous blood was drawn just before medication and 6±1 and 24±1 hours later, for measurement of plasma concentrations of sodium, potassium, chloride, total bicarbonate and magnesium. Serum glucose and uric acid were measured at 0 and 24 ± 1 hours after dosage.

Mathematical methods

The mean experimental values of the urinary volume and solutes, Y, accumulated by the end of each post-dosing period, as functions of time, t, were fitted by a mathematical model:[14]

$$20Y/\log(100\text{-}Y) = \exp\{[2.30(t\text{-}t_1)]/(a+bt)\},$$

where t_1 is the time at which Y=0.1 and a (time) and b (dimensionless) are parameters of the function. The fitting procedure has been described previously.[14]

Flows of urinary variables were defined as:

$$dY/dt=(a+bt_1)/\{(a+bt)^2[(0.43/Y)+(0.43^2)/(100\text{-}Y)\log(100\text{-}Y)]\}.$$

Standard statistical parametrical techniques (paired t-test, linear correlation and regression) were used. Normality of frequency distributions and homoscedasticity of sample variances were evaluated through the chi square and the F test, respectively. However, the non-parametrical sign test was used for evaluating the significance of the differences between the post-amiloride and the post-placebo 24-hour urinary zinc outputs, since no evidence exists that the variable is normally distributed. All tests were two-tailed and p=0.05 was considered the limit of significance.

RESULTS

No significant difference was detected between the 24-hour accumulated urinary outputs of volume and electrolytes after placebo and those yielded on control days.

The timed urinary excretions of volume, sodium, potassium, chloride, magnesium and zinc are shown in Table I. When the subjects failed to void urine just at the end of a collection period, the urines corresponding to that and to the following periods were pooled. Amiloride caused a dose-related

Table I — *Urinary excretory variables measured during different fractioning collection periods after dosing with placebo, amiloride 5 mg (AMI 5) and amiloride 10 mg (AMI 10) thirteen healthy volunteers. Values as means ± S.E.M.*

Variable	Unit	Treatment	Hours post-dosing 0-3	3-6	6-12	12-24
Volume	litre	Placebo	0.23±0.03	0.26 ±0.04	0.33 ±0.06	0.66 ±0.07
		AMI 5	0.33±0.06	0.33 ±0.06	0.45 ±0.06	0.63 ±0.06
		AMI 10	0.41±0.05	0.43 ±0.04	0.57 ±0.08	0.72 ±0.07
Sodium	mmol	Placebo	17.3± 2.1	19.4 ± 2.7	27.0 ± 4.1	58.3 ± 6.3
		AMI 5	30.4± 7.0	26.1 ± 4.1	45.9 ± 4.9	65.9 ± 4.6
		AMI 10	33.9± 3.3	33.3 ± 3.3	60.8 ± 7.2	74.0 ± 7.8
Potassium	mmol	Placebo	8.68±1.05	11.47±1.67	10.74±1.73	14.65±1.13
		AMI 5	9.74±1.20	5.18 ±1.06	6.92 ±0.90	10.38±0.88
		AMI 10	9.31±0.98	5.03 ±1.29	6.76 ±0.76	10.03±1.49
Chloride	mmol	Placebo	23.0± 2.4	28.2 ± 4.0	29.0 ± 4.3	50.6 ± 5.2
		AMI 5	31.9± 5.8	22.2 ± 3.6	33.7 ± 2.8	54.3 ± 4.4
		AMI 10	35.5± 3.5	28.5 ± 3.3	49.2 ± 6.1	59.9 ± 5.7
Magnesium	mmol	Placebo	0.49±0.06	0.48 ±0.06	0.87 ±0.10	2.22 ±0.08
		AMI 5	0.55±0.14	0.28 ±0.06	1.02 ±0.18	2.14 ±0.21
		AMI 10	0.48±0.06	0.35 ±0.06	0.96 ±0.21	1.99 ±0.23
Zinc	mg	Placebo	0.08±0.01	0.07 ±0.01	0.12 ±0.02	0.26 ±0.02
		AMI 5	0.08±0.02	0.04 ±0.01	0.12 ±0.02	0.24 ±0.03
		AMI 10	0.09±0.01	0.03 ± <0.00	0.11 ±0.02	0.23 ±0.03

Data from eleven cases for AMI 10 hours 0-3 and from twelve cases for placebo hours 0-3 and AMI 5 hours 3-6.

Figure 1 — *Mean urinary flow after the administration of placebo, amiloride 5 mg (AMI 5) and amiloride 10 mg (AMI 10) per os to thirteen healthy volunteers. The area under the curve indicates the absolute amount excreted.*

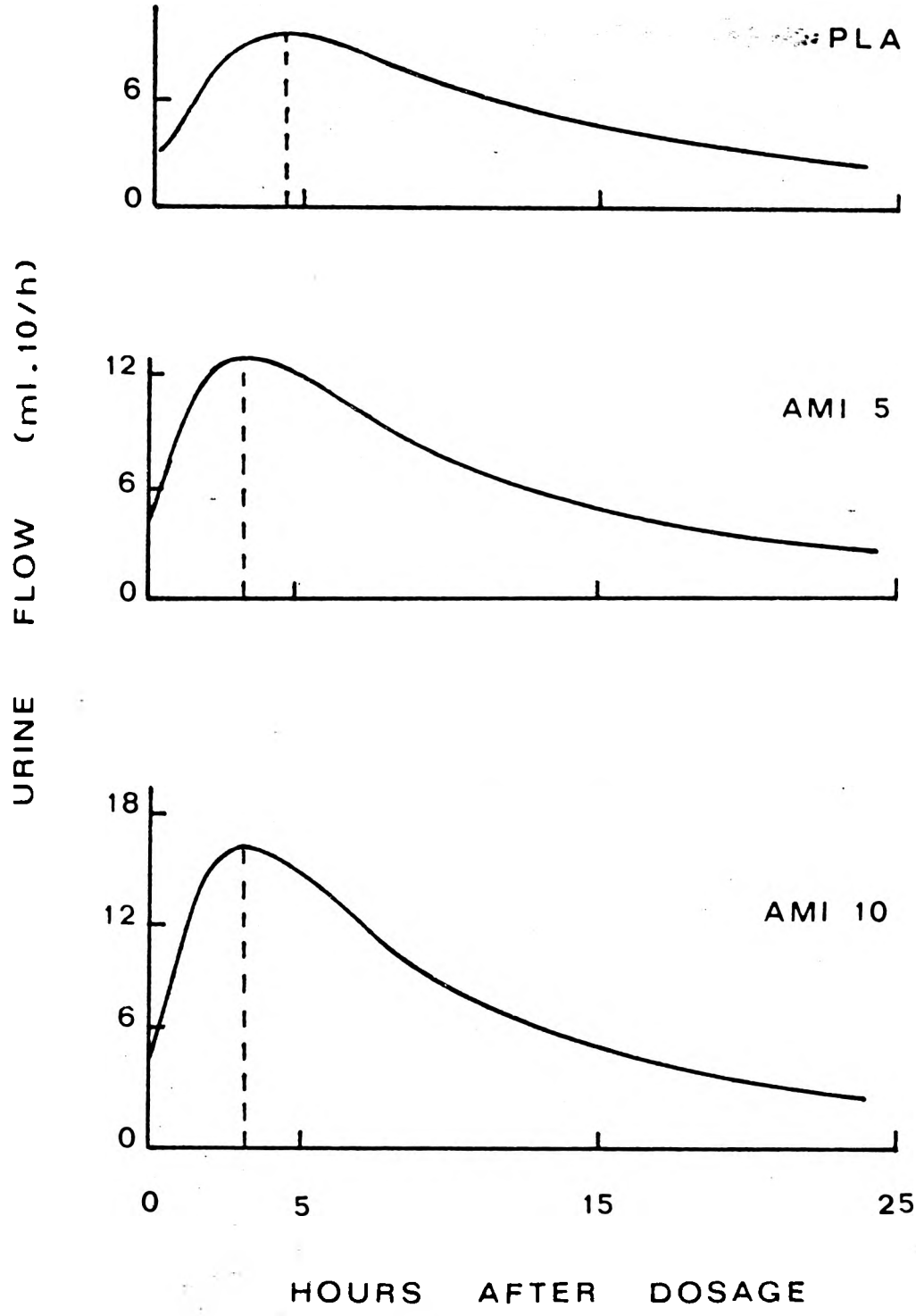

Figure 2 — *Mean urinary sodium flow after the administration of placebo, amiloride 5 mg (AMI 5) and amiloride 10 mg (AMI 10) per os to thirteen healthy volunteers. The area under the curve indicates the absolute amount excreted.*

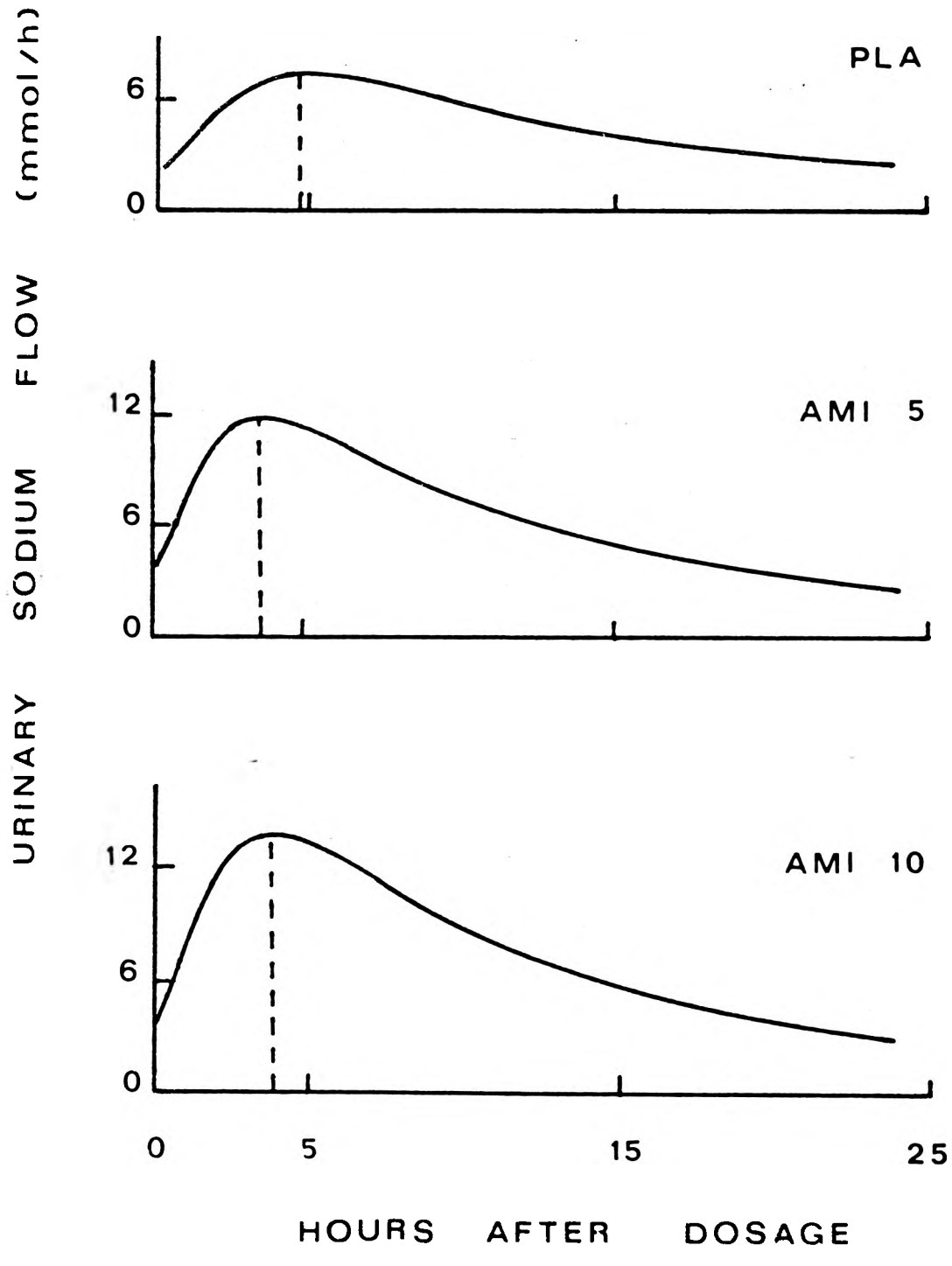

Figure 3 — *Mean urinary potassium flow after the administration of placebo, amiloride 5 mg (AMI 5) and amiloride 10 mg (AMI 10) per os to thirteen healthy volunteers. The area under the curve indicates the absolute amount excreted.*

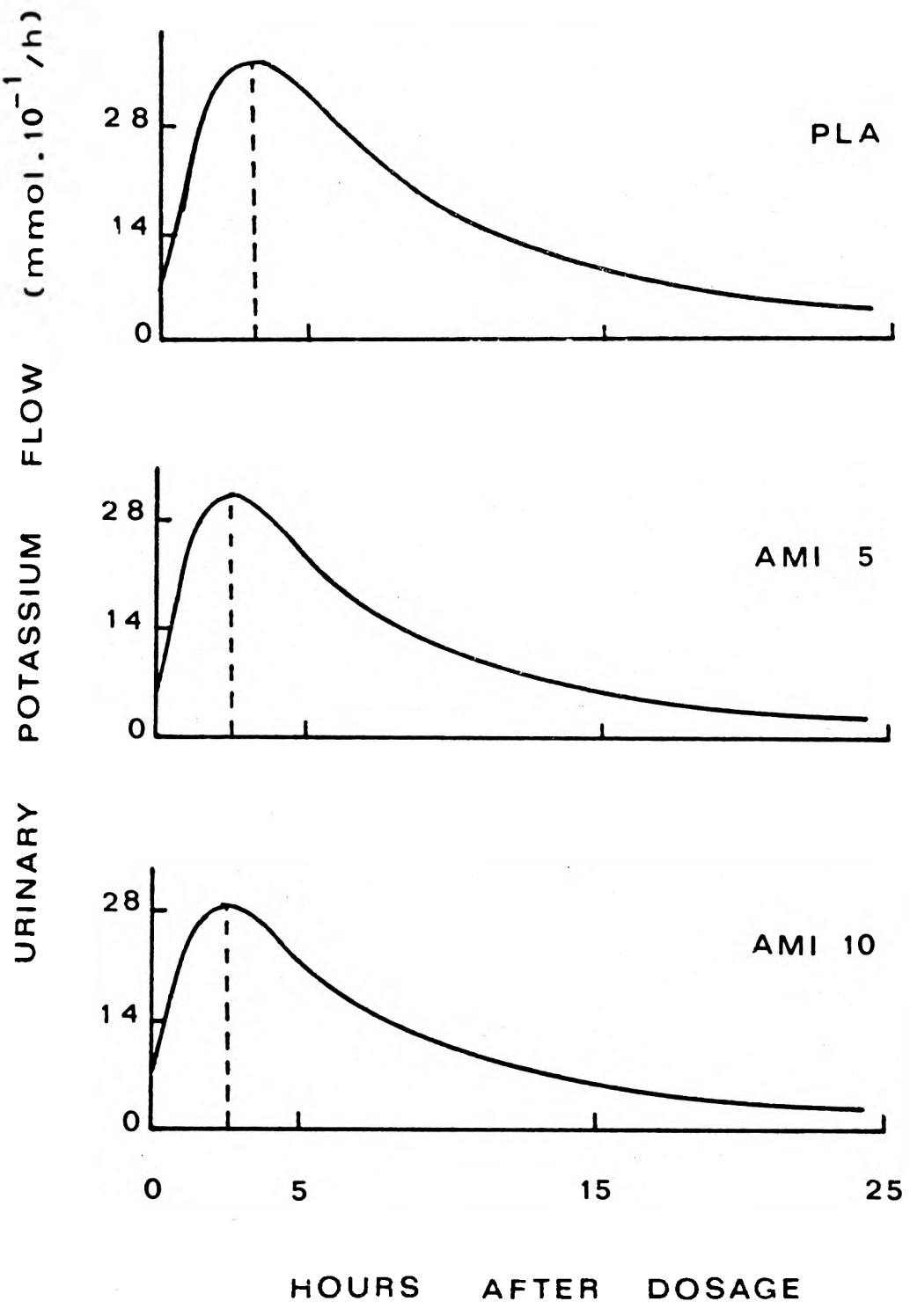

Figure 4 — *Mean urinary chloride flow after the administration of placebo, amiloride 5 mg (AMI 5) and amiloride 10 mg (AMI 10) per os to thirteen healthy volunteers. The area under the curve indicates the absolute amount excreted.*

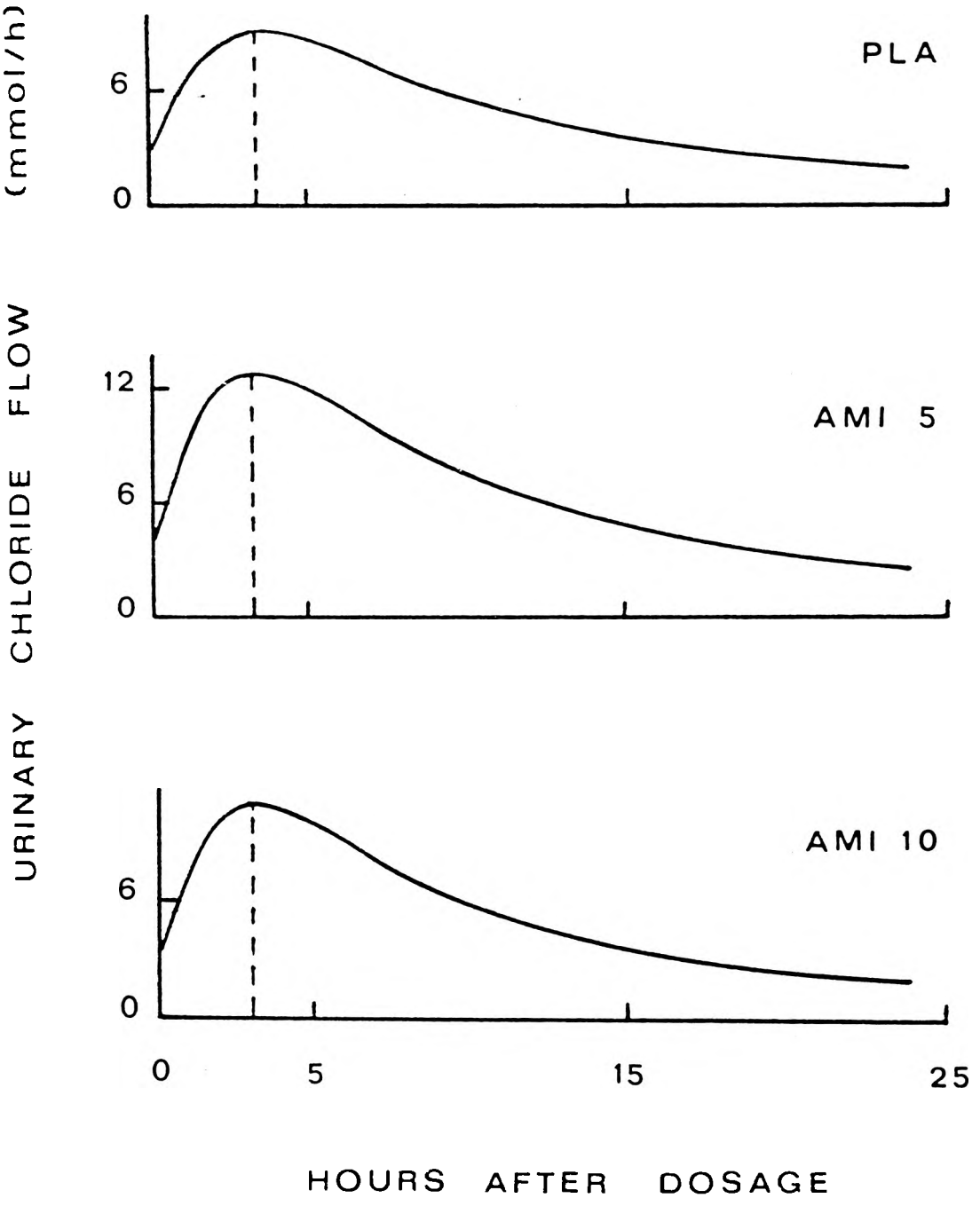

Figure 5 — *Mean urinary magnesium flow after the administration of placebo, amiloride 5 mg (AMI 5) and amiloride 10 mg (AMI 10) per os to thirteen healthy volunteers. The area under the curve indicates the absolute amount excreted.*

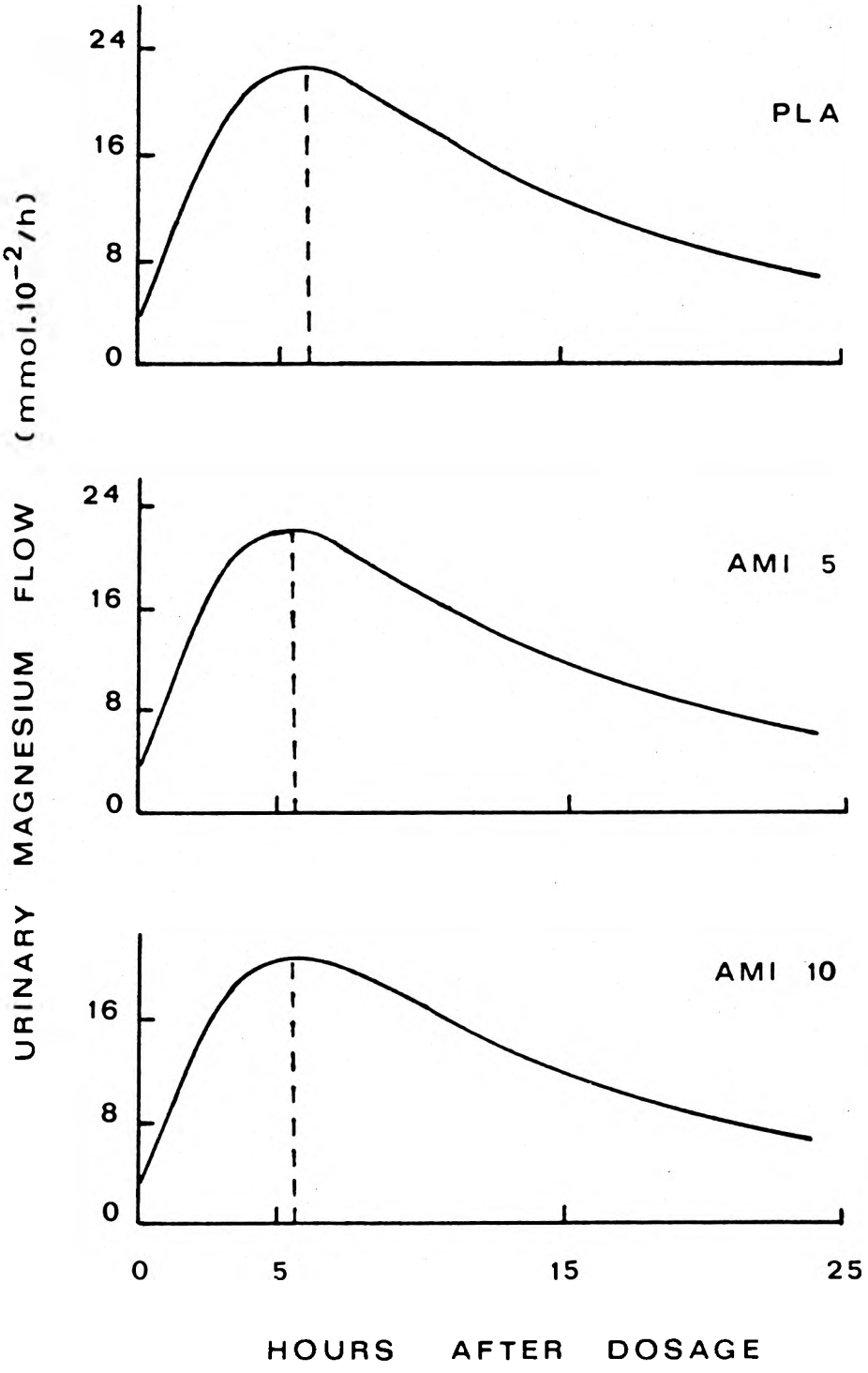

Figure 6 — *Mean urinary zinc flow after the administration of placebo, amiloride 5 mg (AMI 5) and amiloride 10 mg (AMI 10) per os to thirteen healthy volunteers. The area under the curve indicates the absolute amount excreted.*

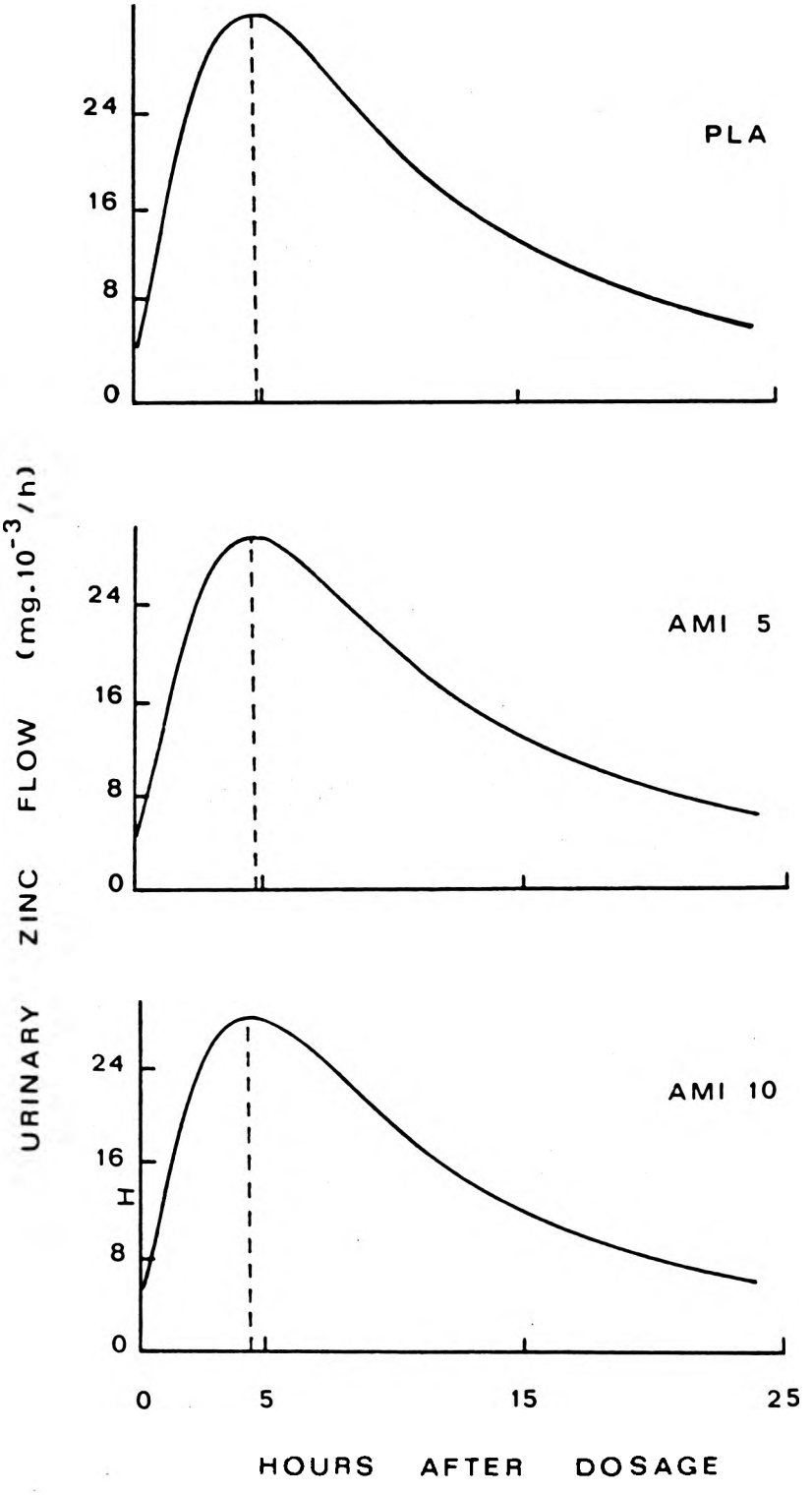

Table II — *24-hour urinary excretory variables measured after dosing with placebo, amiloride 5 mg (AMI 5) and amiloride 10 mg (AMI 10) per os thirteen healthy volunteers. Values as mean ± S.E.M.*

Variable	Unit	Placebo	AMI 5	AMI 10
Volume	litre	1.45 ±0.11	1.72 ±0.14*	2.07 ±0.13*****
Sodium	mmol	119.3± 9.3	166.3±11.7**	196.8±13.9*****
Potassium	mmol	44.2 ± 2.9	31.8 ±3.1***	30.0 ± 2.2****
Chloride	mmol	127.3±7.7	140.5±8.1	168.2±10.0****
Magnesium	mmol	3.99 ±0.12	3.80±0.35	3.71 ±0.38
Zinc	mg	0.52 ±0.05	0.48 ±0.06	0.46 ±0.05*

Significances of the differences with respect to the corresponding placebo mean values:
*$p<0.05$; **$p<0.02$; ***$p<0.01$; ****$p<0.005$; *****$p<0.001$

Table III — *Statistical and parametrical features of the linear transformations of the functions Y(t). AMI 5 = amiloride 5 mg; AMI 10 = amiloride 10 mg.*

Variable	Unit	Treatment	r	p	t_1 (hr)	a (hr)	b
Volume	litre	Placebo	0.9992	<0.001	0.1174	0.9899	0.4198
		AMI 5	0.9997	<0.001	0.0818	0.7723	0.4122
		AMI 10	0.9998	<0.001	0.0659	0.6934	0.3992
Sodium	mmol x 10	Placebo	0.9988	<0.01	0.1563	1.1815	0.4312
		AMI 5	0.9995	<0.001	0.0889	0.8523	0.4122
		AMI 10	0.9997	<0.001	0.0796	0.8217	0.3980
Potassium	mmol	Placebo	0.9999	<0.001	0.0311	0.5132	0.3492
		AMI 5	0.9996	<0.001	0.0277	0.4507	0.3767
		AMI 10	0.9996	<0.001	0.0290	0.4684	0.3804
Chloride	mmol	Placebo	0.9996	<0.001	0.1173	0.8911	0.4356
		AMI 5	0.9994	<0.001	0.0847	0.7847	0.4315
		AMI 10	0.9996	<0.001	0.0760	0.7615	0.4147
Magnesium	mmol x 10^{-1}	Placebo	0.9981	<0.01	0.0551	0.9069	0.3432
		AMI 5	0.9981	<0.01	0.0491	0.8781	0.3475
		AMI 10	0.9980	<0.01	0.0563	0.9341	0.3469
Zinc	mg x 10^{-2}	Placebo	0.9987	<0.01	0.7167	0.7167	0.3317
		AMI 5	0.9984	<0.01	0.0338	0.7354	0.3368
		AMI 10	0.9976	<0.01	0.0300	0.7345	0.3408

increase in 24-hour urinary volume and output of sodium and a dose-related retention of urinary potassium (Table II). The higher dosage induced a significantly greater 24-hour urinary excretion of chloride than placebo and slight, though statistically significant, zinc retention (Table II). 24-hour urinary magnesium output was not affected by either dose of amiloride (Table II).

Urine flow (Fig. 1) and the urinary flows of sodium (Fig. 2) and potassium (Fig. 3), were accelerated by both doses of amiloride, proceeding at higher rates after amiloride than after placebo. These changes were dose-independent. The urinary flows of chloride (Fig. 4), magnesium (Fig. 5) and zinc (Fig. 6) were not significantly affected by either dose of the diuretic. The graphs in Figs. 1 through 6 are the derivatives with respect to time of the Y(t) functions whose statistical and parametrical characteristics are shown in Table III.

Table IV — *Values of several plasma variables after dosing with placebo, amiloride 5 mg (AMI 5) and amiloride 10 mg (AMI 10) in thirteen healthy volunteers. Values in mmol/l as means ± S.E.M.*

Variable	Treatment	Hours post-dosing 0	6	24
Sodium	Placebo	147.4± 0.5	147.2± 0.4	148.1± 0.6
	AMI 5	147.1± 0.4	146.9± 0.4	147.4± 0.4
	AMI 10	147.4± 0.4	146.2± 0.4	146.9± 0.4
Potassium	Placebo	4.11 ±0.05	4.03 ±0.05	4.19 ±0.06
	AMI 5	4.33 ±0.09	4.27 ±0.05	4.34 ±0.06
	AMI 10	4.21 ±0.08	4.23 ±0.04	4.38 ±0.06*
Chloride	Placebo	110.7± 0.9	110.2± 0.8	111.7± 0.8*
	AMI 5	109.1± 0.6	108.9± 1.9	109.7± 1.9
	AMI 10	108.1± 0.4	108.0± 0.5	108.6± 0.5
Total CO_2	Placebo	24.5 ± 0.5(1)	23.7 ± 0.5	25.0 ± 0.6
	AMI 5	24.5 ± 0.5	24.6 ± 0.7	24.1 ± 0.5
	AMI 10	24.8 ± 0.5	22.9 ± 0.5*	22.9 ± 0.4**
Magnesium	Placebo	0.78 ±0.01	0.80 ±0.01	0.81 ±0.01
	AMI 5	0.81 ±0.01	0.81 ±0.01	0.81 ±0.01
	AMI 10	0.80 ±0.01	0.81 ±0.02	0.80 ±0.01
Glucose	Placebo	5.39 ±0.22		5.42 ±0.22
	AMI 5	5.05 ±0.18		5.26 ±0.24
	AMI 10	4.63 ±0.17		5.39 ±0.21***
Uric Acid	Placebo	0.30 ±0.02		0.31 ±0.02*
	AMI 5	0.30 ±0.02		0.31 ±0.02
	AMI 10	0.24 ±0.07		0.24 ±0.02

(1)Data from twelve probands.
Significances of the differences with respect to pre-treatment (0 h) mean values:
*$p<0.05$; **$p<0.02$; ***$p<0.01$

The effects of the medications on blood variables are shown in Table IV. Serum potassium increased significantly 24 hours after amiloride 10 mg. Total serum bicarbonate decreased significantly 6 and 24 hours after amiloride 10 mg. A significant increase in serum glucose occurred 24 hours after amiloride 10 mg and a minor but statistically significant increase in serum uric acid was observed 24 hours after placebo.

DISCUSSION

The mathematical methods used for describing urinary flows have been discussed elsewhere.[14,15]

The increases in 24-hour urinary outputs of fluid and sodium and the retention of urinary potassium provoked by amiloride in a dose-related manner could be expected upon the basis of established knowledge. Flows derived from the data revealed that amiloride accelerated the excretions of volume, sodium and potassium but to a lesser extent than the thiazides do.[4]

Neither the 24-hour urinary magnesium output nor the time-course of its excretion were affected by amiloride, indicating that this diuretic does not affect the renal handling of magnesium. Further investigations are

needed to determine whether amiloride can induce a retention of pre-urinary magnesium when its concentration in the preurine has been increased by the action of a loop or common distal tubule diuretic at nephronal sites proximal to that where amiloride acts.

Amiloride 10 mg provoked a clinically small but statistically significant zinc-retention, without affecting the time-course of zinciuria. This suggests that the zinc-sparing effect of amiloride is not directly dependent upon the renal handling of sodium and potassium after the administration of the diuretic. This issue also merits further investigation.

The effects of amiloride on blood variables could be expected on the basis of established knowledge.

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PAPER C11

Effects of Hydrochlorothiazide Plus Sotalol on Acute Urinary Electrolyte Excretion in Normal Subjects

The finding that a combination of sotalol and hydrochlorothiazide may cause considerable urinary losses of potassium and magnesium is of particular interest and may have a bearing on the cardiovascular effects of this combination. As before Professor Reyes shared the tasks involved and Mr. van der Byl provided computer facilities.

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Effects of hydrochlorothiazide plus sotalol on acute urinary electrolyte excretion in normal subjects

W. P. LEARY, A. J. REYES, K. VAN DER BYL

Summary

Twenty-four-hour urinary outputs, total volume and urinary chlorine (Cl), sodium (Na), potassium (K), calcium, magnesium (Mg), total inorganic phosphate and creatinine levels were measured in 12 biologically equivalent healthy volunteers given single oral doses of placebo, hydrochlorothiazide (HCTZ) 50 mg and a combination of HCTZ and sotalol (STL) 320 mg in a double-blind, random study.

HCTZ and HCTZ + STL increased urinary volume and Na, K, Cl, phosphate and Mg levels significantly and to a similar extent. Since HCTZ causes hyperkaliuresis and hypermagnesiuresis with or without simultaneous administration of STL, the latter does not change the acute effects of HCTZ in healthy subjects.

S Afr Med J 1984; 66: 680-681.

Diuretics are considered drugs of first choice in the treatment of arterial hypertension;[1] however, these drugs have recognized deleterious effects, including somatic depletion of potassium (K)[1-3] and magnesium (Mg)[4-8] and derangements in carbohydrate, purine and lipid metabolism.[9,10] Depletion of K is regarded as particularly important because, although it seldom develops during chronic diuretic treatment unless other causes of K deficiency are also present, serious cardiac arrhythmias may supervene. In addition, it has been suggested that K deficit contributes to the evolution and maintenance of elevated blood pressure,[11] thus hampering the antihypertensive effect of diuretics. Several measures have been proposed to counteract the hyperkaliuretic effects of diuretics, including limitation of dietary sodium (Na) intake and the co-administration of K supplements or K-retaining diuretics such as amiloride, triamterene or spironolactone.[3,4]

The idea that cardiac arrhythmias complicating prolonged diuretic therapy are usually due to K depletion has recently been challenged by evidence in support of the view that urinary Mg loss is the principal causative factor.[4-8] Ionic K is actively pumped into myocardial cells irrespective of the serum K level, provided that normal activity of Na, K-ATPase is maintained.[6] This active process depends upon an adequate intracellular concentration of Mg (acting as a co-factor to the enzyme) and is inhibited when Mg depletion occurs.[6,7,9] Moreover, the myocardial concentration of free cytosolic Ca is increased by Mg depletion, further destabilizing the myocardial electrochemical balance.[6,7,9]

The objective of this study was to determine whether the well-established hyperkaliuretic and hypermagnesiuretic effects of hydrochlorothiazide (HCTZ) 50 mg are modified by the co-administration of the ß-adrenergic blocking agent sotalol (STL) 320 mg to healthy young volunteers.

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A. J. REYES, DR.MED., C.E. CARDIOL., C.P. BIOMAT. BIOEST.

Subjects and methods

Twelve healthy adult male students volunteered to participate in the study after a full explanation of its implications. All were Whites aged 18 - 22 years and none had taken any prescribed medication during the previous 2 months. None was obese or had any history of renal, cardiovascular or metabolic disorder. Smokers were not included in the study.

A standardized diet containing 130 - 160 mmol Na and 3,5-4 litres water (including 2,5 litres tap-water) was given on test days and during the preceding 24-hour control period. Subjects received placebo, HCTZ 50 mg and a combination of HCTZ 50 mg and STL 320 mg (presented in identical form) separately and in random order on 3 different treatment days at least 7 days apart. Medications were given at 08h00 with 100 ml tap-water. Volunteers were confined to a metabolic ward for the first 10 hours after administration of medication. Thereafter they were allowed to return home until 07h00 the following day. During the urine-collection period all exercise and ingestion of alcohol or caffeine and any medicine other than the trial material were forbidden.

On treatment days venous blood was drawn just before medication and 6 ± 1 h and 24 ± 1 h later for measurement of plasma concentrations of chlorine (Cl), Na, K, total calcium (Ca), Mg, carbon dioxide (CO_2), total inorganic phosphate, creatinine and urate.

All laboratory analyses were carried out by technologists unaware of the protocol. All urine passed on control and treatment days was collected at 3, 6, 12 and 24 hours after dosing and analysed accordingly on treatment days. Urinary volume and contents of Cl, Na, K, Ca, Mg, total inorganic phosphate and creatinine were measured.

Solute concentrations in both urine and serum specimens were analysed as follows: the Na, K, Cl and total bicarbonate contents were measured by the ion-selective technique using a Nova 4 analyser. Total inorganic phosphate was measured colorimetrically with a Clinical Sciences kit. Ca and Mg concentrations were measured by atomic absorption using a Varian 275 instrument. Creatinine and urate were measured colorimetrically using Boehringer Mannheim kits. Colorimetrical determinations were carried out on a Beckman 3 spectrophotometer.

Mathematical methods

The mean experimental values for urinary volume and solutes accumulated by the end of each period after administration were

fitted as functions of time by a mathematical model which has been described previously.[10] Urinary flow was calculated and the time to maximal flow after administration evaluated via an iterative computer design.[11]

Standard statistical parametrical techniques, paired *t* test and linear correlation and regression were used. Normality of frequency distributions and homoscedasticity of sample variances were evaluated via the chi-square test and the *F* ratio respectively. All statistical tests were two-tailed and a *P* value of 0,05 was considered the limit of significance.

Results

No significant differences were detected in the 24-hour accumulated mean urinary output as regards volume and electrolytes after placebo and on control days.

Accumulated 24-hour excretions are shown in Table I.

TABLE I. URINARY EXCRETORY VARIABLES MEASURED OVER 24 HOURS AFTER ORAL ADMINISTRATION OF PLACEBO, HCTZ 50 mg OR A COMBINATION OF HCTZ 50 mg AND STL 320 mg IN 12 HEALTHY VOLUNTEERS

Variable	Placebo	HCTZ	HCTZ + STL
Volume (l)	**2,69 ± 0,12**	**3,34 ± 0,12*****	**3,13 ± 0,14***
Na (mmol)	**146,4 ± 9,7**	**306,5 ± 12,8*****	**242,2 ± 16,7*****
K (mmol)	**55,8 ± 4,2**	**70,2 ± 3,4****	**82,5 ± 4,9*****
Cl (mmol)	**184,2 ± 10,1**	**359,5 ± 13,4*****	**305,2 ± 15,0*****
Mg (mmol)	**5,15 ± 0,47**	**6,38 ± 0,41***	**6,16 ± 0,44***
Ca (mmol)	**4,46 ± 0,41**	**4,27 ± 0,34**	**3,89 ± 0,47**
Phosphate (mmol)	**28,2 ± 1,0**	**33,1 ± 2,4***	**33,0 ± 2,3***
Creatinine (mmol)	**19,1 ± 0,8**	**19,4 ± 1,0**	**19,2 ± 1,3**

Values expressed as mean ± SEM.
Significances of the differences with respect to the corresponding mean values on placebo:
*$P < 0,05$;
**$P < 0,005$;
***$P < 0,001$.

Both active treatments caused significant increases in 24-hour urinary outputs in terms of volume and Cl, Na, K, phosphate and Mg content. Ca and creatinine excretions were unchanged.

The time courses for volume, Cl, Na and K practically coincided after HCTZ or HCTZ + STL, as revealed by the times to peak excretions after dosage which were calculated as 2,68, 1,95, 1,96 and 2,66 hours respectively after HCTZ and 3,17, 2,44, 2,45 and 2,90 hours respectively after HCTZ + STL. Peak excretions of Mg, phosphate and creatinine were delayed in relation to those of volume and the other electrolytes, occurring 4,11, 6,30 and 4,39 hours respectively after HCTZ and 4,83, 6,55 and 4,88 hours respectively after HCTZ + STL. These delays were less apparent (although still present) after placebo.

Serum values of the variables measured did not change in a clinically significant manner.

Discussion

The mathematical methods used for describing urinary flows have been discussed elsewhere.[10,11,12] The fact that HCTZ + STL significantly increased urinary output in terms of volume and Cl, Na, K, Mg and phosphate levels confirms that the co-administration of STL 320 mg does not change the acute renal actions of HCTZ 50 mg, despite the fact that non-selective ß-blocking agents usually reduce renal blood flow. This was possibly reflected by the finding that the time to peak excretion of all solutes was slightly longer after HCTZ + STL than after HCTZ alone.

Most antihypertensive diuretics tested under similar conditions so far (chlorthalidone,[13] chlorexolone[14] and HCTZ[11]), cause significant urinary Mg and K losses. The present results from a small sample of healthy, biologically equivalent subjects indicate that the effect of a single dose of HCTZ + STL is indistinguishable from that of HCTZ in this regard.

If the preliminary findings reported in the present study are confirmed, prolonged daily treatment with HCTZ or HCTZ + STL could lead to clinically significant depletion of somatic Mg and K. Serum K and Mg levels should therefore be monitored and the institution of adequate prophylactic measures should be considered.[2-8]

Urinary volume and Cl, Na and K levels approximately coincided after both active preparations, but urinary Mg was delayed with respect to other urinary electrolytes, confirming studies indicating that excretion of this cation is not Cl- or Na-dependent but involves intervention by hormones other than aldosterone.[5]

Diuretics cause a rise in tissue catecholamine levels and Mg depletion which, in turn, further augment each other.[8,12] Myocardial electrolytic imbalance due to a rise in catecholamine levels and Mg depletion is a likely determinant of cardiac arrhythmias.[5-8,12] Whereas ß-adrenergic blockade does not influence diuretic-induced Mg losses, some benefits may be obtained from the co-administration of these agents since ß-blockers should moderate the catecholamine-mediated effects of diuretics.

The changes found in serum variables could be expected on the basis of established knowledge of the acute systemic effects of distal tubular diuretics.

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96 PIONEER

PAPER C12

Effects of Several Diuretic Formulations on Urinary Magnesium Output in Healthy Adults

This paper summarises the results of studies involving several diuretic formulations and includes both previously published and new data. Work was shared, as in the previous collaborative studies, with Professor A.J. Reyes.

EFFECTS OF SEVERAL DIURETIC FORMULATIONS ON URINARY MAGNESIUM OUTPUT IN HEALTHY ADULTS

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ABSTRACT

The effects of monodoses of placebo and a number of diuretic formulations on 24 h urinary excretions of several electrolytes, including Mg, were studied in healthy volunteers. All common (loop and thiazide-like) diuretics, except indapamide 2.5 mg, induced hypermagnesiuresis. Amiloride did not affect 24 h urinary Mg output significantly.

INTRODUCTION

Magnesium depletion caused by diuretics contributes to the pathogenesis of cardiac arrhythmias, sudden death, myocardial infarction, vasospasm in the coronary and cerebral vessels and partially underlies alterations in lipid and carbohydrate metabolism induced by diuretics. The objective of this study was to evaluate the acute effects of several diuretics on the urinary eliminations of Mg and K.

SUBJECTS AND METHODS

Healthy student volunteers aged 18-25 were studied. Monodoses of diuretics or placebo were given double-blind and in random order, at 08h00 and urine was collected at intervals for 24 h thereafter. Seven days elapsed between experiments and a standard diet was prescribed throughout.

RESULTS

The 24 h percentage changes in urinary outputs after medication, in comparison with placebo, are shown in Table I; statistical results refer to mean values.

FIGS. 1, 2 and 3 show the urinary Na and Mg flows after placebo, furosemide 40 mg or hydrochlorothiazide 50 mg respectively. While flows of both cations were almost parallel after placebo, Mg flow was delayed with respect to Na after furosemide or hydrochlorothiazide.

DISCUSSION

All formulations which cause acute hypermagnesiuresis may induce Mg depletion upon prolonged administration. Since urinary Mg flows were delayed with respect to Cl, Na and volume flows after administration of the diuretics concerned, the hypothesis was advanced that slow-acting endocrine-type mechanisms might account, at least partially, for the hypermagnesiuresis. A system analysis of pertinent data reveal loop diuretics

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Diuretics, Jules B. Puschett, Editor

TABLE I. Percentage changes in mean 24-h urinary excretions after monodosing with several diuretic formulations with respect to corresponding outputs after placebo.

N° of cases	Drug and dose (mg)	% change in 24-h renal output				
		Cl	Na	Volume	K	Mg
13	amiloride 5	10^{ns}	39^{b}	19^{a}	-27^{c}	-4^{ns}
13	amiloride 10	32^{d}	65^{e}	43^{e}	-32^{d}	-7^{ns}
9	chlorthalidone 100	152^{e}	165^{e}	69^{d}	69^{d}	87^{d}
9	furosemide 40	49^{b}	48^{d}	49^{d}	21^{a}	52^{a}
19	hydrochlorothiazide 50	63^{e}	61^{e}	38^{e}	31^{a}	24^{a}
9	hydrochlorothiazide 50	59^{a}	63^{d}	35^{a}	22^{a}	44^{a}
7	indapamide 2.5	87^{c}	139^{e}	26^{c}	23^{e}	-8^{ns}
10	muzolimine 30	38^{e}	34^{c}	14^{c}	20^{ns}	8^{ns}
13	xipamide 5	39^{e}	46^{e}	20^{e}	40^{a}	27^{c}
13	xipamide 10	106^{e}	120^{e}	63^{e}	55^{e}	50^{e}
13	xipamide 20	101^{e}	111^{e}	63^{e}	79^{e}	40^{e}

Significances of the differences between mean excretions after drug and after placebo: $^{a}P < 0.05$; $^{b}P < 0.02$; $^{c}P < 0.01$; $^{d}P < 0.005$; $^{e}P < 0.001$; ns: non-significant.

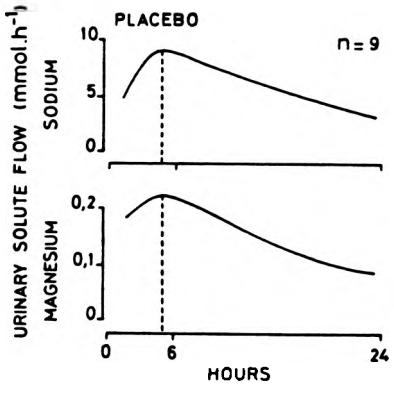

FIG. 1. Urinary Na and Mg flows after placebo administration to healthy volunteers. Adapted from Reyes and Leary [1], by courtesy of Current Therapeutic Research.

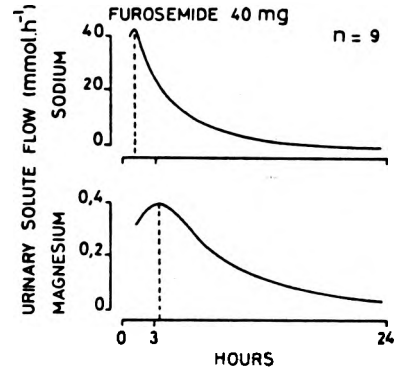

FIG. 2. Urinary Na and Mg flows after administration of furosemide 40 mg to healthy volunteers. Adapted from Reyes and Leary [2], by courtesy of Current Therapeutic Research.

cause hypermagnesiuria by direct blockade of Mg reabsorption which is aggravated by a further increase in Mg filtration at the glomerulus which follows Mg mobilisation from bone secondary to an increase in plasma parathyroid hormone (PTH) level which occurs in response to hypercalciuria provoked by loop diuretics. Thiazide-type diuretics block Mg reabsorption in the early portion of the distal tubule but the principal mechanism whereby they provoke hypermagnesiuresis is a decrease in

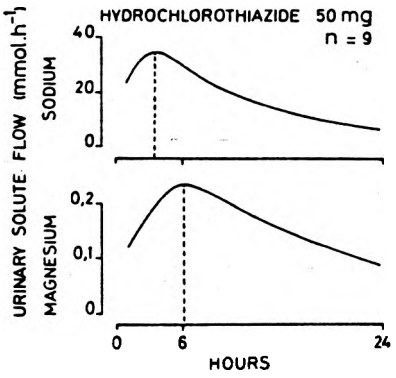

FIG. 3. Urinary Na and Mg flows after administration of hydrochlorothiazide 50 mg to healthy volunteers. Adapted from Leary and Reyes [3], by courtesy of Current Therapeutic Research.

reabsorption of Mg at the loop of Henle. This occurs because decreased Ca excretion induced by these substances reduces plasma PTH and thus diminishes PTH-dependent Mg reabsorption in the loop [4] (FIG. 4).

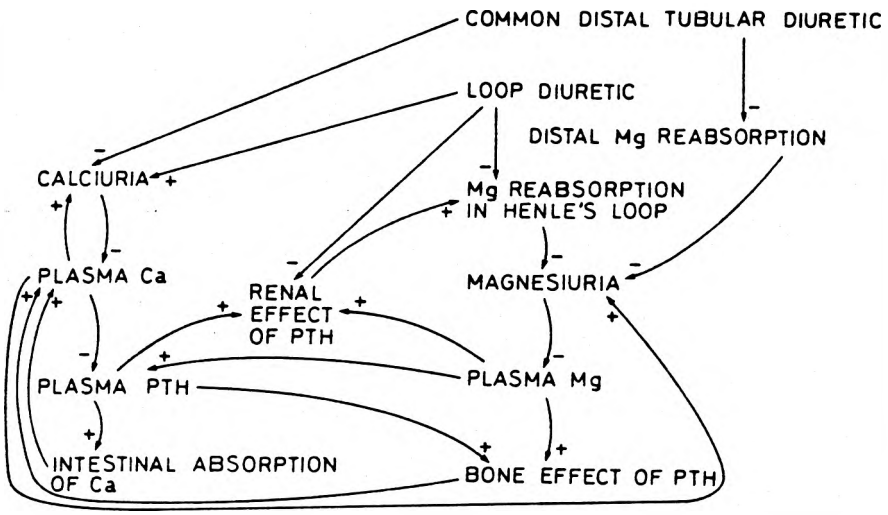

FIG. 4. Causal diagram of the determination of Mg deficiency provoked by loop and distal tubular diuretics. The diagram depicts changes (+: augmentation; -: diminution) resulting from increases in the variables at which arrows start (system dynamics notation). PTH: parathyroid hormone. Adapted from Reyes [4], by courtesy of La Prensa Médica Argentina.

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PAPER C13

Effects of Captopril, Hydrochlorothiazide, and their Combination on Timed Urinary Excretions of Water and Solutes

This provides further data which gives insight into the diuretic effects of captopril an angiotensin converting enzyme inhibitor. Important new findings are described. The third and fourth authors names are included in lieu of a simple acknowledgement which would have been more appropriate.

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Effects of Captopril, Hydrochlorothiazide, and Their Combination on Timed Urinary Excretions of Water and Solutes

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Summary: The effects of single doses of captopril 100 mg, hydrochlorothiazide 25 mg, and a combination of both on 24-h outputs of fluid and several solutes were compared in healthy volunteers. Thirteen subjects were studied in a metabolic ward under strictly controlled conditions. Single doses of placebo, captopril, hydrochlorothiazide, and a combination of captopril and hydrochlorothiazide were given double-blind in random order on 4 separate days. Urine was collected at regular intervals for 24 h after medication. The combination of captopril and hydrochlorothiazide and hydrochlorothiazide alone significantly increased the 24-h urinary outputs of Cl^-, Na^+, fluid, and K^+ compared with placebo and also accelerated the corresponding urinary flows. Captopril did not change the 24-h urinary excretions of Cl^-, Na^+, fluid, and K^+ significantly, though it advanced the time courses of their urinary flows. All medications significantly increased the 24-h renal outputs of Mg^{2+} and creatinine. Captopril significantly increased the 24-h urinary output of urate and advanced its urinary flow. Hydrochlorothiazide significantly decreased the output and retarded the flow. The combination of captopril and hydrochlorothiazide did not change the 24-h urinary output and retarded its flow. It is concluded that the renal excretory actions of captopril are more prolonged than the plasma levels of the drug would indicate. Captopril has diuretic effects which may vary in potency with aldosterone concentrations and uricosuric properties unrelated to aldosterone status. **Key Words:** Angiotensin-I-converting enzyme inhibitors—Captopril—Diuretics—Gout—Hypertension—Magnesium—Uric Acid.

The pharmacologic properties of the angiotensin-I-converting enzyme inhibitor captopril are of both academic and clinical interest. The mechanisms whereby captopril reduces arterial blood pressure when used as monotherapy in essential hypertension remain obscure, however (1–4), and cannot be ascribed solely to inhibition of circulating angiotensin-I-converting enzyme (5–10).

The kidney is of the utmost importance in the regulation of cardiovascular homeostasis. Numerous reports have referred to the renal actions of captopril (11–13), but information on the renal clinical pharmacology of the drug is incomplete, since the effects of captopril on the excretion of various urinary solutes have not been evaluated and the renal pharmacology of captopril has not been compared with that of established antihypertensive diuretics such as the thiazides. Renal actions of captopril merit investigation since there is a dissociation between the time courses of the plasma concentration of the substance, paralleled by circulating angiotensin-I-converting enzyme inhibition, and that of the hypotensive effect of captopril in patients with essential hypertension (14,15). Renal effects might partly explain the mechanism of action of captopril and some of the discrepancies between the known, conventional, time-dependent pharmacokinetics of the drug (14,16) and its pharmacodynamics. Since the coprescription of captopril and hydrochlorothiazide reduces raised blood pressure effectively and safely (17), the renal excretory actions of this combination also merit investigation.

The objective of this study was to compare the effects of 100 mg captopril, 25 mg hydrochlorothiazide, and their combination on the 24-h urinary outputs and flows of fluid and several solutes, after administration of single doses of the formulations.

SUBJECTS AND METHODS

Subjects and operational procedures

Thirteen healthy male adult students volunteered to participate in the study after a full explanation of its im-

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plications. All were aged between 18 and 24 and had taken no other medication within the previous 2 months. None were obese nor had any history of renal, cardiovascular, or metabolic disorders. Smokers were not studied.

A standardized diet containing roughly 160 mmol of Na^+ and 3.5 L of water was prescribed on treatment days and during the 24 h before each of them. Subjects received placebo, captopril 100 mg, a combination of captopril 100 mg and hydrochlorothiazide 25 mg, and hydrochlorothiazide 25 mg, all presented in matching tablets. Formulations were administered separately and in random order on 3 different treatment days, at least 7 days apart. Medications were given at 0800 with 100 ml tap water. Volunteers were confined to a metabolic ward on study days, when ingestion of alcohol, caffeine, or medicines other than the trial formulation was forbidden.

All laboratory analyses were carried out by technologists who were unaware of the protocol being followed. All urine passed on treatment days and during the previous 24 h was collected. Urine collected 0–3, 3–6, 6–9, 9–12, and 12–14 h after dosing on treatment days and pooled urine collected during the previous 24 h were analyzed. Urine volumes were measured at the end of each collection period and samples were kept frozen until chemical analysis. Urine contents of Cl^-, Na^+, K^+, Ca^{2+}, Mg^{2+}, total inorganic phosphate, creatinine, and urate were analyzed.

On treatment days venous blood was drawn by venepuncture just before medication and 6 ± 1 and 24 ± 0.5 h later for measurement of serum concentrations of Cl^-, Na^+, K^+, total Ca^{2+}, Mg^{2+}, total inorganic phosphate, creatinine, urate, and total CO_2. The serum glucose concentration was measured in blood samples withdrawn before and 24 h after medication. Blood was collected in plain glass tubes and serum was separated by centrifugation and frozen until chemical analysis.

Laboratory methods

Urinary and serum variable concentrations were measured as follows: Cl^- and K^+ were evaluated with an IL 943 flame photometer with cesium as diluent. Cl^- and total CO_2 were measured with an IL 446 analyzer with a mercuric diluent and a pCO_2 electrode, respectively. Mg^{2+} and urinary Ca^{2+} were measured by atomic absorption with a Varian 1275 instrument. Total inorganic phosphate, creatinine, urate, glucose, and serum Ca^{2+} were all evaluated colorimetrically on a Model 34

TABLE 1. *Accumulated excretions of urinary variables after monodosing 13 healthy volunteers with placebo, captopril 100 mg, a combination of captopril 100 mg plus hydrochlorothiazide (HZ) 25 mg, and hydrochlorothiazide 25 mg (means ± SEM)*

Urinary variable	Medication	Hours after dosing				
		3	6	9	12	24
Chloride (mmol)	Placebo	29 ± 3	65 ± 4	92 ± 6	118 ± 6	175 ± 8
	Captopril	30 ± 2	66 ± 6	95 ± 8	120 ± 6	159 ± 7
	Captopril + HZ	80 ± 7	162 ± 9	227 ± 8	258 ± 10	299 ± 11
	Hydrochlorothiazide	51 ± 4	111 ± 6	146 ± 7	181 ± 8	225 ± 8
Sodium (mmol)	Placebo	24 ± 3	53 ± 5	76 ± 6	99 ± 6	151 ± 7
	Captopril	25 ± 3	59 ± 6	87 ± 9	110 ± 8	146 ± 9
	Captopril + HZ	71 ± 7	157 ± 8	200 ± 9	227 ± 10	264 ± 11
	Hydrochlorothiazide	48 ± 3	101 ± 6	130 ± 7	161 ± 7	201 ± 7
Volume (L)	Placebo	0.22 ± 0.03	0.86 ± 0.08	1.33 ± 0.10	1.68 ± 0.10	2.44 ± 0.12
	Captopril	0.38 ± 0.05	0.96 ± 0.10	1.47 ± 0.13	1.93 ± 0.15	2.62 ± 0.18
	Captoprol + HZ	0.52 ± 0.04	1.33 ± 0.09	1.95 ± 0.11	2.30 ± 0.13	2.87 ± 0.18
	Hydrochlorothiazide	0.50 ± 0.06	1.30 ± 0.08	1.75 ± 0.11	2.24 ± 0.14	2.94 ± 0.15
Potassium (mmol)	Placebo	7.6 ± 0.8	18.2 ± 1.7	24.1 ± 2.3	31.2 ± 2.4	48.3 ± 3.1
	Captopril	10.1 ± 1.1	19.7 ± 1.8	27.6 ± 2.5	34.2 ± 2.3	46.0 ± 3.0
	Captopril + HZ	15.1 ± 1.6	29.9 ± 2.3	39.3 ± 3.0	46.2 ± 3.2	57.5 ± 3.4
	Hydrochlorothiazide	11.3 ± 1.4	23.5 ± 2.5	30.6 ± 3.0	41.2 ± 4.2	58.2 ± 5.2
Calcium (mmol)	Placebo	0.71 ± 0.10	1.58 ± 0.16	2.22 ± 0.22	2.77 ± 0.29	4.44 ± 0.45
	Captopril	0.62 ± 0.08	1.49 ± 0.15	2.09 ± 0.20	2.73 ± 0.20	4.48 ± 0.28
	Captopril + HZ	1.06 ± 0.12	2.15 ± 0.20	2.90 ± 0.22	3.41 ± 0.28	4.90 ± 0.41
	Hydrochlorothiazide	0.81 ± 0.08	1.72 ± 0.20	2.19 ± 0.22	2.74 ± 0.31	4.12 ± 0.37
Magnesium (mmol)	Placebo	0.65 ± 0.07	1.31 ± 0.07	1.87 ± 0.14	2.37 ± 0.15	4.14 ± 0.35
	Captopril	0.62 ± 0.09	1.33 ± 0.17	2.02 ± 0.21	2.64 ± 0.24	4.87 ± 0.31
	Captopril + HZ	1.02 ± 0.11	2.04 ± 0.13	2.70 ± 0.19	3.47 ± 0.26	6.01 ± 0.40
	Hydrochlorothiazide	0.74 ± 0.08	1.92 ± 0.25	2.69 ± 0.32	3.69 ± 0.31	7.18 ± 0.44
Phosphate (mmol)	Placebo	2.4 ± 0.3	5.6 ± 1.1	7.7 ± 1.2	11.3 ± 1.3	28.5 ± 1.1
	Captopril	4.1 ± 0.7	8.5 ± 1.0	14.1 ± 1.7	21.4 ± 2.1	39.6 ± 3.4
	Captopril + HZ	3.2 ± 0.3	7.1 ± 0.7	11.3 ± 1.0	15.4 ± 1.3	31.8 ± 2.2
	Hydrochlorothiazide	2.7 ± 0.4	6.0 ± 0.9	9.2 ± 1.3	14.9 ± 1.5	34.8 ± 1.9
Creatinine (mmol)	Placebo	1.6 ± 0.2	3.3 ± 0.3	5.6 ± 0.4	8.0 ± 0.4	18.7 ± 0.5
	Captopril	3.4 ± 0.4	6.5 ± 0.5	9.2 ± 0.6	11.7 ± 0.6	20.7 ± 0.7
	Captopril + HZ	3.9 ± 0.3	7.2 ± 0.4	10.1 ± 0.5	13.1 ± 0.5	23.3 ± 1.4
	Hydrochlorothiazide	2.9 ± 0.3	5.3 ± 0.4	7.1 ± 0.6	10.1 ± 0.7	20.5 ± 0.8
Urate (mmol)	Placebo	0.78 ± 0.07	1.58 ± 0.08	2.04 ± 0.09	2.51 ± 0.07	4.49 ± 0.18
	Captopril	0.86 ± 0.07	1.78 ± 0.11	2.51 ± 0.17	3.29 ± 0.14	5.41 ± 0.26
	Captopril + HZ	0.93 ± 0.14	1.71 ± 0.18	2.41 ± 0.19	2.98 ± 0.16	4.36 ± 0.22
	Hydrochlorothiazide	0.65 ± 0.10	1.29 ± 0.20	1.61 ± 0.26	2.17 ± 0.33	3.46 ± 0.45

Beckman Trace II spectrophotometer; Boehringer-Mannheim kits were used for the Ca^{2+} and creatinine measurements and a Clinical Sciences kit for the total inorganic phosphate chemical colorimetric reactions; a Boehringer-Mannheim Peridochrom kit was used for urate determinations and a Beckman endpoint for the glucose enzymatic colorimetric reactions.

Mathematical methods

The mean experimental values, M, of the urinary volume and solutes accumulated by the end of each postdosing collection period, as functions of time, t, were fitted by a mathematical model:

$$20M/\log(100 - M) = \exp\{[2.3 (t - t_1)]/(a + bt)\}, \quad (1)$$

where t_1 is the time at which $M = 0.1$ unit used in the calculations and a (time) and b (dimensionless) are parameters of the function. The fitting procedure has been described previously (18). Flows of urinary variables were defined as

$$dM/dt = (a + bt_1)/\{(a + bt)^2 [(0.43/M) + (0.43)^2/(100 - M)\log(100 - M)]\}. \quad (2)$$

Normality of frequency distributions and homoscedasticity of sample variances were evaluated as appropriate, through the χ^2 test and the F ratio, respectively. Minor deviations from prerequisites for parametric statistics were tolerated. Standard statistical techniques (paired t test and linear correlation and regression evaluated by least squares) were used. All statistical tests were two-tailed and $p = 0.05$ was considered the limit of significance.

Times to peak flow after dosing were evaluated on a computer by an iterative procedure.

RESULTS

No significant differences were found between the mean 24-h urinary outputs of each studied variable on any of the 24-h pretreatment control days and the corresponding mean excretions after placebo.

Table 1 depicts the absolute urinary excretions of fluid and electrolytes accumulated at the end of each fractional collection period after monodosing with placebo, captopril, hydrochlorothiazide, and the combination of captopril and hydrochlorothiazide. The probability values derived from paired t tests contrasting any two mean 24-h urinary outputs of each variable after each formulation are shown in Table 2.

The mean accumulated excretions of all studied variables after all formulations, as functions of time, were satisfactorily fitted by $(M)t$, as shown by the

TABLE 2. *Probabilities corresponding to dependent* t *statistics evaluating the significance of the differences between any two mean 24-h urinary variable outputs after monodosing 13 healthy volunteers with captopril 100 mg, a combination of captopril 100 mg plus hydrochlorothiazide (HZ) 25 mg, and hydrochlorothiazide 25 mg*

Urinary variable	Medication	Medication: Placebo	Medication: Hydrochlorothiazide	Medication: Captopril + hydrochlorothiazide
Chloride	Captopril	0.0962	<0.0001*	<0.0001*
	Captopril + HZ	<0.0001*	0.0001*	
	Hydrochlorothiazide	0.0008*		
Sodium	Captopril	0.7073	<0.0001*	<0.0001*
	Captopril + HZ	<0.0001*	0.0002*	
	Hydrochlorothiazide	0.0006*		
Volume	Captopril	0.0700	0.0809	0.2263
	Captopril + HZ	0.0133*	0.5780	
	Hydrochlorothiazide	0.0009*		
Potassium	Captopril	0.4975	0.0281*	0.0281*
	Captopril + HZ	0.0336*	0.9217	
	Hydrochlorothiazide	0.0873		
Calcium	Captopril	0.8848	0.2430	0.1889
	Captopril + HZ	0.2272	0.0221*	
	Hydrochlorothiazide	0.4140		
Magnesium	Captopril	0.0048*	<0.0001*	0.0258*
	Captopril + HZ	0.0006*	0.0091*	
	Hydrochlorothiazide	<0.0001*		
Phosphate	Captopril	0.0142*	0.3486	0.0843
	Captopril + HZ	0.2857	0.3203	
	Hydrochlorothiazide	0.0026*		
Creatinine	Captopril	0.0008*	0.8335	0.0538
	Captopril + HZ	0.0017*	0.1047	
	Hydrochlorothiazide	0.1227		
Urate	Captopril	0.0348*	0.0070*	0.0290*
	Captopril + HZ	0.5236	0.1285	
	Hydrochlorothiazide	0.0674		

* Significant at $p \leq 0.05$.

r values in Table 3, where the regression parameters of the linearized functions are also shown. The derivations of $(M)t$ with respect to time allowed evaluation of the times to peak excretions after dosing, which are also shown in Table 3. Figures 1 and 2, respectively, depict the $M(t)$ and $(dM/dt)t$-flow-functions for urate.

The evolution of serum variables after dosing and the significances of their differences with respect to pretreatment means are described in Table 4.

No clinical or laboratory abnormalities occurred in any subject during the study period.

DISCUSSION

The mathematical model describing the flows of urinary variables as functions of time has been discussed previously (18,19). The times to peak flows after dosing (Table 3) serve as an overall estimation of the corresponding flow rates.

All the urinary excretory actions of captopril lasted longer than the presence of the drug in the plasma of normal subjects, and times to peak excretions were delayed beyond the time to peak plasma concentration of captopril reported after its oral administration in hypertensive patients (14). Studies are necessary to determine whether the captopril-captopril disulfide and other metabolites of captopril (20), that persist in plasma far longer than free captopril, account for this time shift.

The combination of captopril and hydrochlorothiazide and hydrochlorothiazide alone significantly increased the 24-h urinary excretions of Cl^-, Na^+, fluid, and K^+ (Table 2) and accelerated their corresponding flows with respect to those after placebo dosing (Table 3). Captopril did not change the 24-h renal excretions of Cl^-, Na^+, volume, or K^+ significantly. The fact that captopril did not induce overt natriuresis in this study is at variance with the results of a study in which primary hypertensives were treated with captopril (12). Since Na^+ intake was severely limited to 20 mmol of Na^+ in that trial, whereas Na^+ intake was fairly high in the present investigation, captopril may have an intrinsic natri-

TABLE 3. *Statistical features and parameter values of the linear transformations of the functions M(t). HZ = hydrochlorothiazide*

Urinary variable	Unit used in calculations	Medication	r	p	t_1 (h)	a (h)	b	Time to peak excretion (h)
Chloride	mmol × 10	Placebo	>0.999	<0.0001	0.093	0.7997	0.4079	3.45
		Captopril	>0.999	<0.0001	0.090	0.7072	0.4191	3.21
		Captopril + HZ	>0.999	<0.0001	0.034	0.3744	0.3810	1.96
		Hydrochlorothiazide	>0.999	<0.0001	0.053	0.5093	0.3979	2.46
Sodium	mmol × 10	Placebo	>0.999	<0.0001	0.113	0.8949	0.4174	3.94
		Captopril	>0.999	<0.0001	0.107	0.7610	0.4237	3.46
		Captopril + HZ	>0.999	<0.0001	0.038	0.3681	0.3908	1.96
		Hydrochlorothiazide	>0.999	<0.0001	0.057	0.5158	0.4071	2.22
Volume	litre × 10^{-1}	Placebo	>0.998	<0.0001	0.125	0.8693	0.3734	4.43
		Captopril	>0.999	<0.0001	0.071	0.6982	0.3773	3.67
		Captopril + HZ	>0.999	<0.0001	0.052	0.5204	0.3775	2.70
		Hydrochlorothiazide	>0.999	<0.0001	0.054	0.5734	0.3742	3.18
Potassium	mmol	Placebo	>0.999	<0.0001	0.036	0.6067	0.3391	3.88
		Captopril	>0.999	<0.0001	0.027	0.4752	0.3474	2.91
		Captopril + HZ	>0.999	<0.0001	0.018	0.3641	0.3357	2.19
		Hydrochlorothiazide	>0.999	<0.0001	0.024	0.5175	0.3295	3.39
Calcium	mmol × 10^{-1}	Placebo	>0.999	<0.0001	0.038	0.6322	0.3444	3.88
		Captopril	>0.999	<0.0001	0.043	0.6895	0.3412	4.12
		Captopril + HZ	>0.999	<0.0001	0.026	0.4783	0.3434	2.91
		Hydrochlorothiazide	>0.999	<0.0001	0.033	0.5515	0.3530	3.16
Magnesium	mmol × 10^{-1}	Placebo	>0.998	<0.0001	0.041	0.7063	0.3472	4.12
		Captopril	>0.998	<0.0001	0.043	0.7733	0.3329	4.84
		Captopril + HZ	>0.998	<0.0001	0.026	0.6253	0.3245	3.87
		Hydrochlorothiazide	>0.997	<0.0002	0.036	0.7988	0.3036	5.56
Phosphate	mmol	Placebo	>0.993	<0.0006	0.111	1.2791	0.3536	7.28
		Captopril	>0.998	<0.0001	0.066	0.9625	0.3390	5.82
		Captopril + HZ	0.998	<0.0002	0.083	1.0424	0.3526	6.07
		Hydrochlorothiazide	>0.995	<0.0004	0.101	1.2533	0.3381	7.76
Creatinine	mmol	Placebo	>0.995	<0.0004	0.167	1.4856	0.3770	7.80
		Captopril	>0.998	<0.0001	0.080	0.8818	0.3930	4.39
		Captopril + HZ	>0.998	<0.0001	0.070	0.8557	0.3848	4.39
		Hydrochlorothiazide	>0.995	<0.0004	0.092	1.0823	0.3876	5.36
Urate	mmol × 10^{-1}	Placebo	>0.998	<0.0001	0.034	0.6511	0.3445	3.88
		Captopril	0.999	<0.0001	0.031	0.6110	0.3350	3.87
		Captopril + HZ	>0.999	<0.0001	0.029	0.5314	0.3496	2.91
		Hydrochlorothiazide	>0.999	<0.0001	0.041	0.6490	0.3623	3.64

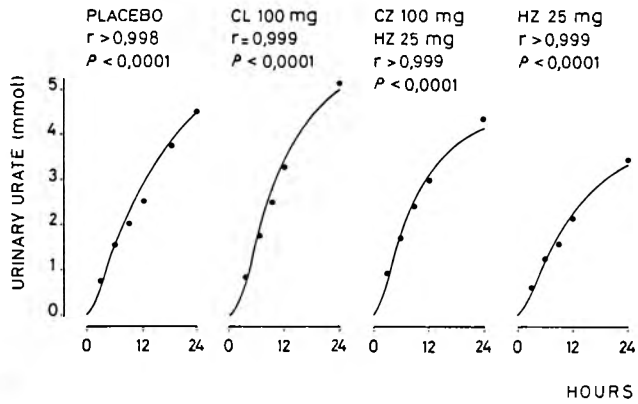

FIG. 1. Accumulated excretions of urate in urine after single administration of placebo, captopril (CL), a combination of captopril and hydrochlorothiazide (CZ) and hydrochlorothiazide (HZ) to 13 healthy volunteers at hour 0 (0800). The mathematical model fitting the data is presented in equation (1).

uretic capacity which becomes apparent when plasma aldosterone concentration is high, as occurs when dietary Na^+ is restricted. Moreover the combination of captopril and hydrochlorothiazide induced significantly higher 24-h renal outputs of Cl^- and Na^+ than hydrochlorothiazide (Table 2) and advanced the urinary Cl^- and Na^+ urinary flows more than hydrochlorothiazide (Table 3). Thus, the chloriuretic and natriuretic properties of captopril are exhibited as a potentiation of the chloriuretic and natriuretic effects of hydrochlorothiazide, possibly because the natriuretic action of the diuretic raises plasma aldosterone. Captopril also accelerated the urinary flows of Cl^- and Na^+,

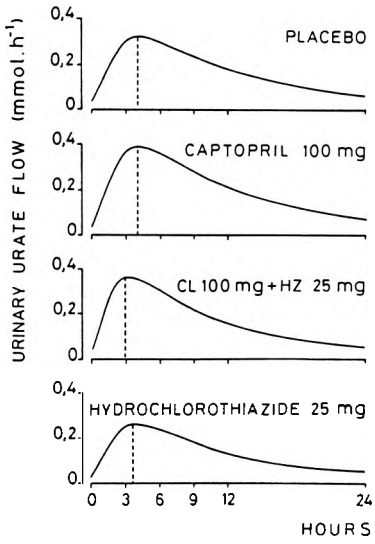

FIG. 2. Urinary urate flows, derived from the curves depicted in Fig. 1, as functions of time. The mathematical model accounting for the functions is presented in equation (2). The areas under the curves between any two times represent the total amount of urate excreted during the interval.

a fact that may be regarded as the expression of chloriuretic and natriuretic capacities of a substance, since the time courses of urinary flows are sensitive indicators of the urinary excretory effects of a drug when small samples are studied.

Captopril did not affect the 24-h urinary excretion of K^+ significantly, whereas the combination of captopril and hydrochlorothiazide and hydrochlorothiazide alone increased it to a similar extent (Tables 1 and 2). All formulations accelerated urinary K^+ flows; the advance provoked by captopril (Table 3) was possibly a consequence of its similar effect on Na^+ flow. Studies with amiloride, following a similar protocol, have shown that both urinary Na^+ and K^+ flows are accelerated by the drug, although Na^+ urinary excretion and K^+ retention are induced (21). Since urinary K^+ output depends largely on the Na^+-K^+, H^+ exchange at the end of the distal convoluted tubule (22), which is under positive control by aldosterone, captopril should act as a urinary-K^+ sparer (23) by depressing angiotensin-I-dependent aldosterone synthesis (24). Some investigations into the chronic effects of captopril on plasma K^+ in hyperaldosteronemic patients confirm this view (12,24,25). Studies in hypertensive patients have shown that captopril attenuates hydrochlorothiazide-induced urinary K^+ losses (12,17,26). These reports are consistent with the present finding that 6 h after dosing plasma K^+ was significantly reduced by hydrochlorothiazide in comparison with pretreatment, but not by the combination of captopril and hydrochlorothiazide (Table 4).

All active formulations significantly increased 24-h magnesiuria compared with placebo (Table 2). Since neither captopril, the combination of captopril and hydrochlorothiazide, nor hydrochlorothiazide alone affected calciuresis, their magnesiuretic effect might be due to direct blockade of Mg^{2+} reabsorption from the nephron, to inducement of Mg^{2+} excretion through the nephronal wall, or to the increase in renal 24-h phosphate excretion which all three active medications induced (Table 2). The magnesiuretic effect of hydrochlorothiazide is well known (27,28). Captopril caused significantly less 24-h magnesiuresis than the combination of captopril and hydrochlorothiazide and hydrochlorothiazide alone, and the combination significantly reduced the 24-h urinary Mg^{2+} losses caused by the diuretic. These results indicate that the combination of captopril and hydrochlorothiazide is less likely to induce somatic Mg^{2+} depletion than hydrochlorothiazide alone, since captopril counteracts the magnesiuretic effect of hydrochlorothiazide to some extent. The acute and chronic effects of captopril and the combination of captopril and hydrochlorothiazide on calciuresis and magnesiuresis merit further detailed assessments (27–30). Mg^{2+} depletion induced by diuretics, especially in pa-

TABLE 4. *Serum variables before (hour 0) and after monodosing 13 healthy volunteers with placebo, captopril 100 mg, a combination of captopril 100 mg plus hydrochlorothiazide (HZ) 25 mg, and hydrochlorothiazide 25 mg (means ± SEM)*

Serum variable	Medication	Hours after dosing		
		0	6	24
Chloride	Placebo	106.1 ± 0.2	104.6 ± 0.2*****	106.0 ± 0.6
(mmol · L^{-1})	Captopril	104.7 ± 0.8	104.6 ± 0.5	105.5 ± 0.4
	Captopril + HZ	104.9 ± 0.7	103.2 ± 0.8	104.1 ± 0.6
	Hydrochlorothiazide	105.4 ± 0.3	103.9 ± 0.5*****	103.6 ± 0.4*****
Sodium	Placebo	145.9 ± 0.5	146.0 ± 0.4	147.8 ± 0.7***
(mmol · L^{-1})	Captopril	146.5 ± 0.6	146.7 ± 0.5	147.1 ± 1.0
	Captopril + HZ	145.3 ± 0.3	144.2 ± 0.8	145.0 ± 0.8
	Hydrochlorothiazide	146.3 ± 0.5	145.0 ± 0.5*	146.5 ± 0.5
Potassium	Placebo	4.32 ± 0.07	4.17 ± 0.06	4.48 ± 0.10
(mmol · L^{-1})	Captopril	4.39 ± 0.13	4.41 ± 0.08	4.62 ± 0.11
	Captopril + HZ	4.05 ± 0.05	4.08 ± 0.08	4.14 ± 0.13
	Hydrochlorothiazide	4.26 ± 0.07	3.93 ± 0.04****	4.30 ± 0.07
Calcium	Placebo	2.49 ± 0.02	2.54 ± 0.02*	2.58 ± 0.03***
(mmol · L^{-1})	Captopril	2.55 ± 0.02	2.58 ± 0.04	2.56 ± 0.05
	Captopril + HZ	2.51 ± 0.02	2.49 ± 0.06	2.57 ± 0.3*
	Hydrochlorothiazide	2.55 ± 0.05	2.66 ± 0.06****	2.71 ± 0.03****
Magnesium	Placebo	0.85 ± 0.01	0.88 ± 0.02*	0.86 ± 0.03
(mmol · L^{-1})	Captopril	0.88 ± 0.02	0.86 ± 0.02	0.88 ± 0.02
	Captopril + HZ	0.82 ± 0.01	0.83 ± 0.01	0.82 ± 0.01
	Hydrochlorothiazide	0.84 ± 0.01	0.82 ± 0.01	0.82 ± 0.01*
Phosphate	Placebo	1.48 ± 0.11	1.47 ± 0.08	1.44 ± 0.07
(mmol · L^{-1})	Captopril	1.32 ± 0.07	2.18 ± 0.12*****	1.46 ± 0.05
	Captopril + HZ	1.35 ± 0.04	1.52 ± 0.04*****	1.39 ± 0.05
	Hydrochlorothiazide	1.29 ± 0.08	1.52 ± 0.07***	1.42 ± 0.07
Creatinine	Placebo	94.1 ± 4.1	96.5 ± 3.7	102.0 ± 3.4**
(μmol · L^{-1})	Captopril	105.4 ± 5.8	100.5 ± 3.7	109.6 ± 3.2
	Captopril + HZ	95.0 ± 2.3	101.5 ± 2.9**	100.7 ± 2.8***
	Hydrochlorothiazide	111.5 ± 9.5	108.5 ± 3.0	109.8 ± 2.6
Urate	Placebo	0.35 ± 0.02	0.32 ± 0.02*****	0.35 ± 0.02
(mmol · L^{-1})	Captopril	0.35 ± 0.02	0.33 ± 0.02*****	0.35 ± 0.02
	Captopril + HZ	0.32 ± 0.01	0.32 ± 0.01	0.35 ± 0.02****
	Hydrochlorothiazide	0.34 ± 0.01	0.34 ± 0.01	0.37 ± 0.01****
Total CO_2	Placebo	22.1 ± 1.1	23.2 ± 1.0**	21.1 ± 1.7
(mmol · L^{-1})	Captopril	22.6 ± 0.6	23.4 ± 0.5**	23.2 ± 0.7
	Captopril + HZ	23.8 ± 0.5	24.7 ± 0.4	24.0 ± 0.6
	Hydrochlorothiazide	24.6 ± 1.8	26.6 ± 0.9	27.5 ± 0.6
Glucose	Placebo	5.2 ± 0.1		5.3 ± 0.1
(mmol · L^{-1})	Captopril	5.3 ± 0.2		5.2 ± 0.2
	Captopril + HZ	5.4 ± 0.1		5.3 ± 0.1
	Hydrochlorothiazide	5.0 ± 0.1		5.3 ± 0.1

Significances of the differences with respect to hour-0 means: *$p < 0.05$; **$p < 0.02$; ***$p < 0.01$; ****$p < 0.005$; *****$p < 0.001$.

tients also treated with cardiac glycosides, is a more important determinant of cardiac arrhythmias and myocardial infarction than K^+ depletion (see 29–30 for reviews). If the magnesiuretic effect of captopril is confirmed, it might explain why patients with cardiac insufficiency have apparently died of cardiac arrhythmias or myocardial infarction within 1–6 months of adding captopril to a regimen that already included diuretics and digoxin (31).

The effects of captopril, the combination of captopril and hydrochlorothiazide, and hydrochlorothiazide alone upon renal creatinine excretion might be expected on the basis of established knowledge.

Captopril significantly increased the 24-h renal output of urate compared with placebo. The mean 24-h excretion after captopril was also significantly higher than those after the combination of captopril and hydrochlorothiazide and hydrochlorothiazide alone. The combination of captopril and hydrochlorothiazide did not affect 24-h urinary urate excretion, and hydrochlorothiazide significantly decreased it (Table 2). Captopril did not affect urinary urate output flow, whereas the combination of captopril and hydrochlorothiazide and hydrochlorothiazide alone accelerated it (Table 3). Captopril decreased mean plasma urate serum concentration 6 h after administration, while the combination of captopril and hydrochlorothiazide and hydrochlorothiazide alone did not affect it (Table 3). Conversely, the mean plasma urate concentration rose significantly 24 h after the combination of captopril and hydrochlorothiazide and hydrochlorothiazide

alone, while it did not change 24-h after captopril. These results identify captopril as an uricosuric agent. Previous studies in hypertensives chronically treated with captopril, hydrochlorothiazide, and captopril plus hydrochlorothiazide have shown that captopril helps attenuate the increase in plasma urate induced by hydrochlorothiazide (17,26). The mechanism by which captopril increases the renal excretion of urate might be an increase of glomerular filtration rate, a decrease in proximal tubular reabsorption of filtered and/or secreted urate, or an increase in urate tubular secretion (32,33).

The uricosuric effect of captopril has important clinical implications. Captopril would be particularly useful in patients with hypertension or heart failure complicated by gout or asymptomatic hyperuricemia, when administered alone or with the purpose of reducing urate retention due to diuretics. Nevertheless, caution should be exercised when using captopril in patients with hyperuricemia due to increased uric acid production (daily urinary excretion of urate higher than 4.76 mmol), with urinary urate stones, or with dehydration (32,34). Increasing fluid intake to at least 2 L daily and alkalinization or urine would prevent the precipitation or urate in the urinary tract of these patients (32).

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PAPER C14

The Effects of Single Doses of Muzolimine Upon Urinary Solute and Fluid Excretion

This paper described the first in-depth study of acute urinary responses to muzolimine, a new loop diuretic. The concept, clinical work, mathematical analysis and preparation of the manuscript were shared by the first two authors. Mr. van der Byl provided computer facilities.

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The effects of single doses of muzolimine upon urinary solute and fluid excretion

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Summary: The natriuretic effect of many loop diuretics is followed by an important decrease – rebound undershoot – in renal Na^+ excretion, which is accompanied by thirst and fluid reposition. Also most loop diuretics increase K^+ and Mg^{2+} urinary outputs, thus leading to somatic depletion of these cations and subsequent cardiac arrhythmias. The objectives of the present study were to describe the urinary outputs and flows of several solutes after various doses of muzolimine.

Experiments were carried out in ten healthy adult volunteers given monodoses of placebo and of muzolimine 20, 30 and 40 mg on separate days and in random order. Urine collected at 3, 6, 9, 12 and 24 h after dosing was analysed for solutes.

Muzolimine 20 mg did not increase mean 24 h urinary Na^+ output significantly with respect to placebo. Muzolimine 30 mg exerted maximal diuretic, chloriuretic and natriuretic effects, while not affecting the mean 24 h urinary outputs of K^+ and Mg^{2+} significantly. Muzolimine 30 mg caused a small undershoot in urinary Na^+ flow after completion of its natriuretic effect.

Muzolimine 30 mg should be considered a first choice loop diuretic since it only causes a mild post-natriuretic undershoot and does not increase urinary Mg^{2+} output significantly.

Key words: diuretic, magnesium, muzolimine, potassium, sodium

Introduction

Urinary flows of fluid and solutes after the administration of placebo and various diuretic formulations have recently been described, as continuous functions of time, through fitting a mathematical model to the corresponding outputs accumulated at definite times after dosing and by evaluating its time derivative (8). This procedure greatly facilitates the analysis and description of the dose- and time-dependent pharmacodynamics of different diuretic formulations (4, 8–13).

The loop diuretic muzolimine has been studied from the point of view of its acute effects on urinary excretion of fluid and solutes in healthy volunteers (2, 3), though not in sufficient detail to cover all essential electrolytes or to allow close comparison between different doses of the diuretic through utilization of the Reyes and Leary mathematical model (8).

The objectives of the present study were to describe the continuous time courses of renal fluid and solute excretion after single doses of muzolimine 20, 30 and 40 mg and to compare these to reference standards collected for furosemide 40 mg. The solutes studied included Mg^{2+}, given that the urinary loss of this cation provoked by furosemide 40 mg constitutes the principal determinant of serious diuretic-induced cardiac arrhythmias (1, 9, 14).

Subjects and methods

Subjects and experimental design

Two separate experiments were conducted each involving ten healthy male adult students who volunteered to participate in the study after a full explanation of its implications had been given. All subjects were aged between 18 and 24 and had taken no other medication within the previous 2 months. None were obese or had a history of renal, cardiovascular or metabolic disorders. Smokers were not studied.

A standardised diet containing 200 to 220 mmol of Na^+ and 2500 ml of water was prescribed on treatment days and during the 24 h preceding each of them (control days). In the first experiment subjects received placebo, 20 mg muzolimine and 30 mg muzolimine separately and in random order on three different treatment days, separated by at least 7 days. Placebo and muzolimine 40 mg were administered alike in the second experiment. Medications were given at 80'clock with 100 ml tap water. Volunteers were confined to a metabolic ward during study days when ingestion of alcohol, caffeine or medicines other than the trial formulations was forbidden.

All laboratory analyses were carried out by technologists who were unaware of the protocol being followed. All urines passed on treatment days and during the previous 24 h were collected. Urine collected from 0–3, 3–6, 6–9, 9–12 and 12–24 h after dosing on treatment days and pooled urine collected during the control days was analysed. Urinary volumes and the concentrations of Cl^-, Na^+, K^+, Mg^{2+}, Zn^{2+}, total inorganic phosphate and creatinine were measured in each urine specimen.

Solute concentrations in urine specimens were analysed as follows: Na^+, K^+ and Cl^- were measured by the ion-selective electrode technique using a Nova 4 analyser. Mg^{2+} and Zn^{2+} were measured by

Table 1. Accumulated excretions of urinary variables after the administration of placebo and of several doses of muzolimine to ten healthy volunteers. Mean ± S.E.M.

Urinary variable	Medication and dose	Hours after dosing				
		3	6	9	12	24
Volume (litres)	Placebo for MUZ 20 & 30	0.30±0.04	0.78 ±0.14	1.45±0.19	1.92±0.17	2.84 ±0.15
	Muzolimine 20	0.60±0.09	1.07 ±0.10	1.63±0.17	2.01±0.19	2.83±0.21
	Muzolimine 30	0.88±0.13	1.44[c] ±0.15	2.05±0.15	2.44±0.17	3.25[c] ±0.15
	Placebo for MUZ 40	0.19±0.02	0.57 ±0.08	1.21±0.15	1.77±0.15	2.54 ±0.14
	Muzolimine 40	0.88±0.11	1.53[b] ±0.12	1.93±0.14	2.17±0.17	2.80 ±0.17
Chloride (mmol)	Placebo for MUZ 20 & 30	38± 4	71 ± 8	113±12	148±11	210 ±12
	Muzolimine 20	70±10	129[c] ±10	166±11	192±12	233 ±12
	Muzolimine 30	117±17	177[c] ±21	221±18	248±20	290[d] ±19
	Placebo for MUZ 40	35± 5	68 ± 9	119±13	170±13	234 ±18
	Muzolimine 40	122±11	191[b] ±11	233±10	254±10	289[b] ±10
Sodium (mmol)	Placebo for MUZ 20 & 30	31± 5	57 ± 8	95±11	128±12	186 ±13
	Muzolimine 20	54± 8	102[c] ± 9	137±11	162±14	199 ±16
	Muzolimine 30	97±16	145[c] ±19	183±18	209±22	248[c] ±21
	Placebo for MUZ 40	31± 5	60 ± 8	111±13	161±14	225 ±18
	Muzolimine 40	102±10	158[b] ±11	199±11	222±11	264[a] ±10
Potassium (mmol)	Placebo for MUZ 20 & 30	11.0±1.8	21.6 ±3.4	31.7±3.3	39.9±3.5	53.7 ±5.4
	Muzolimine 20	13.2±1.9	26.7 ±2.6	34.8±2.8	40.2±2.5	53.8 ±4.1
	Muzolimine 30	19.7±2.8	34.6[b] ±4.0	45.5±3.2	52.7±3.9	65.2 ±5.1
	Placebo for MUZ 40	7.8±1.3	17.3 ±2.7	29.0±3.1	39.7±3.3	40.4 ±4.9
	Muzolimine 40	19.6±2.4	35.7[b] ±3.0	44.7±3.4	50.4±3.7	60.8[a] ±3.9
Magnesium (mmol)	Placebo for MUZ 20 & 30	0.77±0.07	1.28 ±0.12	2.02±0.19	2.69±0.27	4.68±0.48
	Muzolimine 20	1.08±0.19	1.71 ±0.23	2.21±0.23	2.72±0.27	4.38±0.34
	Muzolimine 30	1.30±0.14	1.94[c] ±0.18	2.58±0.17	3.19±0.29	5.08±0.40
	Placebo for MUZ 40	0.76±0.08	1.22 ±0.12	1.96±0.19	2.65±0.28	4.77±0.52
	Muzolimine 40	1.54±0.18	2.24[b] ±0.23	2.78±0.23	3.20±0.27	5.31±0.34
Zinc (mg)	Placebo for MUZ 20 & 30	0.12±0.02	0.20±0.03	0.31±0.04	0.38±0.05	0.62±0.06
	Muzolimine 20	0.15±0.03	0.25±0.03	0.33±0.04	0.39±0.04	0.68±0.07
	Muzolimine 30	0.14±0.02	0.22±0.03	0.31±0.04	0.38±0.04	0.61±0.04
	Placebo for MUZ 40	0.13±0.02	0.19±0.02	0.27±0.03	0.36±0.03	0.66±0.07
	Muzolimine 40	0.16±0.02	0.25±0.02	0.31±0.03	0.35±0.04	0.63±0.05
Total inorganic phosphate (mmol)	Placebo for MUZ 20 & 30	3.3±0.5	7.3±0.9	11.9±0.6	16.3±0.9	29.2±3.0
	Muzolimine 20	4.1±1.0	6.8±1.1	11.2±1.5	15.1±1.8	30.0±3.3
	Muzolimine 30	3.9±0.6	7.5±0.9	13.2±1.0	17.0±1.1	32.9±2.7
	Placebo for MUZ 40	2.8±0.4	6.3±0.6	11.5±0.6	17.6±1.2	30.7±3.4
	Muzolimine 40	4.4±0.9	8.9±1.1	14.1±1.4	18.1±1.5	37.4±1.4
Creatinine (μmol)	Placebo for MUZ 20 & 30	3.3±0.2	5.2 ±0.4	7.4±0.5	10.8±0.6	19.3±1.1
	Muzolimine 20	2.6±0.3	4.7 ±0.5	6.6±0.6	8.9±0.6	16.8±1.4
	Muzolimine 30	3.3±0.3	7.2[c] ±1.3	9.6±1.2	11.9±1.2	20.1±2.1
	Placebo for MUZ 40	3.0±0.3	4.8 ±0.5	7.3±0.5	11.0±0.6	19.5±1.2
	Muzolimine 40	3.3±0.1	5.5 ±0.2	7.7±0.3	9.9±0.5	19.8±0.5

MUZ = Muzolimine.
All values corresponding to hour 3 were derived from nine volunteers ans missing values were added to hour 6 accumulated outputs.
Significances of the differences with respect to mean 6 and 24 hour accumulated values after placebo: [a] $p < 0.05$; [b] $p < 0.02$; [c] $p < 0.01$; [d] $p < 0.001$.

atomic absorption using a Varian 275 275 instrument. Total inorganic phosphate was evaluated colorimetrically (Clinical Sciences kit) on a Beckman Trace 3 spectrophotometer. Creatinine was measured using a Peridochrom (Boehringer Mannheim) kit and the same spectrophotometer.

Mathematical methods

The mean experimental values of the urinary volume and solutes, M, accumulated by the end of each post-dosing collecting period, as functions of time, t, were fitted by a mathematical model:

$$20M/\log(100-M) = \exp\{[2.30(t-t_1)] / (a+bt)\},$$

where t_1 is the time at which M = 0.1 unit used for the fitting and a (time) and b (dimensionless) are regression parameters of the function. The fitting procedure has been described previously (8). Flows of urinary variables were defined as:

$$dM/dt = (a+bt_1)/\{(a+bt)^2\,[(0.43/M) + (0.43)^2/(100-M)\log(100-M)]\}.$$

Times to mean peak excretion of urinary variables after dosing were evaluated on a computer from the flow functions, through an iterative procedure.

Standard parametric statistical techniques, paired t-test and correlation and regression on linearized data (4) were used. Normality of frequency distributions and homoscedasticity of sample variances were evaluated, as appropriate, through the chi square test and the F ratio respectively. All statistical tests were two-tailed and p = 0.05 was considered the limit of significance.

Results

No significant differences were found between the mean 24-h accumulated urinary outputs of fluid and solutes after placebo and those yielded on control days.

The urinary outputs of fluid, Cl^-, Na^+, K^+, Mg^{2+}, Zn^{2+}, total inorganic phosphate and creatinine accumulated at 3, 6, 9, 12 and 24 h after the intake of placebo and of muzolimine 20, 30 and 40 mg are shown in Table 1. The significances of the differences between the post-muzolimine and the post-placebo mean outputs accumulated 6 and 24 h after dosing are also listed in Table 1. Muzolimine 20 mg significantly raised the mean 6 h accumulated outputs of Cl^- and Na^+, but did not affect the mean 24 h outputs of fluid or of any of the studied solutes significantly (Table 1). Muzolimine 30 and 40 mg significantly increased the mean urinary outputs of fluid, Cl^-, Na^+, K^+ and Mg^{2+} produced during the first 6 h after dosing with respect to placebo (Table 1). However, many of these significances were lost for the 24 h outputs (Table 1). Muzolimine 30 mg exhibited significant maximal 24 h diuretic, chloriuretic and natriuretic effects but it did not increase the 24 h outputs of K^+ or Mg^{2+} significantly (Table 1). Muzolimine 40 mg significantly increased the 24 h urinary outputs of Cl^-, Na^+ and K^+, but did not augment those of fluid and Mg^{2+} to a statistically significant extent (Table 1). None of the formulations affected the mean 24 h outputs of Zn^{2+} and creatinine consistently or significantly (Table 1), though they increased the mean 24 h output of inorganic phosphate in a dose-related but insignificant manner (Table 1).

The statistical features of the linearized versions of the M(t) functions and their parameter values are shown in Table 2. The Reyes and Leary mathematical model (8) fitted the data satisfactorily in all cases, thus permitting evaluation of the urinary fluid and solute flows as functions of time and the times to peak flows after dosing (Table 2). Figure 1 depicts the mean urinary Na^+ outputs accumulated after the administration of muzolimine 40 mg and the corresponding urinary Na^+ flow. After an initial natriuretic effect, muzolimine 40 mg gave rise to an important "undershoot" in urinary Na^+ flow,

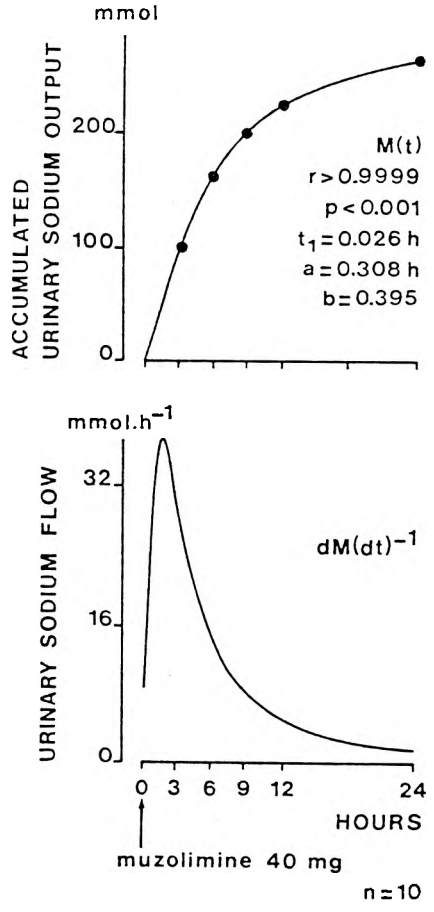

Fig. 1. Top: mean accumulated urinary Na^+ flow after the administration of muzolimine 40 mg per os to ten healthy volunteers at hour 0 (0800 h). The Reyes and Leary (8) mathematical model (continuous-function curve) has been fitted to the experimental means (dots).

Bottom: mean urinary Na^+ flow after the administration of muzolimine 40 mg per os to ten healthy volunteers at hour 0 (0800 h). The graph has been evaluated as the time derivative of the function in the top panel. Any segment of area under the curve represents the amount of electrolytes excreted between the times that constitute the area limits.

Table 2. Numerical features of the functions M(t) accounting for the time courses of accumulated excretions of urinary variables and times from dosing to peak urinary flows to the variables derived as the t values at which $d^2M/dt^2 = 0$

Urinary variable	Unit used in calculations	Medication	Correlation of linearized M(t)	Significance of r with respect to 0		Original ordinates value of linearized M (t)	Slope of linearized M (t)	Time to peak excretion
			r	p	t_l (hour)	a (hour)	b	t_{max} (hour)
Volume	litre × 10^{-1}	Placebo for MUZ 20 & 30	0.9997	< 0.001	0.0912	0.8680	0.3633	4.64
		Muzolimine 20	0.9998	< 0.001	0.0452	0.5957	0.3778	3.17
		Muzolimine 30	0.9998	< 0.001	0.0307	0.4636	0.3730	2.44
		Placebo for MUZ 40	0.9992	< 0.001	0.1427	1.0728	0.3603	6.35
		Muzolimine 40	> 0.9999	< 0.001	0.0308	0.3888	0.3873	1.96
Chloride	mmol × 10	Placebo for MUZ 20 & 30	0.9997	< 0.001	0.0701	0.7381	0.3950	3.43
		Muzolimine 20	> 0.9999	< 0.001	0.0386	0.3972	0.4006	1.73
		Muzolimine 30	> 0.9999	< 0.001	0.0231	0.2866	0.3884	1.47
		Placebo for MUZ 40	0.9997	< 0.001	0.0765	0.8060	0.3822	4.15
		Muzolimine 40	> 0.9999	< 0.001	0.0221	0.2467	0.3901	1.23
Sodium	mmol × 10	Placebo for MUZ 20 & 30	0.9995	< 0.001	0.0878	0.8446	0.4005	3.92
		Muzolimine 20	> 0.9999	< 0.001	0.0498	0.4690	0.4102	1.98
		Muzolimine 30	> 0.9999	< 0.001	0.0278	0.3224	0.3992	1.48
		Placebo for MUZ 40	0.9995	< 0.001	0.0873	0.8785	0.3818	4.64
		Muzolimine 40	> 0.9999	< 0.001	0.0263	0.3083	0.3948	1.47
Potassium	mmol	Placebo for MUZ 20 & 30	0.9999	< 0.001	0.0245	0.4876	0.3357	2.91
		Muzolimine 20	0.9999	< 0.001	0.0204	0.4031	0.3395	2.43
		Muzolimine 30	0.9999	< 0.001	0.0137	0.3369	0.3279	2.18
		Placebo for MUZ 40	0.9998	< 0.001	0.0346	0.5572	0.3371	3.40
		Muzolimine 40	> 0.9999	< 0.001	0.0138	0.2975	0.3346	1.94
Magnesium	mmol × 10^{-1}	Placebo for MUZ 20 & 30	0.9982	< 0.001	0.0349	0.7184	0.3382	4.36
		Muzolimine 20	0.9986	< 0.001	0.0251	0.5409	0.3506	2.91
		Muzolimine 30	0.9985	< 0.001	0.0208	0.5212	0.3408	3.15
		Placebo for MUZ 40	0.9989	< 0.001	0.0355	0.7526	0.3358	4.60
		Muzolimine 40	0.9990	< 0.001	0.0176	0.4804	0.3401	2.90
Zinc	mg × 10^{-2}	Placebo for MUZ 20 & 30	0.9987	< 0.001	0.0216	0.5785	0.3232	3.63
		Muzolimine 20	0.9977	< 0.001	0.0184	0.5583	0.3191	3.62
		Muzolimine 30	0.9985	< 0.001	0.0193	0.5340	0.3273	3.39
		Placebo for MUZ 40	0.9982	< 0.001	0.0213	0.6656	0.3166	4.59
		Muzolimine 40	0.9985	< 0.001	0.0167	0.5266	0.3258	3.38
Total inorganic phosphate	mmol	Placebo for MUZ 20 & 30	0.9992	< 0.001	0.0821	0.9542	0.3610	5.35
		Muzolimine 20	0.9969	< 0.001	0.0663	0.9733	0.3608	5.34
		Muzolimine 30	0.9983	< 0.001	0.0700	0.9527	0.3533	5.10
		Placebo for MUZ 40	0.9995	< 0.001	0.0951	1.0626	0.3514	6.32
		Muzolimine 40	0.9987	< 0.001	0.0609	0.9245	0.3463	5.57

Continued Table 2

Urinary variable	Unit used in calculations	Medication	Correlation of linearized M(t)	Significance of r with respect to 0		Original ordinates value of linearized M (t)	Slope of linearized M (t)	Time to peak excretion
			r	p	t_l (hour)	a (hour)	b	t_{max} (hour)
Creatinine	μmol	Placebo for MUZ 20 & 30	0.9969	< 0.001	0.0825	0.9916	0.3953	4.63
		Muzolimine 20	0.9972	< 0.001	0.1034	1.0577	0.4046	4.89
		Muzolimine 30	0.9991	< 0.001	0.0809	0.8213	0.3972	3.92
		Placebo for MUZ 40	0.9984	< 0.001	0.0896	1.0514	0.3914	5.36
		Muzolimine 40	0.9980	< 0.001	0.0821	0.9975	0.3942	4.87

MUZ = Muzolimine

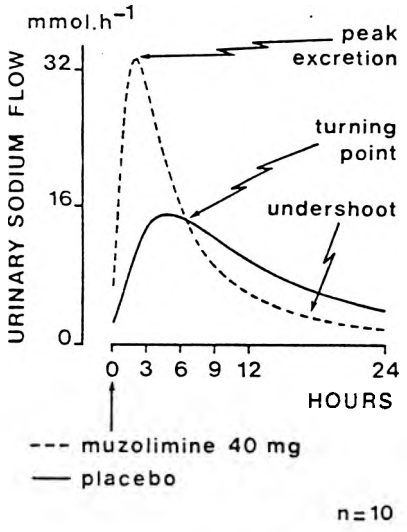

Fig. 2. Mean accumulated Na^+ flows after the separate administration of placebo and of muzolimine 40 mg to ten healthy volunteers at hour 0 (0800 h). Any segment of area between one of the curves and the time axis represents the amount of electrolyte excreted between the times that constitute the area limits. The flow function after muzolimine 40 mg shows a natriuretic phase, where it is above the after-placebo Na^+ flow curve, and an undershoot phase which follows, where the postmuzolimine urinary sodium flow is below its after-placebo counterpart. Peak excretions occurred 4.64 h after placebo and 1.47 h after muzolimine 40 mg. The turning point is the first point after peak excretions where the functions after each formulation assume an equal value; this point marks the end of the natriuretic phase and the beginning of the undershoot phase of the post-muzolimine urinary Na^+ flow.

i.e., to a Na^+ flow level below that after placebo (Figure 2). This undershoot counterbalanced the earlier natriuresis induced by muzolimine 40 mg to a great extent. All formulations of muzolimine accelerated the flows of fluid and of all solutes with respect to placebo (Table 2). However, fluid, Cl^-, Na^+ and K^+ flows were accelerated more than the urinary Mg^{2+} flows; muzolimine 20, 30 and 40 mg decreased the times to peak Mg^{2+} flow by 1.4, 1.2 and 1.7 h with respect to placebo, whereas the corresponding values for urinary Na^+ flows, which were dose-related, were 1.9, 2.4 and 3.2 h respectively (Table 2).

Discussion

The mathematical method used for describing urinary flows has been discussed elsewhere (8). When all other parameter values are kept constant, higher t_l and a and lower b values indicate lower rates of urinary flow. Time to peak excretion stands for a fixed relationship between t_l, a and b, since it is the time at which $d^2M/dt^2 = 0$. Thus, time to peak excretion is an overall expression of the rate of urinary flow, and consequently has clear physiological and mathematical meaning.

The fact that monodosing with muzolimine 20 mg did not increase the mean 24 h urinary output of fluid, Cl^- and Na^+ significantly, in this standard sample of healthy volunteers, disqualifies the formulation as an effective diuretic.

Muzolimine 30 mg induced significant increases in mean 24 h excretions of fluid, Cl^- and Na^+, and thus appears to be an effective diuretic formulation. The time-related pharmacodynamics were those of a loop diuretic (4) and consistent with the pharmacokinetics of the drug (5), although the effects of muzolimine 30 mg on urinary excretion of fluid and electrolytes were slightly more prolonged than those of the loop diuretics furosemide 40 mg (11) and piretanide 12 mg (4).

Muzolimine 30 and 40 mg significantly increased urinary mean Na^+ output with respect to placebo during the first 6 h after dosing (154% and 163% respectively, Table 1). However, both formulations reduced the urinary mean Na^+ output between 6 and 24 h (−20% and −35% respectively, Table 1), as depicted in Figure 2 for muzolimine 40 mg. This undershoot in urinary Na^+ flow, which occurs after the initial natriuretic response to all loop diuretics, would be caused principally by secondary hyperaldosteronism and by an increase in serum anti-

diuretic hormone (ADH), since the renin-angiotensin-aldosterone system and ADH secretion are activated by the prime renal effects of diuretics, and aldosterone and ADH attain a maximal concentration in plasma 4–6 h after the administration of a loop diuretic (6). The undershoot in natriuresis is usually accompanied by thirst and fluid reposition by the patients (A. J. Reyes, unpublished). Appropriate manoeuvres during the natriuretic response to loop diuretics might speed the relief of oedema, and minimize the hormonal response and therefore the undershoot. If patients with gravitational oedema remain supine for 2 to 3 h starting 1 h after the intake of muzolimine 30 mg, an increase in the amount of fluid and Na^+ within the vascular compartment could result when the diuretic-induced haemodynamic and metabolic shifts are stimulating the hormonal response; thus, natriuresis would be increased and the hormone-induced undershoot decreased.

Although muzolimine 30 mg did not increase the mean 24 h urinary K^+ and Mg^{2+} output significantly, it accelerated their corresponding flows (Table 2). These accelerations, which were also present after muzolimine 20 and 40 mg, were dose-related. Perhaps if the sample size had been larger, muzolimine 30 mg would have provoked significant 24 h hyperkaliuresis and hypermagnesiuresis; for this reason, the possibility that chronically administered muzolimine 30 mg could provoke K^+ and Mg^{2+} depletion when should be considered and plasma concentrations of these cations should be monitored during treatment.

After an initial rise in Mg^{2+} output with respect to placebo (52% increase during the first 6 h after dosing, Table 1), muzolimine 30 mg caused an undershoot in magnesiuresis (8% decrease in output between 6 and 24 h post dosing, Table 1). This counterbalance could be due to increased parathormone (PTH)-dependent reabsorption of Mg^{2+} in the loop of Henle (9), because loop diuretics augment renal Ca^{2+} excretion and thus reduce plasma free Ca^{2+} and elevate plasma PTH (7). In any event, comparisons between the present results and those of similar experiments where the loop diuretic furosemide 40 mg and the distal tubular diuretics clorexolone 10 mg, chlorthalidone 100 mg, hydrochlorothiazide 50 mg and xipamide 5, 10 and 20 mg were studied under similar experimental circumstances (9), indicate that muzolimine 30 mg may be safer than the above formulations, all of which provoked mean increases in 24 h urinary Mg^{2+} excretion which were statistically significant and of a greater magnitude than the small percentual rise induced by muzolimine 30 mg.

All doses of muzolimine delayed urinary Mg^{2+} flows with respect to urinary Na^+ flow. This phenomenon occurs after all hypermagnesiuretic diuretics, whether they be loop or distal tubular substances (9), and confirms Mg^{2+} and Na^+ are handled to a great extent independently by the kidney. The processes underlying this delay have not been fully elucidated though they would appear to be, at least partly, endocrine in nature (8).

The fact that muzolimine did not affect 24 h urinary Zn^{2+} output could be expected on the basis that loop diuretics, unlike distal tubular drugs (13), do not cause hyperzinciuria.

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SECTION D : CARDIOVASCULAR EFFECTS OF MEDICINES

Many medicines used in the treatment of a variety of conditions also have some effect upon arterial blood pressure. The papers which follow describe the cardiovascular effects of drugs used in the course of general anaesthesia, the treatment of bronchial asthma and angina or hypertension. Clinically significant changes in blood pressure, cardiac output, pulse rate or peripheral resistance are possible when these medicines are administered to patients with healthy cardiovascular systems and it is important that medical practitioners be aware of the possible occurrence of such changes both in normotensive and hypertensive patients.

PAPER D1

Cardiovascular Effects of Salbutamol: A Comparison with Isoprenaline

The technical expertise required to measure cardiac output was provided by Professor A.J. Coleman. Intubation of arteries was a shared procedure as was the analysis of data and preparation of the publication. The protocol followed was a joint venture.

Cardiovascular Effects of Salbutamol: A Comparison with Isoprenaline*

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SUMMARY

The immediate cardiovascular effects of inhaled salbutamol (200 μg) and an isoprenaline-phenylephrine (80 μg, 120 μg) mixture have been compared. Both drugs were administered by pressurized aerosol to normal volunteers. Significant increases in pulse rate and cardiac output and a decrease in peripheral vascular resistance followed inhalation of the isoprenaline-phenylephrine mixture. No such changes were noted after salbutamol.

S. Afr. Med. J., **46**, 1177 (1972).

In recent years it has been suggested that a link exists between deaths from bronchial asthma and the use of pressurized aerosols containing sympathomimetics.[1-3] The precise nature of this link has not been established, but the cardiac effects of nonselective β-adrenergic stimulants such as isoprenaline, may be involved.[2,4]

Salbutamol is a potent bronchodilator with little effect upon the β-adrenoreceptors of the heart at clinically effective doses.[5,6] The effects of salbutamol and isoprenaline on pulse rate and arterial pressures are well documented,[6-7] as is the effect of isoprenaline on cardiac output, but the effects of *inhaled* salbutamol on cardiac output have not been adequately studied in man. In this study a comparison has been made between the effects on pulse, blood pressure, and cardiac output of salbutamol, and a preparation containing isoprenaline hydrochioride and phenylephrine bitartrate, when administered by inhalation.

*Date received: 15 February 1972.

METHODS

Cardiac output, arterial pressure, and pulse rate were measured in 5 healthy volunteers, by previously described techniques.[5] Pressures were recorded continuously by Statham gauge and cardiac output was measured by dye dilution using a Philips XO 1000 oximeter/densitometer. These measurements were recorded 5 and 10 minutes after a 15-minute rest in the laboratory, and again 5, 10 and 15 minutes after the inhalation of placebo, 0,2 mg salbutamol, or a mixture of 0,08 mg isoprenaline hydrochloride and 0,12 mg phenylephrine bitartrate. This represented twice the normally recommended dose of each agent, and was given to simulate the kind of misuse that may commonly occur during a severe episode of bronchospasm. The bronchodilators were used in random order, 4 hours apart. Each subject was carefully instructed in the correct technique of inhalation. Rest periods and control measurements, as described above, preceded the inhalation of each sympathomimetic.

Stroke volume and total systemic arterial resistance were calculated mathematically. Tests of statistical significance were applied to the mean differences in measurements made before and 5, 10 and 15 minutes after inhalations of the drugs studied. Student's *t*-test was applied to the paired comparisons.

RESULTS

Detailed results are set out in Tables I - IV.

Placebo effect: No changes in pulse rate, mean arterial pressure, cardiac output, stroke volume, and peripheral vascular resistance, were noted after inhalation of placebo.

Salbutamol: No significant changes in mean arterial pressure, cardiac output, pulse rate, peripheral vascular resistance, and stroke volume, were noted.

Isoprenaline with phenylephrine: No significant changes in stroke volume, and mean arterial pressure occurred.

TABLE I. THE EFFECT OF ISOPRENALINE-PHENYLEPHRINE MIXTURE UPON HEART RATE, BLOOD PRESSURE AND CARDIAC OUTPUT

	Heart rate (beats/min)				Mean blood pressure (mmHg)				Cardiac output (L/min)			
		Min after inhalation				Min after inhalation				Min after inhalation		
Subject No.	Control	+5	+10	+15	Control	+5	+10	+15	Control	+5	+10	+15
1	71	75	72	71	77	80	76	72	7,5	7,0	7,4	7,4
2	71	83	83	88	86	84	80	80	4,4	5,9	6,0	6,7
3	58	71	64	65	93	100	100	94	4,7	6,9	6,1	6,9
4	69	79	79	75	95	76	78	76	6,0	7,5	6,8	6,1
5	80	100	83	83	107	100	100	100	5,0	5,9	6,6	4,4
Mean	70	82	76	76	92,0	88,0	87,0	84,0	5,5	6,6	6,6	6,3
SD	7,9	11,2	8,2	9,2	11,1	11,3	12,1	12,0	1,3	0,7	0,6	1,2
SEM	3,1	4,5	3,3	3,7	4,5	4,5	4,9	4,8	0,5	0,3	0,2	0,5
t		5,121	3,466	2,568		0,892	1,365	2,468		2,758	3,667	1,431
Significance of diff. from control		$P<0,01$	$P<0,05$	NS		NS	NS	NS		NS	$P<0,025$	NS

TABLE II. THE EFFECT OF ISOPRENALINE-PHENYLEPHRINE MIXTURE UPON PERIPHERAL VASCULAR RESISTANCE AND STROKE VOLUME

	Peripheral vascular resistance (dynes/sec cm^{-5})				Stroke volume (ml)			
		Min after inhalation				Min after inhalation		
Subject No.	Control	+5	+10	+15	Control	+5	+10	+15
1	837	914	822	778	103	93	103	104
2	1 572	1 139	1 067	955	62	71	72	76
3	1 599	1 159	1 312	1 090	100	87	83	90
4	999	811	918	997	88	95	86	81
5	1 719	1 356	1 212	1 818	62	59	80	53
Mean	1 345	1 075	1 066	1 128	83	81	85	81
SD	398,0	215,5	202,0	402,0	20,2	15,5	11,4	18,8
SEM	159,2	86,2	80,8	160,8	8,1	6,2	4,6	7,5
t		3,080	3,029	1,586		0,546	0,301	0,602
Significance of diff. from control		$P<0,05$	$P<0,05$	NS		NS	NS	NS

TABLE III. THE EFFECT OF SALBUTAMOL UPON HEART RATE, BLOOD PRESSURE AND CARDIAC OUTPUT

	Heart rate (beats/min)				Mean blood pressure (mmHg)				Cardiac output (l/min)			
Subject		Min after inhalation				Min after inhalation				Min after inhalation		
No.	Control	+5	+10	+15	Control	+5	+10	+15	Control	+5	+10	+15
1	60	58	56	54	77	76	74	76	6,2	5,6	5,4	5,6
2	79	79	83	75	73	80	78	76	5,2	5,9	5,5	5,0
3	64	63	63	65	104	100	100	100	5,5	5,8	6,4	6,0
4	74	75	68	71	87	88	84	84	6,5	6,0	6,2	6,3
5	63	68	58	63	97	100	100	102	4,8	5,2	4,9	6,3
Mean	68	69	66	66	88	89	87	88	5,6	5,7	5,7	5,8
SD	8,1	8,6	10,8	8,0	13,1	11,1	12,2	12,7	0,7	0,3	0,6	0,6
SEM	3,2	3,4	4,3	3,2	5,2	4,4	4,9	5,1	0,3	0,1	0,2	0,2
t		0,555	1,486	2,083		0,723	0,244	0,000		0,260	0,157	0,606
Significance of diff. from control		NS	NS	NS		NS	NS	NS		NS	NS	NS

TABLE IV. THE EFFECT OF SALBUTAMOL UPON PERIPHERAL VASCULAR RESISTANCE AND STROKE VOLUME

	Peripheral vascular resistance (dynes sec cm^{-5})				Stroke volume (ml)			
		Min after inhalation				Min after inhalation		
Subject No.	Control	+5	+10	+15	Control	+5	+10	+15
1	996	1 086	1 096	1 086	104	97	96	104
2	1 123	1 085	1 135	1 216	67	75	66	67
3	1 517	1 379	1 250	1 333	86	92	102	92
4	1 074	1 173	1 050	1 067	88	80	91	89
5	1 637	1 537	1 633	1 295	76	77	85	100
Mean	1 269	1 252	1 233	1 199	84	84	88	90
SD	287,6	200,1	235,7	120,1	13,9	9,7	13,8	14,4
SEM	115,0	80,0	94,3	48,1	5,6	3,9	5,5	5,8
t		0,39	0,666	0,925		0,000	1,032	1,511
Significance of diff. from control		NS	NS	NS		NS	NS	NS

An increased pulse rate was noted at 5, 10 and 15 minutes. This change was significant at 5 ($P<0,01$) and 10 minutes ($P<0,05$). Peripheral vascular resistance fell, but this, too, was significant only 5 and 10 minutes after using the aerosol ($P<0,05$).

There was a mean increase in cardiac output of 18,2% during the 15-minute period after inhalation of the isoprenaline-phenylephrine mixture. This change was significant at 10 minutes ($P<0,025$).

DISCUSSION

The efficacy of *inhaled* salbutamol as a bronchodilator is well established. Some of its effects on the cardiovascular system have been reported,[5-7] but to date cardiac output has not been measured directly in normal subjects.

Positive inotropic and chronotropic stimulation of the heart increases the metabolic demand for oxygen, which may not be met by patients with impaired gaseous exchange. Low arterial oxygen tensions may be worsened in asthmatics, even though airway obstruction is reversed,[6,9] thereby further aggravating the situation, particularly in patients with limited cardiac reserve. In anaesthetized animals these effects may lead to cardiac arrest.[10] In the experiments reported here, salbutamol, unlike isoprenaline, did not stimulate cardiac β-adrenoreceptors and thus appears unlikely to cause serious cardiac arrhythmias when it is given by aerosol inhalation. It must be stressed, however, that the relevance of such arrhythmias has recently been questioned,[11] and that the development of resistance to bronchodilators may also contribute to the death rate from asthma.

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PAPER D2

The Immediate Cardiovascular Effects of Pancuronium, Alcuronium and Tubocurarine In Man

The clinical work described was shared by the authors as was preparation of the manuscript.

The immediate cardiovascular effects of pancuronium, alcuronium and tubocurarine in man

A. J. Coleman J. W. Downing W. P. Leary D. G. Moyes M. Styles

Previous studies in man on the cardiovascular effects of the muscle relaxant pancuronium have been conflicting. Kelman & Kennedy[1] found a significant increase in heart rate, blood pressure and cardiac output. McDowell & Clark[2] and Kolliker[3], reported a fall in blood pressure with no change in heart rate. Baird & Reid[4] and Fastner & Agoston[5], found no significant effect on the heart rate and blood pressure and Loh[6] found that pancuronium increased both blood pressure and heart rate.

The discrepancies between their reports can be reasonably attributed to differences in methods of study. There were differences in premedication and in the degree of surgical stimulation. Measurements were sometimes made during endotracheal intubation[7], which is a procedure well known to cause tachycardia[8] and several patients were receiving digitalis. Arterial carbon dioxide levels were not always controlled and some studies were performed upon patients receiving volatile anaesthetic agents. There have also been discrepancies too, for similar reasons, in reports on the cardiovascular effects of tubocurarine and alcuronium[9–13].

In evaluating a new drug it is useful to compare it with established agents which fulfil similar roles in clinical practice. In this study pancuronium, a relatively new drug, has been compared with tubocurarine and alcuronium administered in similar circumstances. There are no previous reports of this nature so far as the authors are aware and, to our knowledge, no data on the *absolute level* of cardiac output in man following the administration of muscle relaxants has been revealed by a study of the literature.

MATERIAL

Twenty–eight unpremedicated patients, who gave their consent, were investigated prior to elective minor surgery. All patients were males aged

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25 to 36, who weighed between 52 and 67kg and were between 160 and 170cm in height. No clinical evidence of cardiopulmonary disease was found in any case.

METHOD

Radial artery and right atrial catheters were inserted in all patients under local analgesia when they arrived in the operating theatre and the patients were then left undisturbed for a period of twenty minutes prior to the induction of anaesthesia.

Pressures were recorded continuously by Statham gauge, and cardiac output was measured at 5 minute intervals by dye dilution, using a Philips XO 1000 Oximeter/Densitometer. Blood required for estimating acid base and blood gas status was drawn from the arterial catheter 10 and 25 minutes after induction of anaesthesia.

Anaesthesia was induced in all patients by giving 200mg of 2.5% thiopentone intravenously over a period of twenty seconds. All patients received 50mg suxamethonium intravenously to facilitate spraying of the vocal cords and glottis with 1ml 4% lignocaine, and intubation of the trachea. Continued apnoea was achieved by giving the patients 0.2mg of fentanyl initially, followed by 0.05mg every 15 minutes. Patients were connected to a Manley respirator delivering an expired minute volume of 5 litres (3.5 litres of nitrous oxide, 1.5 litres of oxygen).

Patients were then assigned to one of five groups in random fashion. Three patients (group 1) served as controls and received no further muscle relaxant drugs. Ten patients (group 2) received 6mg of pancuronium (approximately 0.1mg/kg). Five patients (group 3) received atropine 1.2mg and 6mg of pancuronium. Five patients (group 4) received 30mg of tubocurarine (approximately 0.5mg/kg). Five patients (group 5) received 15mg of alcuronium (approximately 0.25mg/kg). The muscle relaxants used in this study were supplied in ampoules and contained no preservatives. All drugs were administered intravenously.

The doses of muscle relaxant (groups 2–5), were considered as representative of the usual intubating dose, and were given upon completion of a 20 minute control period following induction of anaesthesia. Observations continued thereafter for a further period of 15 minutes.

Patients in group 3 were managed in identical manner to group 2 except 1.2mg atropine was given intravenously 10 minutes after induction of anaesthesia.

At the conclusion of each experiment the cardiac output densitometer was calibrated using the patient's own blood and known doses of cardiac green. Cardiac output was calculated using the method of Williams *et al*[14], to prepare an appropriate programme for the Olivetti 101 computer.

Total peripheral resistance (PVR) was calculated from the formula of Aperia[15].

$$PVR = \frac{\text{mean arterial pressure (mm Hg)}}{\text{cardiac output (litres/min)}} \times 80 \text{ dynes sec cm}^{-5}$$

Tests of statistical significance were applied to the mean differences in measurements made before and after injection of muscle relaxants by applying Student's t–test to the paired comparisons.

RESULTS

Blood gases and pH measurements

In all groups the mean arterial pH, arterial carbon dioxide tension ($PaCO_2$) and arterial oxygen tensions PaO_2 taken 10 minutes after the induction of anaesthesia were within normal limits for patients under anaesthesia. The values were as follows: pH 7.35 to 7.46 (mean 7.39), $PaCO_2$ 30 to 45mm Hg (mean 35), and PaO_2 130 to 180 (mean 150). Comparison with measurements taken 15 minutes later revealed no greater changes than 0.05 in pH, 4mm Hg in $PaCO_2$ and 30mm Hg in PaO_2.

Cardiovascular measurements in group 1 (control patients)

There were no significant changes in the three control patients.

Cardiovascular measurements in groups 2 to 5 (relaxant patients)

The results in the relaxant groups are set out in tables 1 to 4. Control values are the mean of observations made in the anaesthetised patients 0, 5, 10 and 15 minutes prior to the administration of the muscle relaxant drugs.

Changes in the measurements began 20 seconds after the administration of the relaxant drugs and were complete in 45 seconds; thereafter a new steady state existed until the studies were discontinued 15 minutes later.

Group 2 Pancuronium alone (table 1). There was a significant increase in heart rate (7 to 10 beats per minute) and a significant increase in mean blood pressure (13 to 14mm Hg). The mean central venous pressure (CVP) fell significantly by 2mm Hg, and cardiac output increased significantly by 1 litre/minute. Neither systemic vascular resistance nor stroke volume changed.

Group 3 Pancuronium after atropine (table 2). The administration of 1.2mg atropine produced a significant increase in heart rate, blood pressure and cardiac output. Central venous pressure and peripheral vascular resistance were significantly reduced. No further changes were seen after administration of pancuronium.

TABLE 1

Observation	Control	Minutes after pancuronium			Mean maximum change
		+5	+10	+15	
Heart rate (beats/min)	60.5	67.8‖	68.5‖	70.2‖	+ 9.9
SEM	2.3	2.4	2.6	2.4	1.2
Mean blood pressure (mm Hg)	73.8	86.0‡	86.7‡	87.2¶	+14.8
SEM	3.2	5.1	4.9	4.8	3.1
Central venous pressure (mm Hg)	5.7	4.1‖	3.5‖	3.9‖	— 2.3
SEM	0.67	0.6	0.6	0.5	0.4
Cardiac output (litres/min)	5.6	6.7‖	6.7‖	6.7‖	+ 1.2
SEM	0.37	0.5	0.5	0.5	0.2
Peripheral vascular resistance (dynes/sec^{-5})	1083	1026	1035	1044	— -45
SD	218	233	237	234	8.0
Stroke volume (ml)	92	99	98	95	+ 5
SD	8	7	8	8	1.0

Significance levels: $P<0.05$*; $P<0.025$†; $P<0.01$‡; $P<0.005$¶; $P<0.001$‖
The mean maximal change in individual patients is given without regard for time

TABLE 2

Observation	Control period one	Minutes after atropine		Minutes after pancuronium		
		+5	+10 control period (two)	+5	+10	+15
Heart rate (beats/min)	69.3	101.2¶	101.0¶	101.6	100.0	100.0
SEM	6.0	7.0	6.9	8.9	8.0	8.0
Mean blood pressure (mm Hg)	78.8	95.0*	86.8	89.4	87.0	87.6
SEM	6.2	6.6	3.2	5.3	4.8	4.3
Central venous pressure (mm Hg)	7.1	4.6*	5.3	4.8	4.8	4.8
SEM	1.3	0.5	0.7	0.7	0.7	0.7
Cardiac output (litres/min)	4.4	6.4‖	6.6‖	6.6	6.4	6.3
SEM	0.5	0.4	0.5	0.6	0.3	0.3
Peripheral vascular resistance (dynes/sec^{-5})	1432	1188*	1052*	1084	1088	1099
SD	231	202	217	178	184	200
Stroke volume (ml)	63	63	65	65	64	63
SD	10	12	14	13	12	14

Significance levels: $P<0.05$*; $P<0.025$†; $P<0.01$‡; $P<0.005$¶; $P<0.001$‖
The mean maximal change in individual patients is given without regard to time

Group 4 Alcuronium alone (table 3). There was a significant increase in heart rate of 13 beats/minute and a significant drop of mean blood pressure of 9 to 10mm Hg. The mean central venous pressure fell significantly by 0.7mm Hg. Cardiac output increased but this was not significant. Peripheral vascular resistance fell significantly by 150 to 250 dynes sec cm^{-5}. The stroke volume declined insignificantly.

Group 5 Tubocurarine alone (table 4). There was a significant increase

TABLE 3

Observation	Control	Minutes after alcuronium			Mean maximum change
		+5	+10	+15	
Heart rate (beats/min)	71.7	84.2¶	84.9¶	84.5¶	+13.5
SEM	6.9	6.5	6.5	6.3	1.1
Mean blood pressure (mm Hg)	85.1	74.6‡	75.0†	76.0	-8.7
SEM	5.1	4.0	4.1	5.0	2.7
Central venous pressure (mm Hg)	5.3	4.6†	4.6†	4.6†	— 1.9
SEM	0.9	0.6	0.6	0.6	0.3
Cardiac output (litres/min)	4.6	4.8	5.0	5.0	+ 0.6
SEM	0.3	0.4	0.4	0.3	0.1
Peripheral vascular resistance (dynes/sec^{-5})	1486	1243*	1200*	1216*	—250
SD	150	157	200	120	20.0
Stroke volume (ml)	65	57	59	60	— 3
SD	7	6	4	5	0.5

Significance levels: $P<0.05$*; $P<0.025$†; $P<0.01$‡; $P<0.005$¶; $P<0.001$‖.
The mean maximal change in individual patients is given without regard for time

TABLE 4

Observation	Control	Minutes after tubocurarine			Mean maximum change
		+5	+10	+15	
Heart rate (beats/min)	68.1	74.0†	74.4†	71.4*	+7.9
SEM	2.4	3.7	4.2	3.6	1.5
Mean blood pressure (mm Hg)	77.1	63.4*	65.8*	65.5*	—17.5
SEM	2.8	6.2	3.2	4.1	5.0
Central venous pressure (mm Hg)	4.3	2.6*	2.3*	2.5*	—2.3
SEM	1.1	1.0	1.1	1.2	0.7
Cardiac output (litres/min)	3.6	3.6	3.7	3.6	0.1
SEM	0.2	0.6	0.5	0.4	0.01
Peripheral vascular resistance (dynes/sec^{-5})	1713	1408*	1422*	1456*	—300
SD	150	120	170	145	35.0
Stroke volume (ml)	53	49	49	50	—5
SD	7	8	8	10	0.5

Significance levels: $P<0.05$*; $P<0.025$†; $P<0.01$‡; $P<0.005$¶; $P<0.001$‖.
The mean maximal change in individual patients is given without regard for time

in heart rate of 3 to 6 beats/minute and a significant drop in mean blood pressure of 12 to 14mm Hg. Central venous pressure fell significantly about 2mm Hg. There was no change in cardiac output. The peripheral vascular resistance fell by about 300 dynes sec cm^{-5}. Stroke volume fell insignificantly.

DISCUSSION

The absence of systematic changes after induction of anaesthesia in group 1 patients, who received no muscle relaxant, confirms that changes recorded in groups 2 to 5 were due to the muscle relaxants; with the exception of Kelman & Kennedy[1,9] few previous clinical investigators have presented data on control groups but, if valid conclusions are to be drawn, such studies appear essential in view of the many factors which may influence cardiovascular responses in anaesthetised man.

In a study on pancuronium in ten patients premedicated with papaveretum and hyoscine, Kelman & Kennedy[1] reported a 25% rise in heart rate, 9% rise in arterial pressure and an 8% increase in cardiac output with peripheral vascular resistance unchanged. In this study slightly larger doses of pancuronium in unpremedicated patients (group 2), produced increases of 16% in pulse rate, 20% in blood pressure and 19% in cardiac output. These changes did not occur when the pancuronium was preceded by atropine (group 3); this suggests that the cardiovascular effects of pancuronium are mainly due to vagolytic activity, which supports the hypothesis first advanced by Kelman & Kennedy[1].

Kelman & Kennedy[9] have also investigated the cardiovascular effects of alcuronium. In five premedicated patients they reported no change in arterial pressure, 9% increase in heart rate, and an initial 10% increase in cardiac output followed 8 minutes later by a 6% reduction. They reported a 12% reduction in peripheral vascular resistance which returned to control levels after 5 minutes. The results reported above are different. There was an 18% increase in pulse rate, a 14% reduction in arterial blood pressure and a 17% fall in peripheral vascular resistance after the injection of alcuronium. It should be realised, however, that larger doses of alcuronium (0.2mg/kg) were given in the present investigation in keeping with current clinical practice, that patients were unpremedicated and that cardiac output was measured directly and a possible source of inaccuracy in the method used by Kelman & Kennedy[1,9] was thus avoided. In their study relative changes in cardiac output were determined from the dye curves of a non-calibrated earpiece densitometer which is a method liable to errors consequent upon changes in pinna blood volume.

Interpretation of previous reports on the cardiovascular effects of tubocurarine is difficult because other factors known to influence cardiovascular stability were present. In many investigations no attempts were made to

control alterations in cardiac output consequent upon changes in arterial carbon dioxide level[16], and frequently cardiovascular effects have been attributed to tubocurarine in patients anaesthetised with halothane or pethidine, either of which might have confused the results. In the present study tubocurarine was associated with a 9% increase in pulse rate, 18% fall in arterial pressure, 17% fall in peripheral vascular resistance, and no change in cardiac output. It was not possible to demonstrate significant differences between the cardiovascular effects of tubocurarine and alcuronium.

The ideal muscle relaxant would be a drug which produces relaxation of skeletal muscle with no side effects whatsoever. If its cardiac accelerator action is disregarded pancuronium emerges as closest to this ideal at the present time and, relative to tubocurarine and alcuronium, appears to be the best non–depolarising muscle relaxant currently available.

SUMMARY

The cardiovascular effects of pancuronium, alcuronium and tubocurarine have been investigated in unpremedicated patients prior to elective minor surgery.

Heart rate, arterial and central venous pressures and cardiac output were measured in 28 patients after induction of anaesthesia and after the intravenous injection of clinically equivalent doses of one of three muscle relaxants in 25 patients. A control group of three patients were given no muscle relaxant drugs and served as controls.

There was no significant change in measurements made after induction of anaesthesia in the control patients. Pancuronium was associated with a significant increase in heart rate, blood pressure and cardiac output, but did not effect peripheral vascular resistance. These responses were completely inhibited by intravenous atropine. Alcuronium and tubocurarine were both associated with a significant increase in heart rate and falls in blood pressure and peripheral resistance.

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PAPER D3

The Immediate Cardiovascular Effects of Althesin (Glaxo CT 1341), a Steroid Induction Agent, and Thiopentone in Man

The clinical work described was shared by the authors as was preparation of the manuscript.

The immediate cardiovascular effects of Althesin (Glaxo CT 1341), a steroid induction agent, and thiopentone in man

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The steroid anaesthetic agent, Althesin (Glaxo CT 1341), was first described in 1971 by Child *et al.*[1]. Campbell *et al.*[2] introduced the drug into clinical practice and reported its effects upon the cardiovascular system of six patients with major cardiac disease. Cardiovascular effects were also studied by Savege *et al.*[3] in healthy unpremedicated patients prior to surgery.

In this study some acute effects of Althesin upon the cardiovascular system have been investigated in healthy unpremedicated patients prior to surgery and compared with the effects of thiopentone in similar circumstances.

METHODS

Twenty–three unpremedicated patients, who gave their consent, were investigated prior to elective minor surgery. All were males, aged between 25 and 35 years, who weighed between 52 and 68kg, and were between 165 and 175cm in height. No clinical evidence of cardiopulmonary disease was found in any case.

Two dose levels of Althesin were used. Ten patients (group 1) received 0.15ml/kg of Althesin (approximately 10ml) and five patients (group 2) were given 0.06ml/kg (approximately 4ml). A further eight patients (group 3) were given thiopentone 4mg/kg (approximately 250mg).

The dose levels of Althesin used in this study were based on our initial experience with the drug and upon the sleep dosages suggested by others (0.06ml/kg by Clarke *et al.*[4] and 0.15ml/kg by Campbell *et al.*)[2]. The 4mg/kg dose level of thiopentone was adopted because it is regarded as equi–

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potent with 0.06ml/kg Althesin[5]. Both drugs were given intravenously over a period of 20 seconds.

Pressures were measured electrometrically by Statham gauge and cardiac output by dye dilution, using a Philips XO 1000 Oximeter/Densitometer. Arterial oxygen saturation was measured at the same time as cardiac output. A lead 1 electrocardiograph and arterial and venous pressures, were recorded on a Philips 3T Cardiopan recorder. Radial artery and right atrial catheters were inserted in all patients under local analgesia on arrival in theatre. Patients were left undisturbed for a period of ten minutes following introduction of the catheters. Control measurements were then made at three minute intervals for a period of nine minutes.

Anaesthesia was induced by giving an intravenous injection of either Althesin (groups 1 and 2), or thiopentone (group 3). The patients breathed air throughout the investigation and airways were carefully maintained until completion of the subsequent surgical procedures.

Measurements were made 1, 3, 6 and 9 minutes after induction before routine inhalational anaesthesia was introduced prior to surgery. Some patients began to regain consciousness before the end of the nine minute period; in such cases measurements were discontinued and inhalational anaesthesia was started.

Aliquots of each patient's blood containing known doses of indocyanine green (cardiac green) were used to calibrate the cardiac output densitometer at the end of each study under conditions prevailing during the actual experiment. Cardiac output was calculated from dye curves using the method of Williams *et al.*[6] to prepare the appropriate programme for an Olivetti 101 computer.

Total peripheral resistance (PVR) was calculated from the formula of Aperia[7].

$$\text{PVR} = \frac{\text{mean arterial pressure (mm Hg)}}{\text{cardiac output (litres/min)}} \times 80 \text{ dynes sec cm}^{-5}$$

Tests of statistical significance were applied to the mean differences in measurements made before and after induction of anaesthesia by applying Student's t–test to the paired observations.

Differences between CT 1341 and thiopentone were sought by comparing the recorded changes consequent upon induction of anaesthesia by the two drugs.

RESULTS

Results are set out in tables 1, 2 and 3. The control values shown in the tables are the mean of observations made in each patient 9, 6, 3 and 1 minutes prior to induction of anaesthesia.

Group 1, Althesin 0.15ml/kg (table 1)

There was a significant increase in heart rate at 1, 3 and 6 minutes after injection. The maximum increase in heart rate (mean 21.8 beats per minute) occurred in all patients within three minutes of the injection of the drug. There was a consistent fall in arterial blood pressure which was significant at each period of measurement. The drug was generally associated with a fall in central venous pressure, but this was only significant at the end of the ninth minute. The cardiac output increased insignificantly by a mean amount of 200ml per minute.

Peripheral vascular resistance was significantly reduced at each period of measurement. Arterial oxygen saturation fell significantly at one and three minutes after induction, but returned to pre-induction values in every patient after the elapse of nine minutes.

TABLE 1

Observation	Control	Minutes after injection				Mean maximum change
		+1	+3	+6	+9	
Heart rate (beats/min)	85.6	107.3††	99.2¶	99.8¶(9)	103.4(7)	+21 8
SEM	6.56	6.25	4.39	4.59	3.49	2.79
Mean blood pressure (mm Hg)	96.1	74.9††	77.4††	75.0††(9)	75.7‡(6)	—24.1
SEM	3.1 8	3.24	2.76	3.18	4.82	3.0
Mean venous pressure (mm Hg)	4.2	4.0	4.0	4.5(6)	2.4†(7)	—0.86
SEM	0.47	0.57	0.44	0.38	0.49	0.02
Cardiac output (litres/min)	6.75	6.9	7.0	6.8(7)	7.1(4)	+0.2
SEM	0.48	0.46	0.42	0.51	0.67	0 01
Peripheral vascular resistance (dynes/sec^{-5})	1205	915††	939††	917††(7)	901††(4)	—325
SEM	108.9	81.8	86.6	112.6	124.5	48.9
Arterial oxygen saturation %	95.6	88.1¶	89.3‡	93.8	95.6	—8.0
SD	1.8	4.1	4.6	2.3	2.7	2.1

Significance levels: P<0.05*; P<0.025†; P<0.01‡; P<0.005¶; P<0.001††

Heart rate, mean arterial blood pressure, central venous pressure, cardiac output, total systemic peripheral vascular resistance and arterial oxygen saturation before and after injection of Glaxo CT 1341 (0.15ml/kg) in 10 patients expressed as mean values (±SEM). The mean maximum change in individual patients without regard to time is given. Mean values are based upon observations in all 10 patients except when studies terminated prior to elapse of 9 minutes; in these instances, statistical comparison is based upon paired t-test of the lesser number indicated within brackets.

TABLE 2

Observation	Control	Minutes after induction				Maximum change
		+1	+3	+6	+9	
Heart rate (beats/min)	88.5	86.0	94.3(4)	90.3(3)	88.0(2)	—1.2
SEM	7.3	7.8	6.0	6.4	6.4	
Mean blood pressure (mm Hg)	104.3	83.8††	71.0††(4)	75.6¶(3)	99.0(2)	—26.7
SEM	8.1	8.4	0.9	1.9	3.5	
Central venous pressure (mm Hg)	4.8	3.0	3.7(3)	3.0(4)	—	—2.0
SEM	0.98	0.7	0.34	1.1		
Cardiac output (litres/min)	7.1	7.6	7.2	6.6	—	—0.4
SEM	0.68	1.2	0.54	0.73		
Peripheral vascular resistance (dynes/sec^{-5})	1292	802	800	869	—	—268
SEM	276.0	111.4	67.5	69.3		

Significance levels: P<0.05*; P<0.025†; P<0.01‡; P<0.005¶; P<0.001††

Heart rate, mean arterial blood pressure, central venous pressure, cardiac output, total systemic peripheral vascular resistance and arterial oxygen saturation before and after injection of Glaxo CT 1341 (0.06ml/kg) in 5 patients expressed as mean values (± SEM). The mean maximum change in individual patients without regard to time is given. Mean values are based upon observations in all 5 patients except when studies terminated prior to elapse of 9 minutes; in these instances, statistical comparison is based upon paired t-test of the lesser number indicated within brackets.

Group 2, Althesin 0.06ml/kg (table 2)

There was no systematic change in pulse rate following injection of Althesin in this group. A consistent fall in arterial mean blood pressure occurred which was significant at the end of the 1st, 3rd and 6th minute. There was no significant change in cardiac output or central venous pressure. Peripheral vascular resistance fell in every patient but this was not statistically significant.

Thiopentone, 4mg/kg (table 3)

A consistent increase in heart rate occurred and this was significant at each period of observation. Mean arterial blood pressure fell significantly in all patients, and mean central venous pressure fell but was significant only at the sixth minute after injection of thiopentone. Peripheral resistance fell and was significant at the end of the third and sixth minute following the injection of thiopentone. There was no change in cardiac output. There was no significant change in the arterial oxygen saturation after thiopentone.

TABLE 3

Observation	Control	Minutes after induction +1	+3	+6	+9	Maximum change
Heart rate (beats/min)	92.7	106.9¶	103.8¶	100.4†(7)	99.1†(7)	+17.0
SEM	5.03	4.6	5.2	4.2	5.6	
Mean blood pressure (mm Hg)	95.9	86.0¶	82.0‡	82.0†(7)	80.3‡(6)	—16.9
SEM	4.2	4.4	3.7	4 3	4.1	
Central venous pressure (mm Hg)	2.2	1.6	1.1	0.7*(7)	0.8(5)	—1.3
SEM	0.57	0.3	0.3	0.3	0.8	
Cardiac output (litres/min)	8.96	9.0	9.1	8.6	—	—0.2
SEM	0.8	0.8	1.1	0 8		
Peripheral vascular resistance (dynes/sec^{-5})	911	882	827*	835*(7)	—	—157
SEM	115.2	146.8	138.2	99.3		
Arterial oxygen saturation %	95.0	91.1	95.6(5)	94.2(6)	—	
SD	2.9	3.6	2.1	2.7		

Significance levels: P<0.05*; P<0.025†; P<0.01‡; P<0.005¶; P<0.001††

Heart rate, mean arterial blood pressure, central venous pressure, cardiac output, total systemic peripheral vascular resistance and arterial oxygen saturation before and after injection of thiopentone (4.0mgm/kg) in 8 patients expressed as mean values (±SEM). The mean maximum change in individual patients without regard to time is given. Mean values are based upon observations in all 8 patients except when studies terminated prior to elapse of 9 minutes; in these instances, statistical comparison is based upon paired t-test of the lesser number indicated within brackets.

Althesin, 0.15ml/kg versus Althesin 0.06ml/kg

Similar changes in blood pressure, central venous pressure, cardiac output and peripheral vascular resistance occurred after injection of 0.06ml/kg or 0.15ml/kg Althesin. At the lower dosage, however, the changes in heart rate and the peripheral vascular resistance were not significant.

Althesin versus thiopentone

There was a greater fall in mean arterial blood pressure following the administration of Althesin than after thiopentone, however the differences were not significant. There was a fall in peripheral vascular resistance

following the administration of both drugs, but this was significantly greater after Althesin at the 1, 3, 6 and 9 minute intervals ($P < 0.05$). There was a fall in arterial oxygen saturation after both drugs – this was significantly greater after Althesin ($P < 0.05$).

DISCUSSION

The cardiovascular effects of intravenous anaesthetic agents have been extensively studied both in man and animals. Comparison between these studies and the investigation reported here cannot readily be made because of species variation and differences in experimental techniques adopted. Dobkin[8] reported a 16% rise in heart rate, 17% reduction in mean arterial blood pressure, 10% reduction in peripheral vascular resistance and no significant change in cardiac output, when twice the sleep dose of thiopentone was given to unpremedicated healthy volunteers. In our patients sleep doses of thiopentone resulted in similar changes; heart rate increased 25%, mean blood pressure fell 22%, peripheral vascular resistance fell by 24% and there was no significant change in cardiac output. A reduction of cardiac output below the normal range after thiopentone anaesthesia has been observed by Etsten[9], Fieldman[10] & Flickinger *et al.*[11], but premedicant drugs were given prior to the induction of anaesthesia in these studies and might explain this finding.

Observations made in regard to Althesin are similar in every respect to the changes in cardiovascular status reported by Campbell *et al.*[2], and, except in regard to heart rate at the low dose range, to the report of Savege *et al*[3].

The observations made after induction of anaesthesia in group 2 patients (low dose of Althesin) differed little from those made in patients who had received almost treble the sleep dose of Althesin (group 1); this confirms the high therapeutic index of the drug which was suggested by animal experiments[1].

The exact mechanism of the changes in cardiovascular status after induction of anaesthesia is uncertain, but a definite pattern follows injection of both drugs. Peripheral resistance and blood pressure fall, heart rate increases and the cardiac output either increases or is unchanged. These are changes which suggest a peripheral site of action, the hypotension being due to peripheral vasodilatation which produces a reflex tachycardia and consequent maintenance of cardiac output.

The immediate cardiovascular effects of the two drugs appear to be remarkably similar except that the fall in peripheral vascular resistance is significantly greater following Althesin and consequently it may be tentatively predicted that induction of anaesthesia with Althesin will be more hazardous in patients suffering from diseases which restrict or 'fix' the cardiac output. Patients suffering from mitral stenosis or constrictive

pericarditis are intolerant to sudden vasodilatation and fatal arterial hypotension might occur if Althesin were administered to such a patient. In all other respects, however, Althesin offers an acceptable alternative to thiopentone for induction of anaesthesia.

SUMMARY

The acute cardiovascular changes have been measured in 23 healthy unpremedicated patients following induction of anaesthesia with either Althesin, a steroid anaesthetic agent, or thiopentone. Two dose levels of Althesin were used: 0.15ml/kg (group 1) and 0.06ml/kg (group 2). The dose of thiopentone was 4mg/kg (group 3). In all three groups the blood pressure fell, the pulse rate increased and cardiac output was either slightly increased or unchanged by comparable amounts, but the fall in peripheral vascular resistance was significantly greater in the Althesin groups.

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PAPER D4

Cardiovascular Effects of Acebutolol (M & B 17803A), in Exercising Man; A Comparative Study with Practolol and Propranolol

The clinical work described was shared by the authors as was preparation of the manuscript.

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CARDIOVASCULAR EFFECTS OF ACEBUTOLOL (M & B 17803A), IN EXERCISING MAN; A COMPARATIVE STUDY WITH PRACTOLOL AND PROPRANOLOL

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ABSTRACT

A new drug, M & B 17803A, was found intrinsically more potent than practolol as a β-receptor blocking agent and, unlike propranolol, did not reduce the cardiac output in exercising man.

DL — (2-acetyl-4-butyramidophenoxy)-2 hydroxy-3-isopropylamino-propane hydrochloride (acebutolol) is a potent new sympathetic β-receptor antagonist. Preliminary investigations in animals indicate that acebutolol selectively blocks myocardial sympathetic β-receptors and has little effect on bronchial or vascular muscle.[1] In addition, the drug has quinidine-like and local anaesthetic properties.[2]

This paper records the findings of a study in which equal doses of acebutolol, practolol, or propranolol were given intravenously to healthy volunteers during sustained sub-maximal exercise.

METHODS

A total of 19 subjects was studied. All were male students or technicians aged 27 ± 5 years, weighing 65 ± 10 kg., and being 71 ± 6 cm. in height. No clinical evidence of cardiopulmonary disease was found in any case and all investigations were carried out under aseptic conditions in an operating theatre, with their consent.

Radial artery and right atrial catheters were inserted in all patients under local analgesia. Subjects then rested for 10 minutes before commencement of the study. Pressures were recorded by Statham gauge, and cardiac output was measured by dye dilution, using a Philips XO 1000 Oximeter/Densitometer. Pulse rate and arterial pressure were recorded on a Philips 3T Cardiopan recorder.

When recording devices had been calibrated and resting pulse rate noted, subjects began to exercise seated on a bicycle ergometer. During the first 5 minutes, workload was adjusted to produce a 50 to 75 percent increase in pulse rate. Cardiac output, arterial pressure and pulse rate were measured at least twice in the ensuing 6 minutes. An injection of 10 mg. acebutolol, (6 subjects), propranolol, (8 subjects), or practolol, (5 subjects), in 10 ml. normal saline was then given intravenously over 15

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seconds. Cardiac output, pulse and arterial pressure were measured 2, 4 and 6 minutes after injecting the drug, while exercise continued as before. The cardiac output densitometer was calibrated at the end of each study with aliquots of the patients' blood containing known amounts of indocyanine green.

Cardiac output was calculated from dye curves using the method of Williams, O'Donovan and Wood,[3] to prepare an appropriate programme for the Olivetti 101 computer. Total peripheral resistance (P.V.R.) was calculated by the formula of Aperia.[4] Tests of statistical significance were applied to the mean differences in measurements made before and after injection of M & B 17803A, practolol or propranolol by applying student's t-test to the paired observations.

RESULTS

Presentation of data has been simplified by comparing the mean of observations made during exercise *before* injection of M & B 17803A, practolol or propranolol with the mean of measurements made *after* injecting the drug, since no significant differences were noted between measurements made 2, 4 and 6 minutes after administration of each drug.

The individual values for heart rate, mean arterial pressure, cardiac output, stroke volume and systemic vascular resistance appear in Tables I to III.

Acebutolol

A significant fall in pulse rate followed intravenous injection of acebutolol ($P < 0.01$). Stroke volume increased in all but one subject. This was statistically significant, ($P < 0.05$) there were no significant changes in mean arterial pressure, ($P > 0.100$), cardiac output, ($P > 0.200$), or systemic vascular resistance, ($P > 0.200$).

Propranolol

There were statistically significant falls in mean arterial pressure, ($P < 0.001$), pulse rate, ($P < 0.001$) and cardiac output ($P < 0.025$) following the administration of propranolol to exercising man.

No significant change in stroke volume ($P > 0.500$) or peripheral resistance was recorded ($P > 0.200$).

Practolol

Intravenous administration of 10 mg. practolol during sub-maximal exercise produced significant falls in mean arterial pressure ($P < 0.001$), pulse rate ($P < 0.025$) and peripheral resistance ($P < 0.05$). Stroke volume increased by 2.5 percent and cardiac output fell by 2.4 percent, but these changes were not statistically significant ($P > 0.200$).

Table I — *Effects of 10 mg. Acebutolol Given by Intravenous Injection to Exercising Man.*

Subject	PRE-INJECTION Pulse Rate (Beats/min)	Cardiac Output (L/min)	Stroke Volume ml.	Mean Arterial Pressure (mm. Hg)	P.V.R. (Dynes sec cm-5)	POST-INJECTION Pulse Rate (Beats/min)	Cardiac Output (L/min)	Stroke Volume ml.	Mean Arterial Pressure (mm. Hg)	P.V.R. (Dynes sec cm-5)
1	125	11.50	92.0	110	765.2	117	11.7	100.3	110	750.2
2	135	15.90	117.8	136	684.3	112	14.4	128.6	118	655.6
3	107	14.31	133.7	120	670.9	104	14.2	137.4	115	638.9
4	112	17.15	153.1	104	485.1	102	15.3	150.5	104	542.0
5	107	14.51	135.6	104	573.4	95	14.6	153.9	104	569.1
6	88	8.70	98.9	112	1029.9	83	9.3	112.4	106	908.9
Mean	112.3	13.57	121.9	114.3	701.5	102.2	13.28	130.5	109.5	677.5
± Std. Dev.	±14.9	±2.82	±21.4	±11.1	±171.5	±11.1	±2.1	±19.3	± 5.4	±123.1

Table II — *Effects of 10 mg. Propranolol Given by Intravenous Injection to Exercising Man.*

Subject	PRE-INJECTION					POST INJECTION				
	Pulse (beats/min)	Cardiac Output (L/min)	Stroke Volume ml.	Mean Arterial Pressure (mm. Hg)	Peripheral Resistance (dynes/sec cm-5)	Pulse (beats/min)	Cardiac Output (L/min)	Stroke Volume (ml.)	Mean Arterial Pressure (mm. Hg)	Peripheral Resisitance (dynes/sec cm-5)
7	125	15.98	127.8	93	465.6	94	12.5	133.8	85	540.5
8	115	14.37	125.0	94	523.3	99	13.0	132.2	83	507.3
9	112	15.25	136.2	106	556.1	99	11.8	119.2	95	644.1
10	109	13.25	121.6	96	579.6	103	12.6	122.7	90	569.6
11	150	11.08	73.9	101	729.2	122	12.4	101.6	87	561.3
12	167	13.30	79.6	108	649.6	132	11.2	85.4	103	731.1
13	132	9.30	70.5	108	929.0	107	7.6	71.0	94	989.5
14	115	12.90	112.2	120	744.2	102	9.27	90.8	99	854.3
Mean	128.1	13.18	105.9	103.3	647.1	107.3	11.30	107.1	92.0	674.7
± Std. Dev.	±19.3	±2.0	±25.0	±8.5	±140.0	±12.2	±1.8	±21.8	±6.5	±160.2

Table III — *Effects of 10 mg. Practolol Given by Intravenous Injection to Exercising Man.*

Subject	PRE-INJECTION					POST INJECTION				
	Pulse Rate	Cardiac Output (L/min)	Stroke Volume (ml.)	Blood Pressure	P.V.R.	Pulse Rate	Cardiac Output (L/min)	Stroke Volume (ml.)	Blood Pressure	P.V.R.
15	112	14.29	127.6	114	638.2	110	13.44	122.2	102	607.1
16	136	10.73	78.9	112	835.0	136	10.37	76.3	103	794.6
17	122	17.81	146.0	107	480.6	115	18.30	159.1	93	406.6
18	110	13.86	126.0	112	646.5	102	12.58	123.3	104	661.4
19	117	12.03	102.8	111	738.2	109	12.45	114.2	101	649.0
Mean	119.4	13.74	116.3	111.2	667.7	114.4	13.42	119.0	100.6	623.7
± Std. Dev.	±9.3	±2.4	±23.2	±2.3	±117.7	±11.6	±2.6	±26.4	±3.9	±125.4

DISCUSSION

It may be argued that comparing equal doses of drugs with different potencies limits the practical value of data collected. This difficulty was partly overcome in the study described here by injecting doses within the therapeutic range of each drug. The experiments reported here indicate that acebutolol is intrinsically more potent that practolol, as a β-receptor blocking agent and, unlike an equal dose of propranolol, does not reduce cardiac output.

No change in airways resistance occurs in asthmatics given 100 or 200 mg. acetutolol by mouth (leary and Coleman, unpublished) and animal studies show that acebutolol has marked quinidine-like effects and is significally more potent than practolol as an anti-arrhythmic agent.[1,2]

These studies suggest that, like practolol, acebutolol has a high degree of cardioselectivity and may, therefore, be administered to patients with obstructive airways disease and as an antiarrhythmic agent following myocardial infarction or cardiac surgery, with comparative safety.

Further clinical trials of acebutolol are indicated to determine the efficacy of this agent in various cardiac arrhythmias in man and its relative safety in patients with cardiac insufficiency.

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PAPER D5

Some Hemodynamic Effects of Sodium Nitroprusside

This study was conceived by Dr. M. Styles and Professor A.J. Coleman. Clinical work was shared and the paper was drafted and edited by all three authors.

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Some Hemodynamic Effects of Sodium Nitroprusside

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Arterial hypotension to about 45 mm Hg below control was induced in 12 patients by infusion of a 0.01 per cent solution of sodium nitroprusside. The drug was found reliable, free of tachyphylaxis, and evanescent of action. During the hypotensive phases arterial oxygen saturation was maintained at 95–99 per cent. Cardiac output decreased insignificantly by a mean value of 200 ml/min in conscious individuals, but increased by about 850 ml/min in anesthetized subjects. Blood pressures returned to 90 per cent of control values within 120 seconds of discontinuation of sodium nitroprusside. The use of the drug merits further investigation. (Key words: Hypotension; Sodium nitroprusside; Available oxygen.)

THE CARDIOVASCULAR EFFECTS of sodium nitroprusside in normotensive and hypertensive patients have been described by Schlant *et al.*,[1] and those following acute myocardial infarction, by Franciosa *et al.*[2] Two clinical studies of the use of this drug as a hypotensive agent in anesthetic practice have been published.[3, 4] Schlant *et al.*[1] reported a 37.2 per cent reduction in mean arterial blood pressure in normotensive patients given intravenous doses of 0.61–3.87 μg/kg/min sodium nitroprusside. This was associated with a mean decrease of peripheral vascular resistance of 31.1 per cent, an 8.8 per cent decline in cardiac index, and an increase in heart rate of 5.5 per cent. These effects and the hemodynamic changes found in patients given sodium nitroprusside after myocardial infarction[2] suggest that the drug is a pure vasodilator, without action upon the heart or sympathetic nervous system.[1, 5] Reports of the use of sodium nitroprusside as a hypotensive agent in clinical anesthetic practice[3, 4] stress the potency, evanescence of action, and absence of tachyphylaxis of the drug, and suggest that further evaluation is indicated.

The availability of oxygen to the tissues, an important factor in clinical anesthetic practice, merits special attention during induced hypotension, because in this situation cardiac output is commonly reduced.[6–8] There are no published reports of the effects of sodium nitroprusside on cardiac output or arterial oxygenation in anesthetized man. In this study an attempt has been made to augment available knowledge by providing this information.

Methods

Twelve patients, five women and seven men, were investigated, with their consent. Patients were 20 to 50 years old and had no clinical evidence of cardiopulmonary or central nervous system disease. In every case elective surgery involving structures close to the body surface was indicated, and the surgeons had requested that arterial blood pressures be controlled during these procedures. Surgical procedures included excision of facial keloid malformations and lipomata and a salivary gland exploration. All studies were performed with the patients supine in the horizontal position.

Patients received 100 mg pethidine (meperidine) and 25 mg promethazine intramuscularly 30 to 60 minutes before the study. Using local analgesia, plastic catheters were inserted percutaneously into the radial artery, and into the right atrium from a right antecubital vein. Pressures were measured by Statham strain-gauge transducers supported 3 inches above the surface of the operating table and recorded continuously on a Philips 3T recorder. Heart rate was measured using a SAN-El-

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TABLE 1. Hemodynamic Data from Five Patients (Group I) Given Sodium Nitroprusside after Control Periods before and after Induction of Anesthesia*

	Control, Awake	Sodium Nitroprusside Hypotension, Awake	Control, Awake	Control, during Anesthesia	During Anesthesia and Sodium Nitroprusside	During Anesthesia after Sodium Nitroprusside
Blood pressure (mm Hg)	92.4 ± 7.3	48 ± 8.7‖	79.5 ± 5.7	89.6 ± 6.4	46.5 ± 4.6‖	90 ± 14.1
Heart rate (beats/min)	70.6 ± 11.8	70.2 ± 19.7	51 ± 2.2	80.6 ± 11.1	93.2 ± 10.3‡	66.7 ± 12.3
Central venous pressure (mm Hg)	4 ± 0.3	1 ± 0.25†	4 ± 0.21	2 ± 0.2	0 ± 0.13†	2 ± 0.14
Cardiac output (l/min)	4.3 ± 0.3	4.1 ± 0.3	3.6 ± 0.2	4.4 ± 1.2	5.3 ± 0.9	3.8 ± 0.4
Stroke volume (ml)	63 ± 13.2	62 ± 15	70 ± 7.5	54 ± 10.5	57 ± 10.7	49 ± 3.7
Peripheral vascular resistance (dynes/sec/cm^{-5})	1,721 ± 226	931 ± 156§	1,708 ± 61	1,722 ± 333	712 ± 79¶	1,913 ± 180
Oxygen saturation (per cent)	97 ± 2.1	97 ± 1.3	97 ± 2.4	99 ± 3.5	99 ± 3.7	98 ± 3.1
Available oxygen (ml/min)	766 ± 93	751 ± 108	665 ± 90	824 ± 315	904 ± 196	683 ± 71
Pa_{O_2} (mm Hg)	95 ± 10.5	93 ± 11.2	95 ± 11.1	120 ± 28.2	110 ± 17.9	135 ± 25.3
Pa_{CO_2} (mm Hg)	40 ± 3.7	37 ± 4.2	41 ± 4.7	40 ± 4.5	39 ± 3.9	42 ± 4.2
*p*H	7.41 ± 0.07	7.39 ± 0.06	7.4 ± 0.08	7.37 ± 0.05	7.35 ± 0.06	7.40 ± 0.07
$P\bar{v}_{O_2}$ (mm Hg)	45 ± 3.7	47 ± 3.8	45 ± 3.0	50 ± 4.3	49 ± 4.8	45 ± 7.1
$P\bar{v}_{CO_2}$ (mm Hg)	47 ± 5.0	42 ± 2.7	47 ± 4.5	48 ± 4.7	4[illegible] ± 5.0	47 ± 3.7
*p*H	7.37 ± 0.03	7.30 ± 0.05	7.30 ± 0.04	7.30 ± 0.05	7.3[illegible] ± 0.05	7.35 ± 0.07

* Results are means ± SD. Statistical data relate to comparison of values obtained during infusion of sodium nitroprusside with those obtained in the preceding control period.
† $P < 0.05$; ‡ $P < 0.025$; § $P < 0.01$; ¶ $P < 0.005$; ‖ $P < 0.001$.

2D16 pulsemeter and a finger photocell. Cardiac output was measured by dye dilution using indocyanine green and the Philips XO-1000 combined oximeter/densitometer cuvette. Arterial oxygen saturation and hemoglobin were measured during the cardiac output estimation. Blood needed for estimating acid–base and blood–gas status was obtained from the arterial and venous catheters at regular intervals during the studies, and analyzed in duplicate, using the Beckman modular blood–gas assembly.

At the conclusion of each experiment the cardiac output densitometer was calibrated using the patient's own blood and known doses of indocyanine green. Cardiac output was calculated using the method of Williams *et al.*[9] to prepare an appropriate program for an Olivetti digital computer. Total peripheral vascular resistance (PVR) was calculated from the formula of Aperia [10]:

$$PVR = \frac{\text{mean arterial pressure (mm Hg)}}{\text{cardiac output (l/min)}} \times 80 \text{ dynes sec cm}^{-5}$$

Available oxygen was calculated from the formula:

$$\text{Available oxygen (ml/min)} = \frac{\text{cardiac output ml/min}}{100} \times (\text{hemoglobin} \times 1.34) \times \% \text{ oxygen saturation}$$

Tests of statistical significance were applied to the mean differences between values obtained in the control periods preceding hypotension and during the hypotensive phases, induced by halothane on the one hand, and sodium nitroprusside on the other, by applying Student's t test to the paired comparisons.

Five patients (Group I) received sodium nitroprusside after control periods both before and after induction of anesthesia. Patients were left undisturbed following introduction of the catheters until heart rates and arterial pressures were steady. Cardiac output, arterial oxygen saturation and hemoglobin estimations were then made at about 3-minute intervals until the areas of at least two dye-dilu-

TABLE 2. Hemodynamic Data from Seven Patients (Group II) Given Sodium Nitroprusside after Induction of Anesthesia

	Control during Anesthesia	Value during Sodium Nitroprusside Hypotension	Control
Blood pressure (mm Hg)	99.7 ± 13.6	54.6 ± 11.7‖	95.7 ± 10.8
Heart rate (beats/min)	88.0 ± 22	113.0 ± 25†	78.4 ± 20.6
Central venous pressure (mm Hg)	2 ± 0.3	1 ± 0.2†	4 ± 0.5
Cardiac output (l/min)	4.3 ± 1.1	5.1 ± 1.0	4.0 ± 0.8
Stroke volume (ml)	50 ± 10	47 ± 14.8	48 ± 9.3
Peripheral vascular resistance (dynes sec cm^{-5})	1,930 ± 485	917 ± 386‖	1,913 ± 456
Oxygen saturation (per cent)	95 ± 2.1	95 ± 2.3	96 ± 2.3
Available oxygen (ml/min)	665 ± 82	803 ± 220	632 ± 72
Pa_{O_2} (mm Hg)	92 ± 17.1	85 ± 18.3	95 ± 13.7
Pa_{CO_2} (mm Hg)	35 ± 7.4	30 ± 7.7	37 ± 8.0
*p*H	7.37 ± 0.05	7.39 ± 0.04	7.37 ± 0.06
$P\bar{v}_{O_2}$ (mm Hg)	41 ± 9.1	40 ± 7.1	42 ± 5.3
$P\bar{v}_{CO_2}$ (mm Hg)	42 ± 7.3	37 ± 5.8	49 ± 3.7
*p*H	7.31 ± 0.02	7.30 ± 0.07	7.30 ± 0.06

* Results are means ±SD. Each period of hypotension was preceded by a control period. Statistical data relate to comparison of values obtained during infusion of sodium nitroprusside with those obtained in the preceding control period.

† $P < 0.05$; ‡ $P < 0.025$; § $P < 0.01$; ¶ $P < 0.005$; ‖ $P < 0.001$.

tion curves appeared to agree within 10 per cent and the arterial oxygen saturation was steady to within 1 per cent. Blood pressures and heart rates at this moment were recorded and served as control measurements. Infusion of a 0.01 per cent solution of sodium nitroprusside in 5 per cent dextrose in water was then commenced, at a rate adjusted to maintain the mean arterial blood pressure between 40 and 50 mm Hg below the control level. When the hypotensive state had been established for at least 5 minutes, all hemodynamic measurements were repeated. Infusion of sodium nitroprusside was then discontinued and a second series of control estimations was made when the blood pressure returned to control levels.

Anesthesia was induced by 100 to 200 mg of 2.5 per cent thiopental given intravenously over a period of 30 seconds. All patients received 50 mg succinylcholine intravenously to facilitate intubation of the trachea. Continued apnea was achieved by giving each patient either alcuronium or pancuronium. Patients were connected to a Manley respirator delivering an expired minute volume of about 5 liters (70 per cent nitrous oxide and 30 per cent oxygen). The minute volume was adjusted as necessary to Pa_{CO_2} between 30 and 40 mm Hg. A period of 10 minutes was allowed to elapse following induction of anesthesia and then control hemodynamic measurements were made. After incision of the skin, sodium nitroprusside infusion was commenced, and when mean blood pressure was 40–50 mm Hg below the control level, all hemodynamic measurements were repeated. Surgical intervention was interrupted 3 minutes prior to measurement periods. Sodium nitroprusside infusion was discontinued to coincide with surgical needs, and when the arterial blood pressure had approximated control values for at least 15 minutes, a final series of control measurements was made.

Seven patients (Group II) were investigated after induction of anesthesia. The initial anesthetic management and methods of study were identical to those described above.

Results

Hypotension was readily obtained following the infusion of sodium nitroprusside, although some difficulty in obtaining a steady level of reduced mean pressure was experienced initially. The surgeons were well satisfied with operating conditions in all cases.

Group I patients were unaware of the reductions in their blood pressures preoperatively, and experienced no ill effects whatever.

The average reduction of mean arterial pres-

sure during sodium nitroprusside infusion (44 mm Hg) was associated with significant decreases in central venous pressure and peripheral vascular resistance (table 1). Cardiac output and arterial oxygen saturation were not changed, indicating that available oxygen was maintained at control levels.

Responses to sodium nitroprusside during anesthesia differed significantly from those in the conscious state in respect to heart rate, which increased by 13 beats/min, only. In addition, mean cardiac output increased by 900 ml/min and mean available oxygen increased by more than 75 ml/min, but these changes were not significant.

In Group II, responses to sodium nitroprusside were similar in every respect to those in Group I following induction of anesthesia (table 2).

There was no notable change in the blood–gas or acid–base status of patients during this study except a slight increase in Pa_{CO_2} following induction of anesthesia (tables 1 and 2). Arterial hypotension, once established, was readily maintained by a fixed rate of sodium nitroprusside infusion, indicating that there was no early tendency to develop tachyphylaxis.

Following discontinuation of sodium nitroprusside, arterial blood pressure returned to within 90 per cent of control in less than 120 seconds in all cases.

Discussion

Major considerations in induced hypotension are oxygenation and the adequacy of blood flow, particularly to the brain. A technique which provides good surgical conditions without a reduction in available oxygen is preferable to one known to reduce cardiac output and available oxygen.

Clinical studies [3, 4] indicate that sodium nitroprusside provides good surgical conditions. Results of the present study indicate that these conditions are obtained without jeopardizing oxygen delivery, and further suggest that reduction of *arterial pressure* rather than *blood flow* is the factor that facilitates surgery in induced hypotension. Venous blood *p*H and blood–gas, although not strictly representative of mixed venous blood, were maintained at control levels during the hypotensive phases of the study, suggesting that total-body perfusion was adequate. In contrast, when hypotension is induced by ganglionic blocking drugs, spinal anesthesia, or deep halothane anesthesia, cardiac output is reduced.[6, 7, 8, 11] Halothane and trimethaphan, probably the most popular sequence, were associated in one study with a significant 11.8 per cent reduction in cardiac output,[6] and in another with 33–41 per cent reductions in cardiac index.[7]

It is concluded that sodium nitroprusside may offer important advantages as a hypotensive agent and merits further scrutiny in depth regarding, for example, adequacy of regional perfusion of vital organs.

The authors are grateful to Dr. T. H. Taylor of the Anaesthetic Department, The London Hospital, for initiating this study and for supplies of sodium nitroprusside. and to the surgeons of the Professorial Surgical Units of King Edward Hospital for their unstinting cooperation and assistance with the project.

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PAPER D6

Isoproterenol Blockade in Man; Comparison Between Penbutalol and Acebutolol

The technical expertise required to measure cardiac output was provided by Professor A.J. Coleman. Intubation of arteries was a shared procedure as was the analysis of data and preparation of the publication. The protocol followed was a joint venture. Professor Asmal provided editorial assistance.

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ISOPROTERENOL BLOCKADE IN MAN; COMPARISON BETWEEN PENBUTALOL AND ACEBUTOLOL

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ABSTRACT

Penbutalol and acebutolol are new β-adrenergic blocking agents. The haemodynamic effects of isoproterenol, infused at several rates, have been measured before and after intravenous penbutalol 0.2 and 0.3 mg. or acebutolol 5 and 10 mg. Isoproterenol infusion increased heart rate, cardiac output, stroke volume and peripheral bloodflow in healthy subjects. Central venous pressure and peripheral vascular resistance fell. These effects were partly blocked by both acebutolol and penbutalol.

The effects of penbutalol upon peak flow, FEV_1 and FVC were assessed in 7 students with bronchial asthma. Five developed bronchospasm after penbutalol 0.15-0.2 mg. I.V.I. or oral doses of 2 to 8 mg.

Therapeutic doses of both agents effectively block cardiac adrenergic receptors, but acebutolol seldom precipitates bronchospasm in asthmatics.

INTRODUCTION

Penbutalol and acebutolol (Fig. 1) are potent new β-adrenergic blocking agents. The clinical pharmacology of penbutalol has not been fully described in man, but preliminary experiments indicate that acebutolol is effective in a variety of clinical situations and resembles practolol in its relative selectivity for β_1 receptors.[1-2]

In this series of studies, the haemodynamic effects of isoproterenol were determined before and after administration of penbutalol or acebutolol. In addition, the effects of penbutalol have been measured in a group of asthmatics.

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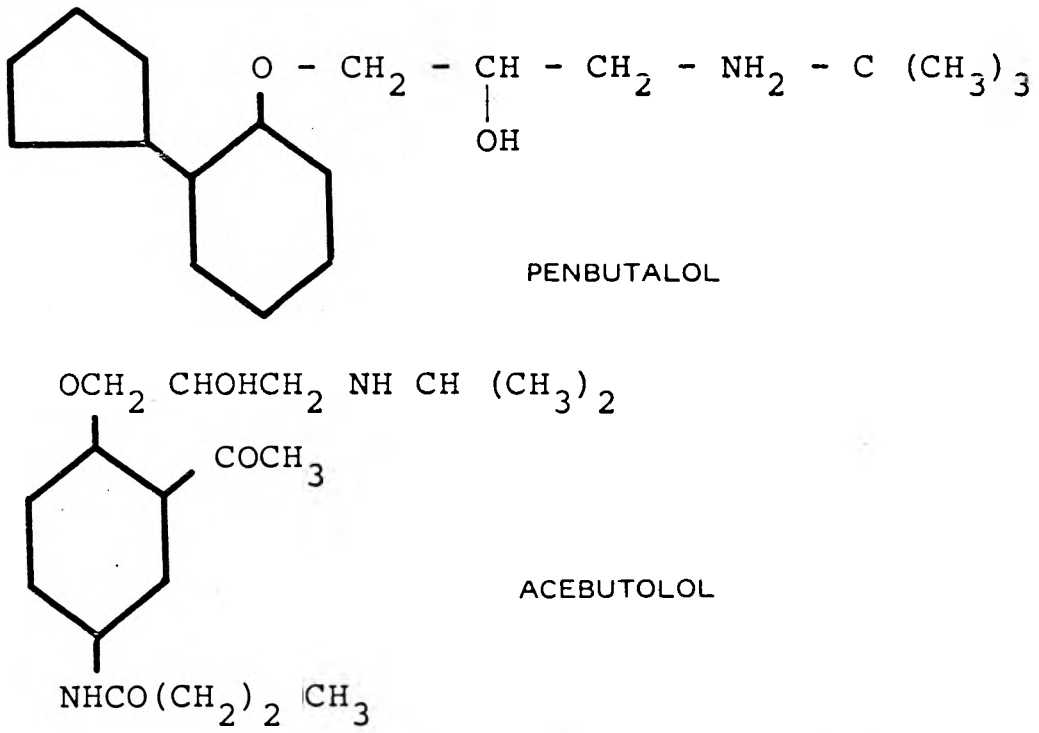
Figure 1 — *Structural formulas of Penbutalol and Acebutolol*



METHODS

Medical technicians or senior medical students volunteered for this study. All were given a detailed explanation of the techniques involved so that informed consent could be obtained. All studies took place in a laboratory equipped for human experimentation, under the uninterrupted supervision of 3 senior clinicians and a registered nurse.

Haemodynamic Studies

Subjects weighed 60 to 75 kg. and were 20 to 35 years old. No clinical evidence of cardiopulmonary disease was present. Plastic catheters were inserted percutaneously, under local analgesia, into the right radial artery, and via a suitable vein in the right cubital fossa, into the right atrium. Pressures were measured electrometrically by Statham strain gauge transducers, supported 7.5 cm. above the surface of the operating table, and recorded continuously on a Philips 3T recorder. Heart rate was measured using the SAN-El-2D 16 pulse meter and a finger photo cell. Cardiac output was measured by dye dilution, using indocyanine green and the Philips XO-1000 combined oximeter/densitometer cuvette. Oxygen saturation and haemoglobin were measured during the cardiac output estimations. Subjects were supine and horizontal throughout the study.

At the conclusion of each experiment, the cardiac output densitometer was calibrated, using each subject's own blood and known doses of cardiac green. Cardiac output was calculated by the method of Williams *et al.*,[3] on an appropriate programme prepared for an Olivetti digital computer. Peripheral vascular resistance (PVR) was calculated from the formula of Aperia.[4]

$$PVR = \frac{\text{mean arterial pressure (mm. Hg)}}{\text{cardiac output (litre/min.)}} \times 80 \text{ dynes sec./cm-5}$$

Peripheral blood flow in the mid-calf region of the left leg was measured at 3 minute intervals by venous occlusion plethysmography using mercury in silastic strain gauge transducers. Change in resistance of the gauge was measured on a Parks 270 plethysmograph, and recordings were made on a 25 cm. Beckman potentiometric recorder at a paper speed of 10 cm. per minute.

Subjects were left undisturbed after introduction of the catheters until heart rates and arterial pressures were steady. Cardiac output, arterial oxygen saturation and haemoglobin estimations were then made at 3-minute intervals until the areas of at least 2 dye dilution curves appeared to agree within 10 percent and the arterial oxygen saturation was steady to within 1 percent. Blood pressures and heart rates were noted at this moment and served as control measurements.

Isoproterenol was then infused at several rates between 0.24 and 2.0 μg./min., in 6 subjects, so that dose-response curves could be plotted. Measurements were made 5 minutes after each change in isoproterenol infusion rate and increasingly doses were given to raise heart rate by at least 25 percent or to a maximum of 110 beats per minute. Isoproterenol infusion was then discontinued.

Subjects remained supine, and cardiovascular parameters were measured at 3-minute intervals until the heart rate and cardiac output were within 10 percent of control values. Penbutalol 0.2 mg. was then administered intravenously over a two-minute period and after a lapse of 15 minutes the infusion of isoproterenol was resumed at the rates as before.

Tests of statistical significance were applied to the mean differences in measurements made in the control period preceding administration of drugs, and during infusion of isoproterenol by applying a Student's t-test to the paired comparisons. The same comparison was applied to mean differences in measurements made during isoproterenol infusions before and after administration of penbutalol 0.2 mg.

In 4 further subjects, all measurements described above were recorded at rest and 30 minutes after 0.3 mg. penbutalol. Isoproterenol was then infused at rates of 0.69, 1.0 and 1.5 μg./min. and measurements made 5 minutes after each change in infusion rate. The response to isoproterenol administered under these circumstances was compared to that when isoproterenol was given alone or after penbutalol 0.2 mg. Statistical significance was tested by applying Student's t-test to the comparison of sample means.

In 4 experiments, similar studies were done on acebutolol. Isoproterenol was infused at rates of 0.69, 1.0 and 1.5 μg./min. before and after intravenous injections of acebutolol 5 and 10 mg.

Pulmonary Studies

The effects of penbutalol upon peak flow, FEV_1 and FVC were assessed in 7 students with allergic Type I asthma. All were males aged 19 to 27 years and 55 to 70 kg. in weight. No subject had used bronchodilators in the 12-hour period before the investigation. Following a 10-minute rest period, peak flow, FEV_1 and FVC were measured using a Wright peak flow meter and a Vitallograph wedge spirometer. Penbutalol 0.05 mg. was then injected intravenously and measurements repeated 90 minutes later. Further injections of 0.05 mg. were given every 90 minutes to a total dose of 0.2 mg. penbutalol. Measurements were repeated before each injection.

Six asthmatics took placebo and oral penbutalol 2, 4 or 8 mg. in random order.

Table I — *Effects of Penbutalol 0.2 mg. I.V.I. on Isoproterenol Infusion*

Observations	Control	Isoproterenol Infusion μg./min.					Isoproterenol Stopped 10 min.	Isoproterenol Infusion μg./min. after Penbutolol 0.2 mg.			
		0.24	0.48	0.69	1.0	1.5		0.48	0.69	1.0	1.5
Heart rate beats/min.	69.6 (3.16)	76.0 (3.21)	73.4 (3.12)	77.6** (3.75)	82.6*** (4.61)	89.0*** (4.69)	70.6 (2.98)	69.8 (2.63)	66.0* (2.04)	71.2*** (2.71)	76.0† (5.47)
Mean arterial pres. min./Hg (S.E.M.)	83.4 (2.89)	83.7 (2.60)	82.6 (3.31)	81.4 (3.34)	80.6 (2.99)	82.0 (3.90)	83.6 (2.04)	86.8 (2.98)	84.0 (2.16)	85.0* (2.63)	86.0 (2.53)
Central Venous Pres. mm.Hg	4.56 (0.50)	3.63 (0.63)	3.63 (1.01)	3.06 (1.23)	2.56 (1.49)	2.56 (1.58)	4.00 (0.74)	3.50 (1.26)	2.50 (1.40)	1.81 (1.52)	1.75 (1.66)
Cardiac Output 1/min. (S.E.M.)	7.26 (0.67)	9.3 (0.42)	8.52 (0.85)	9.64** (0.64)	10.53†† (0.80)	11.52†† (0.52)	7.72 (0.35)	8.15 (0.38)	8.05* (0.68)	9.38 (0.67)	9.90* (0.68)
Stroke Volume ml. (S.E.M.)	103.2 (5.43)	122.3 (8.35)	115.6 (10.75)	124.4 (7.19)	133.0*** (8.22)	133.0*** (4.80)	109.4 (4.75)	116.0 (2.48)	121.3 (7.28)	131.4 (9.65)	131.2 (10.34)
Peripheral Vascular Resistance dynes cm^{-5} (S.E.M.	948 (85)	721 (21)	812 (100)	685** (44)	624† (58)	576† (45)	874 (47)	853 (26)	851* (67)	734 (35)	694* (31)
Flow	2.49 (0.90)	3.66 (0.76)	2.95 (0.51)	3.28 (0.66)	3.71 (0.80)	3.92 (1.00)	2.87 (0.48)	3.17 (0.38)	3.20* (0.64)	3.09 (0.42)	3.27 (0.55)

Data expressed as means ± SEM. Statistical significance tested using paired comparisons. Effects of penbutalol 0.2 mg. I.V.I. assessed by comparisons with responses to isoproterenol infusion before blockade. Effects of isoproterenol assessed by comparisons with control measurements.

* $P < 0.050$ ** $P < 0.025$ *** $P < 0.010$ † $P < 0.005$ †† $P < 0.001$

Peak flow, FEV_1 and FVC were measured immediately before taking the capsules and 2, 4, 6, and 8 hours later. The respiratory effects of acebutolol were investigated separately and have been reported elsewhere.[5]

RESULTS

Results appear in Tables I to III and Figures 2 to 9, inclusive.

Table II — *Effects of Penbutalol 0.3 mg. I.V.I. on Isoproterenol Infusion*

Observation	Control	Isoproterenol Infusion μg./min. after Penbutalol 0.3 mg. 0.69	1.0	1.5
Heart rate beats/min. (S.E.M.	73.2 (3.15)	68.5 (2.22)	68.7 (2.10)*	70.7 (2.66)***
Mean arterial Pressure mm. Hg (S.E.M.)	94.7 (1.44)	95.2 (1.80)	95.0 (2.04)	96.5 (1.71)
Central Venous Pressure	2.62 (1.30	1.62 (0.90)	1.25 (0.78)	1.00 (1.50)
Cardiac Out-Put 1/min. (S.E.M.)	7.85 (0.78)	7.07 (0.88)*	7.65 (0.45)**	8.65 (1.43)
Stroke Volume (S.E.M.) ml.	107 (11.77)	102 (9.75)	111 (3.71)*	121 (15.62)
Peripheral Vascular Res. dynes cm^{-5} (S.E.M.)	993 (94)	1119 (115)***	1003 (62)†	951 (119)**
Flow	4.04 (0.03)	2.97 (0.09)	3.62 (0.51)	4.11 (0.59)

Data expressed as means ± S.E.M. Tests of significance refer to differences between response to isoprenaline alone and isoprenaline and penbutalol 0.3 mg.

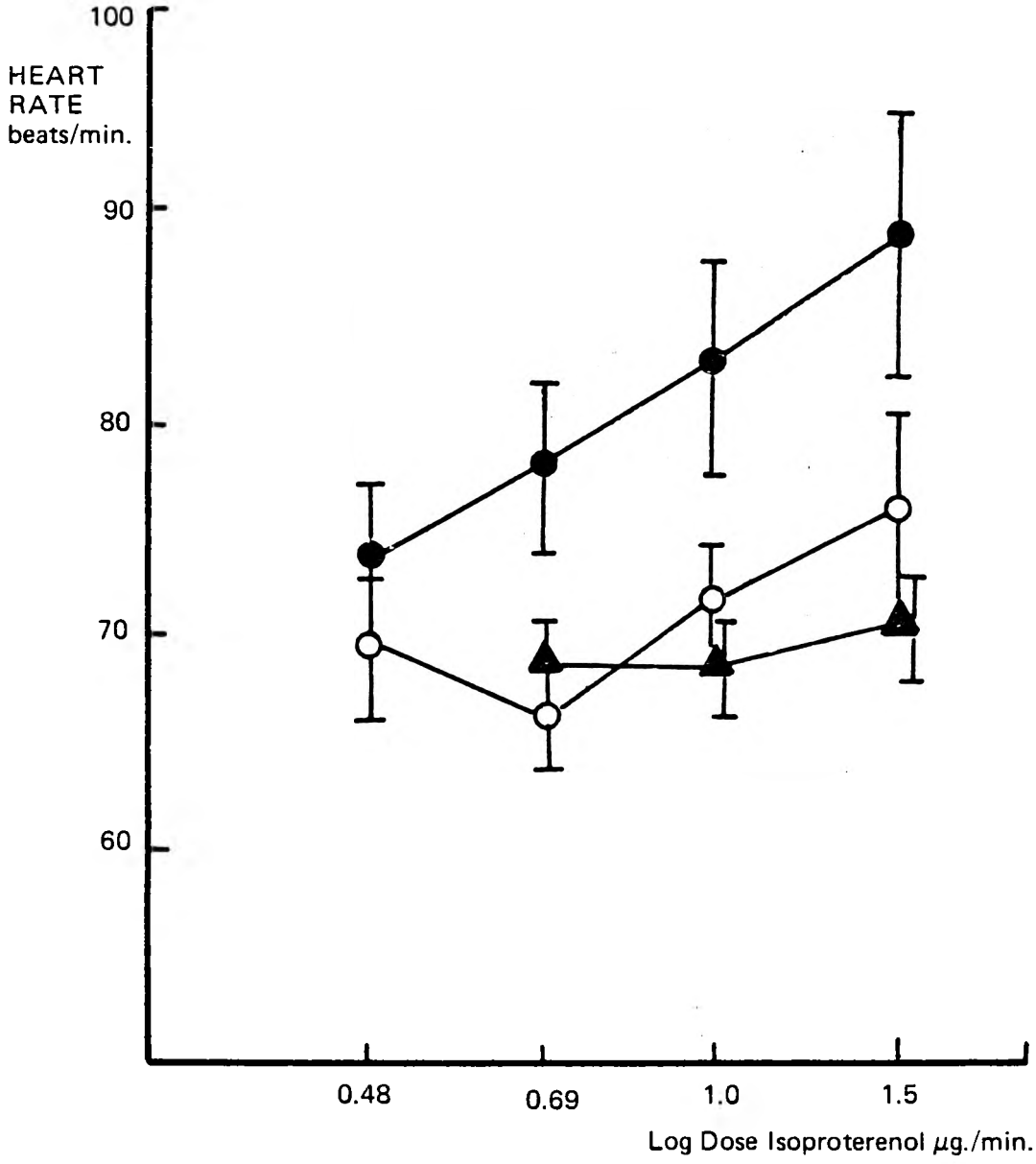
* $P < 0.05$ ** $P < 0.025$ *** $P < 0.010$ † $P < 0.005$

(a) *Haemodynamic Studies*

Isoproterenol caused significant increases, at various infusion rates, in heart rate, cardiac output and stroke volume. Peripheral vascular resistance fell, but no statistically significant changes in central venous pressure, mean arterial pressure or peripheral blood flow occurred (Table I, Fig. 2-5). Many of the effects of isoproterenol were much reduced by penbutalol 0.2 and 0.3 mg. by intravenous infusion (I.V.I.) Valid comparisons between subjects given 0.2 and 0.3 mg. penbutalol could not be made with respect to arterial and venous pressures or peripheral flow because of

differences in control measurements. (Tables I and II).

Figure 2 — *Blockade of isoproterenol by penbutalol. Closed circles represent isoproterenol infusion. Open circles isoproterenol after penbutalol 0.2 mg. I.V.I. and triangles isoproterenol after penbutalol 0.3 mg.*



There was no significant difference between the effects of acebutolol 5 mg. and penbutalol 0.2 mg. I.V.I. Acebutolol 10 mg. was more potent than penbutalol 0.2 mg. in blocking the effects of isoproterenol on cardiac output, stroke volume and peripheral vascular resistance, ($P = < 0.05$), but this comparison is possibly invalid in that control measurements were different in the groups studied. (Table III).

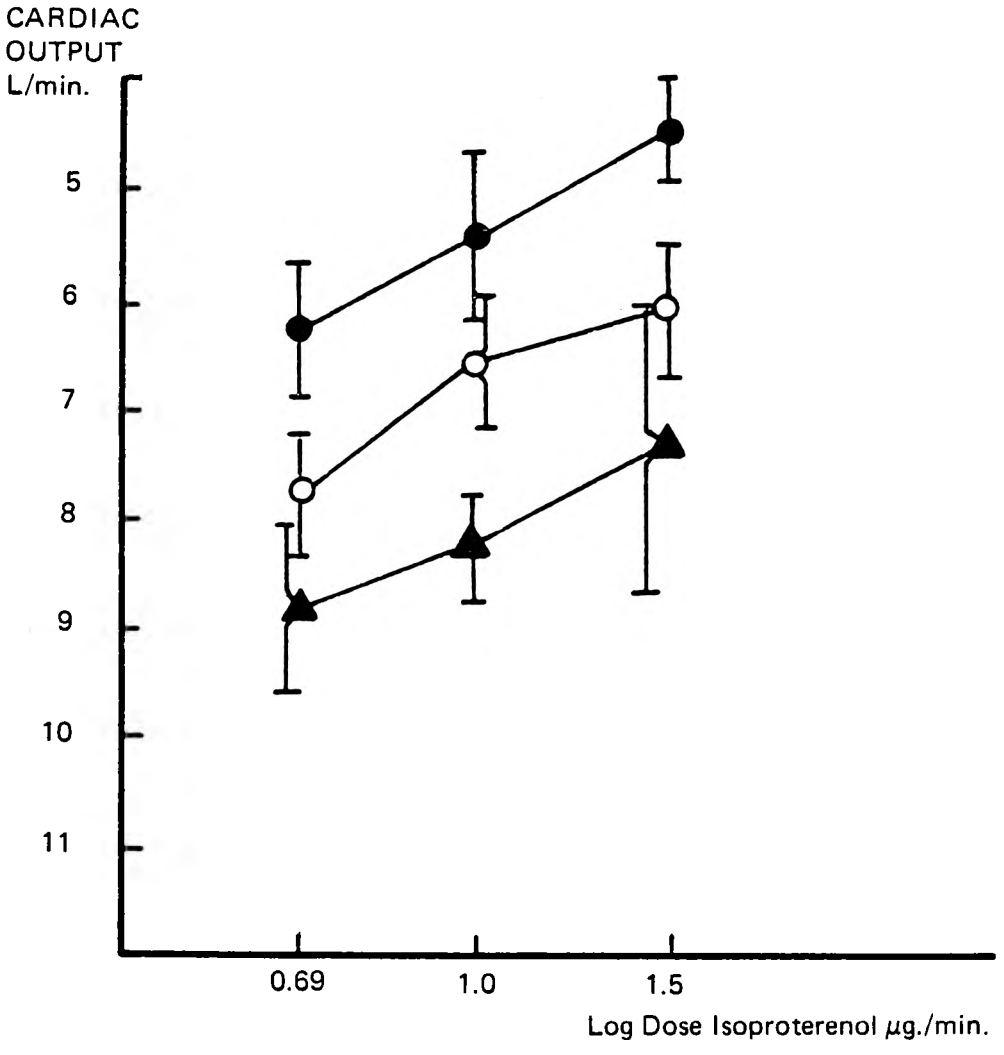
Table III. — *Effects of Acebutolol on Isoproterenol Infusion*

Observations	Control	Isoproterenol Infusion μg./min. 0.69	1.0	1.5	Isoproterenol Infusion μg. /min. after Acebutolol 5 mg. 0.69	1.0	1.5	Isoproterenol Infusion μg. /min. after Acebutolol 10 mg. 0.69	1.0	1.5
Heart rate beats/ min (S.E.M.)	67.8 (5.60)	76.5*** (5.52)	83.3* (6.05)	92.3* (10.9)	63.5 (3.50)	71.0 (1.53)	71.5 (1.50)	67.5 7 (2.53)	70.5* (2.96)	76.0 (4.16)
Mean Arterial Pres. mm.Hg (S.E.M.)	91.5 (2.33)	86.0* (2.35)	85.0* (2.12)	88.7* (3.53)	89.0 (1.00)	88.3 (1.67)	85.0 (3.00)	93.5 (1.66)	91.0 (1.68)	91.3 (1.89)
Central Venous Pres. mm.Hg (S.E.M.)	4.88 (0.80)	3.13*** (0.99)	2.81*** (0.86)	3.33** (0.93)	4.38 (1.87)	4.08 (0.87)	3.75 (0.25)	5.06 (0.74)	4.75** (0.77)	5.33** (0.60)
Cardiac Output 1/min. (S.E.M.)	5.70 (0.20)	7.65* (0.73)	9.55† (0.61)	9.90*** (1.32)	6.60 (0.60)	7.50 (0.51)	8.10 (0.70)	6.15 (0.40)	6.93 (0.57)	7.77 (1.01)
Stroke Vol. Ml. (S.E.M.)	85.8 (8.70)	101.3 (12.59)	115.8** (11.37)	111.7 (25.73)	103.5 (3.50)	104.7 (4.81)	112.5 (7.50)	91.3 (7.09)	98.5† (10.19)	109.0 (16.62)
Peripheral Vascular Resistance dynes cm^{-5} (S.E.M.)	1288 (54)	926† (98)	723†† (58)	743† (110)	1087 (87)	950 (61)	848 (103)	1232*** (98)	1078*** (111)	981** (146)
Flow	2.37 (0.18)	2.67 (0.32)	2.93* (0.22)	3.30* (0.17)	3.36* (1.27)	2.34 (0.60)	2.86 (0.96)	2.41 (0.59)	2.32 (0.48)	2.59 (0.74)

Data expressed as means ± S.E.M. Statistical significance tested using paired comparisons as Table I. Effects of 5 mg. Acebutolol not analysed statistically, because whole sample did not receive this dose.

* $P < 0.050$ ** $P < 0.025$ *** $P < 0.010$ † $P < 0.005$ †† $P < 0.001$

Figure 3 — *Blockade of isoproterenol by penbutalol. Closed circles represent isoproterenol infusion. Open circles isoproterenol after penbutalol 0.2 mg. I.V.I. and triangles isoproterenol after penbutalol 0.3 mg.*



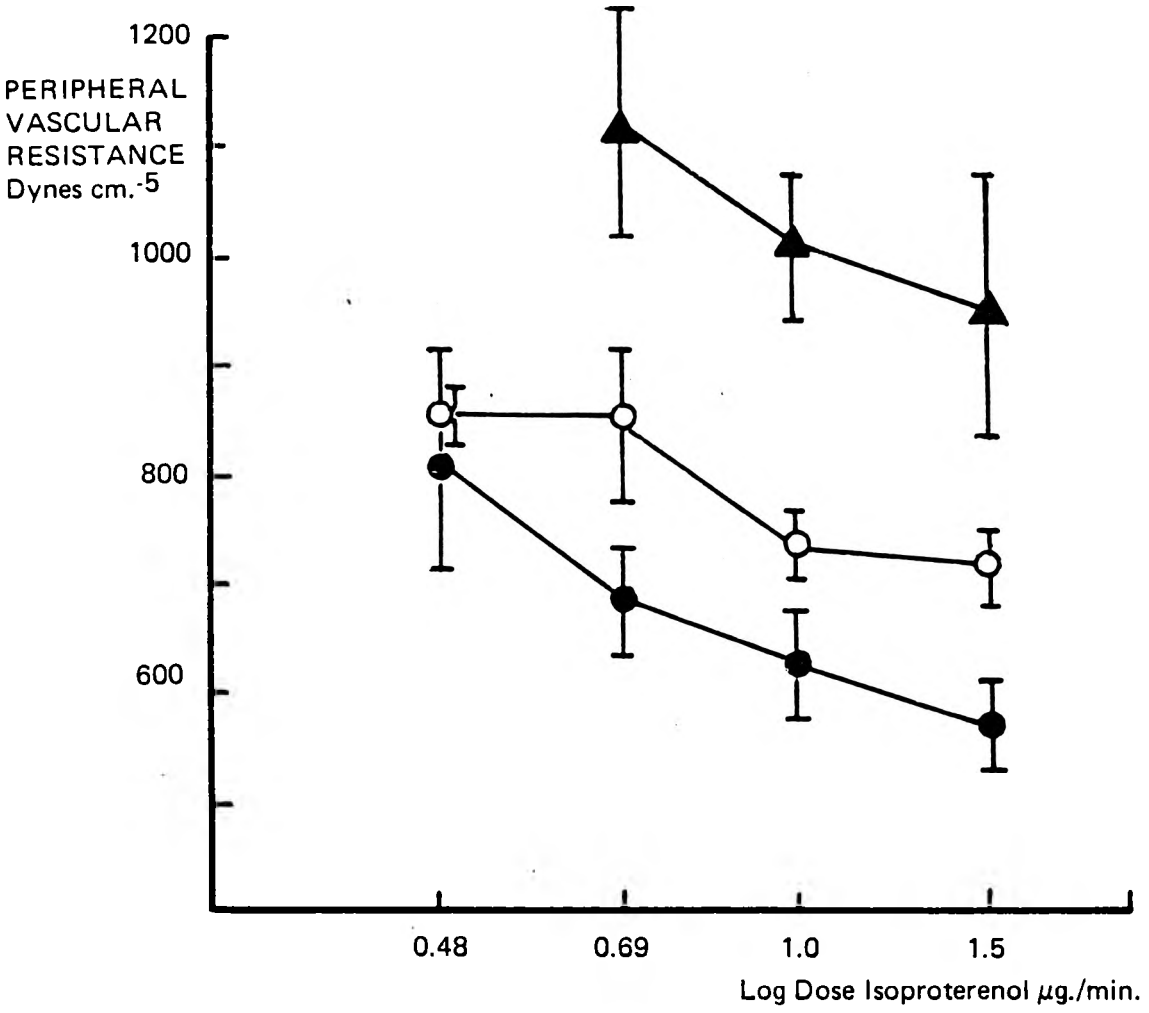
(b) *Pulmonary Studies*

Results are set out in Tables IV and V. Two asthmatics developed bronchospasm 90 minutes after 0.15 mg. penbutalol I.V.I. and within 4 hours of a 4-mg. dose given by mouth. Two others reacted unfavourably to oral penbutalol 2 mg. and 1 became breathless 4 hours after taking 8 mg.

DISCUSSION

A number of β-adrenergic blocking agents have been synthesized in recent years. These preparations are much alike in their pharmacological charac-

Figure 4 — *Blockade of isoproterenol by penbutalol. Closed circles represent isoproterenol infusion. Open circles isoproterenol after penbutatol 0.2 mg. I.V.I. and triangles isoproterenol after penbutalol 0.3 mg.*



teristics although slight differences in clinical efficacy are reported. Two compounds, practolol and acebutolol,[5] appear to be partly selective for β_1 receptors.

Intravenous penbutalol 0.1 to 0.3 mg. or acebutolol 5 to 10 mg. block the cardiovascular actions of isoproterenol effectively. The duration of this blockade was not investigated, but experiments elsewhere indicate that the biological half-life of pentubalol I.V.I. is about 10 hours and that of acebutolol 6 hours.[6-7]

Penbutalol precipitates bronchospasm in asthmatics, a tendency shared with other non-selective β-adrenergic blocking agents. Previous

Figure 5 — *Blockade of isoproterenol by penbutalol. Closed circles represent isoproterenol infusion. Open circles isoproterenol after penbutalol 0.2 mg. I.V.I. and triangles isoproterenol after penbutalol 0.3 mg.*

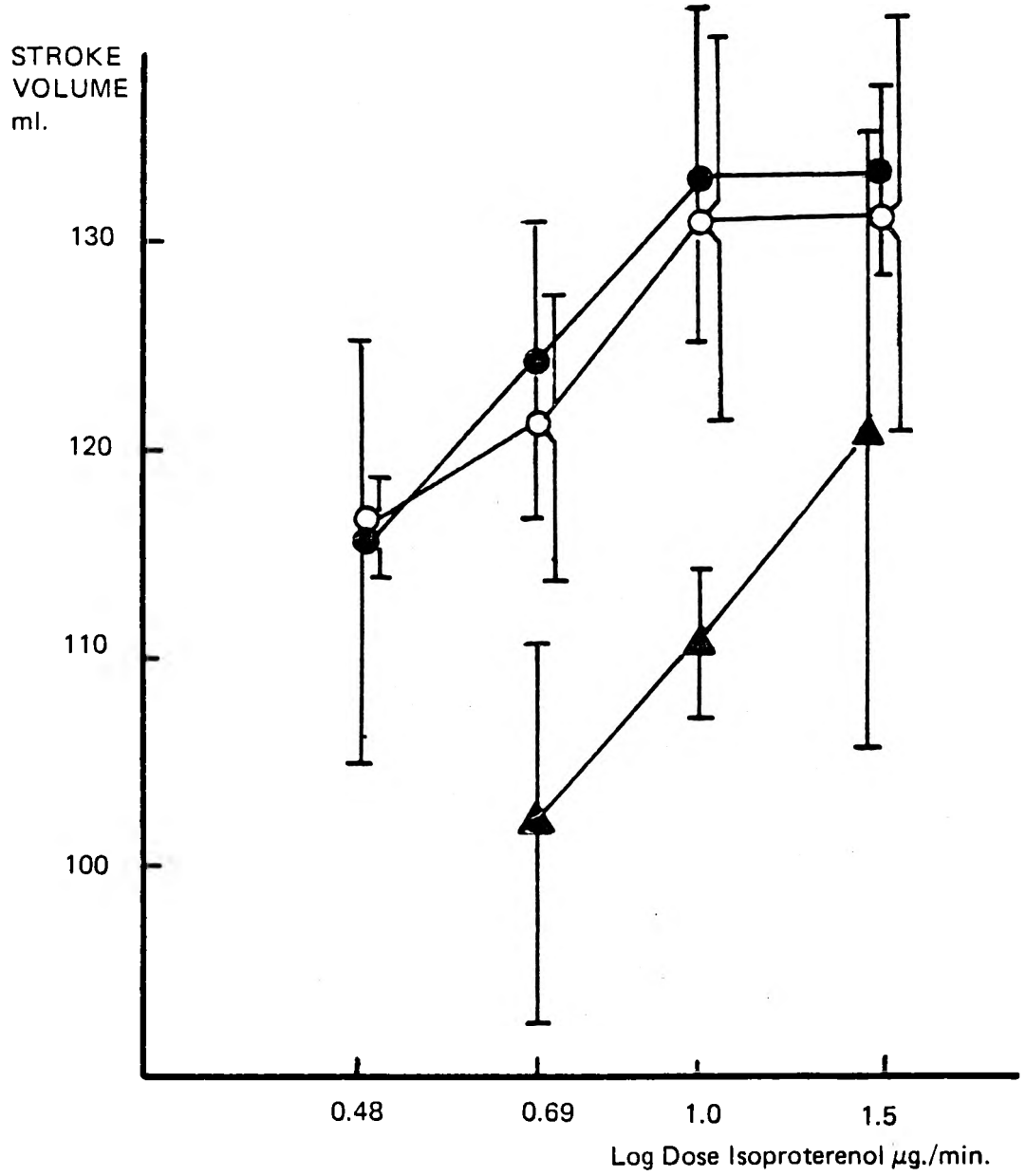


Figure 6 — *Blockade of isoproterenol by acebutolol. Closed circles represent isoproterenol infusion. Open circles isoproterenol after acebutolol 5 mg. I.V.I., triangles isoproterenol after acebutolol 10 mg. I.V.I.*

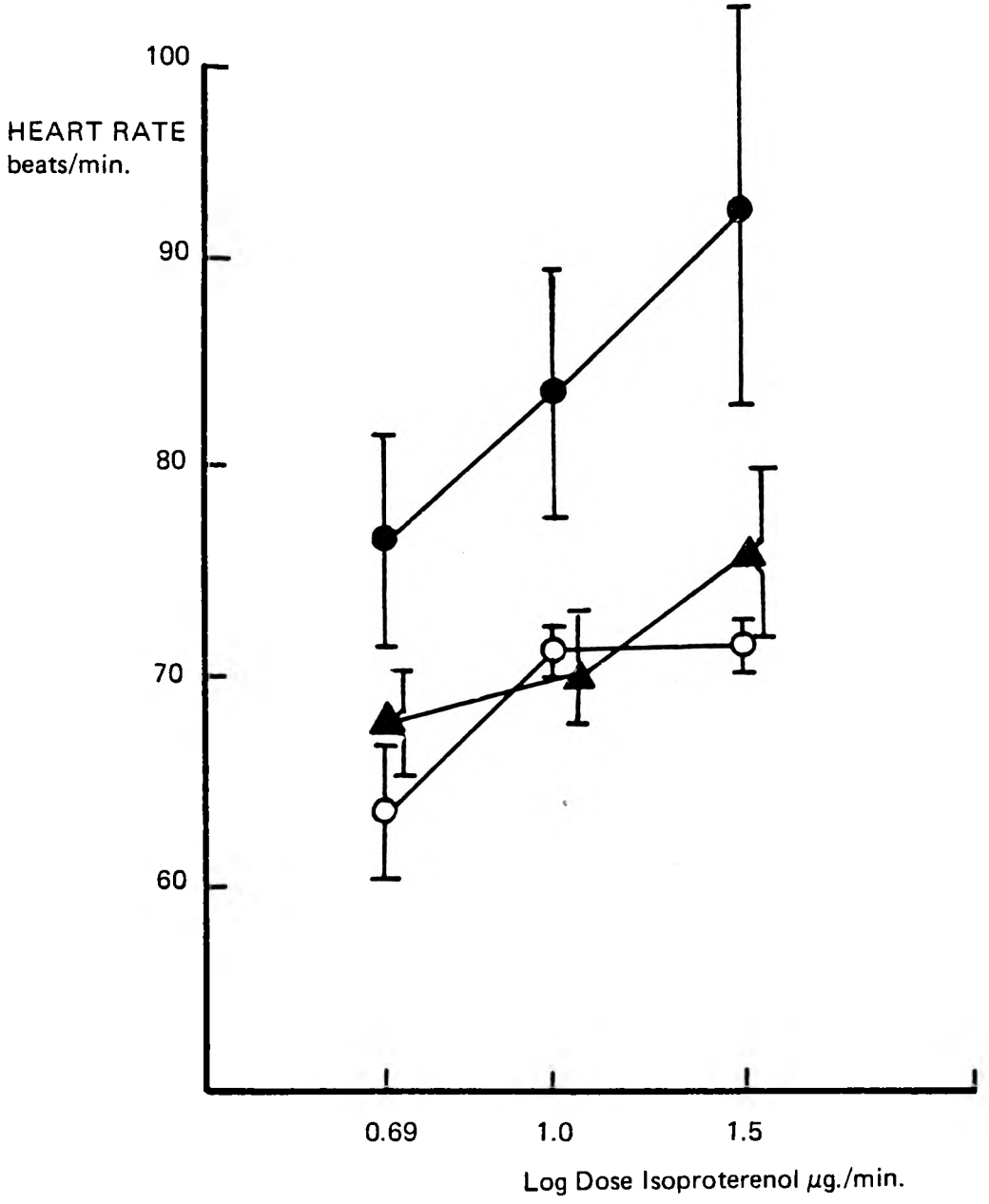
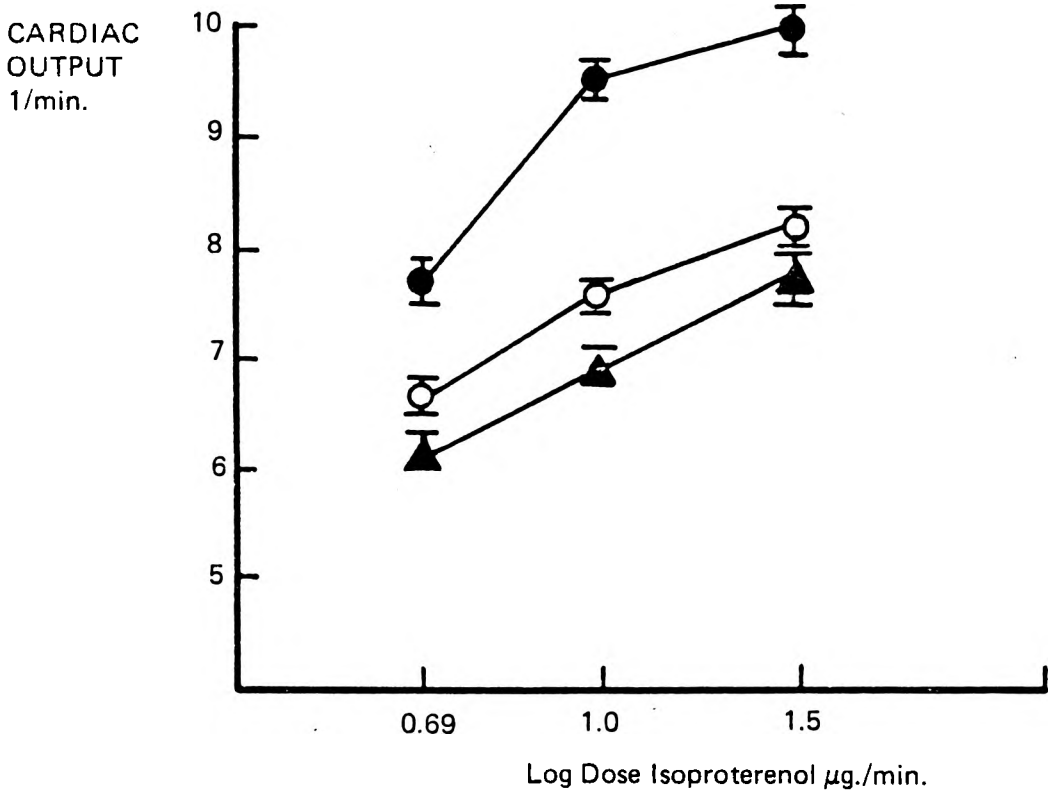


Figure 7 — *Blockade of isoproterenol by acebutolol. Closed circles represent isoproterenol infusion. Open circles isoproterenol after acebutolol 5 mg. I.V.I., triangles isoproterenol after acebutolol 10 mg. I.V.I.*



studies indicate that acebutolol and practolol are usually not associated with respiratory side effects and may be used when asthmatics require β blockade.

Our preliminary studies indicate that penbutalol is a potent adrenergic blocking agent likely to resemble propranolol in the clinical situation, rather than practolol or acebutolol.

Acknowledgements

We wish to thank Miss L. Bees and Mr. C. Lockett for technical assistance and Mrs. Y. Potgieter for typing the manuscript. We are grateful to Dr. C. Venter of Hoechst Pharmaceuticals and to May & Baker Limited for supplies of penbutalol and acebutolol respectively.

Table IV — *Effects of Intravenous Penbutalol in Asthmatic Subjects*

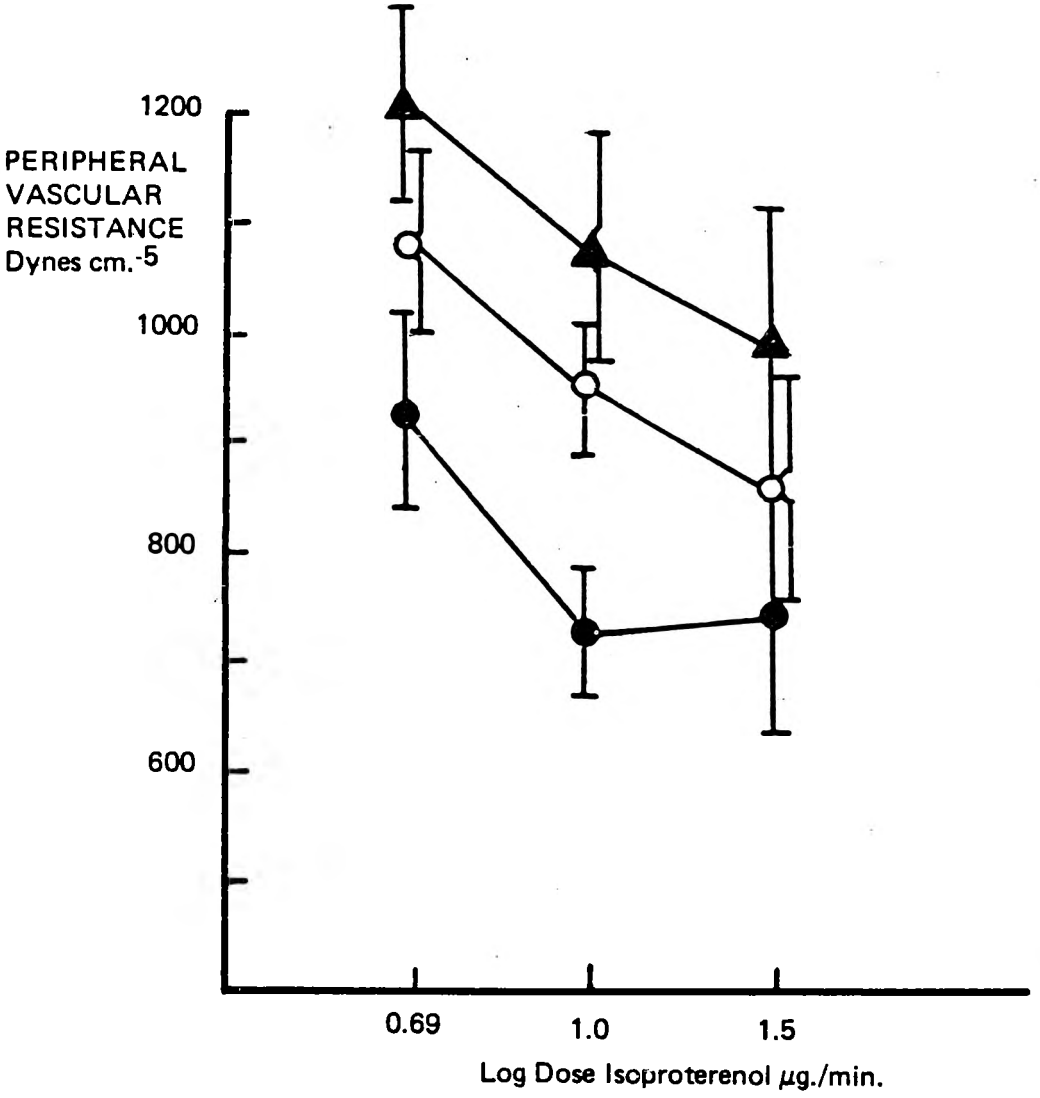
Subject No.	Control Measurements			0.1 mg. Penbutalol			0.15 mg. Penbutalol			0.2 mg. Penbutalol		
	FEV_1	FVC	Peak Flow	FEV_1	FVC	Peak Flow	FEV_1	FVC	Peak Flow	FEV_1	FVC	Peak Flow
1	3.2	5.05	500	3.2	4.75	515	3.1	4.75	450	3.1	4.65	485
2	2.9	5.35	460	3.55	5.15	520	3.5	5.35	580	3.5	5.05	520
3	1.55	3.05	325	1.45	3.15	240	STUDY ABORTED					
4	1.9	3.6	190	1.9	3.45	220	1.55	2.95	120	ABORTED		
5	2.5	4.2	450	2.5	3.8	440	2.2	3.4	410	2.5	3.5	390
6	4.7	6.9	500	4.55	6.5	470	4.45	6.5	450	INCOMPLETE		
Mean	2.79	4.69	404.17	2.86	4.47	400.83	2.96	4.59	402.0	3.03	4.40	465.0
S.E.M.	0.46	0.56	50.2	0.47	0.51	55.4	0.5	0.65	76.1	0.29	0.47	38.8

Table V — *Effects of Oral Penbutalol in Asthmatic Subjects*

Dosage	Subject No.	Control Measurement			2 hr.			4 hr.			6 hr.			8 hr.		
		FEV_1	FVC	Peak Flow	FEV_1	FVC	Peak Flow	FEV_1	FVC	Peak Flow	FEV_1	FVC	Peak Flow	FEV_1	FVC	Peak Flow
2 mg.	1	3.05	4.75	495	3.05	4.65	480	2.9	4.55	480	2.9	4.55	430	2.85	4.65	455
	2	3.05	5.2	465	2.7	5.0	420	3.05	5.0	420	3.4	5.15	480	3.1	4.9	480
	3	1.7	3.1	315	1.75	2.9	280	—	—	—	1.2	2.7	260	1.55	3.0	260
	4	2.0	3.4	220	1.5	2.8	185	1.5	2.75	170	1.15	2.25	95	A B O R T E D		
	5	2.15	3.4	360	1.4	2.45	220	E X P E R I M E N T		A B O R T E D						
	6	4.5	7.1	490	4.55	6.9	470	4.5	6.7	450	4.3	6.7	440	4.6	6.7	440
	Mean	2.74	4.49	390.8	2.49	4.12*	342.5*	2.99	4.75	380.0	2.59	4.27	341.0	3.03	4.81	408.8
	S.E.M.	0.42	0.62	45.49	0.49	0.70	53.19	0.61	0.81	71.1	0.62	0.82	72.2	0.63	0.76	50.26
4 mg.	1	3.35	4.4	515	3.25	4.8	460	3.25	4.75	490	3.15	4.45	460	2.95	4.55	440
	2	3.3	3.3	520	2.8	2.8	450	E X P E R I M E N T		A B O R T E D						
	3	2.0	3.6	400	1.0	2.15	160	E X P E R I M E N T		A B O R T E D						
	6	4.5	6.6	450	4.2	6.5	440	4.35	6.5	440	4.2	6.5	430	4.3	6.5	440
	Mean	3.29	4.48	471.25	2.81	4.06	377.5									
	S.E.M.	0.51	0.75	28.6	0.67	0.99	72.6									
8 mg.	1		4.75	460		4.35	350	E X P E R I M E N T		A B O R T E D						
	6		6.65	420		6.65	440		6.7	400		6.5	405		6.5	390

Asterisks signify significant group change in paired comparisons ($P < 0.05$).
Mean and S.E.M. not calculated for $<$ 3 measurements.

Figure 8 — *Blockade of isoproterenol by acebutolol. Closed circles represent isoproterenol infusion. Open circles isoproterenol after acebutolol 5 mg. I.V.U., triangles isoproterenol after acebutolol 10 mg. I.V.I.*



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PAPER D7

The Cardiovascular Effects of Etilefrine

The technical expertise required to measure cardiac output was provided by Professor A.J. Coleman. Intubation of arteries was a shared procedure as was the analysis of data and preparation of the publication. The protocol followed was a joint venture. Professor A.C. Asmal provided editorial assistance.

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The Cardiovascular Effects of Etilefrine

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Summary. Intravenous etilefrine increases the pulse rate, cardiac output, stroke volume, central venous pressure and mean arterial pressure of healthy individuals. Peripheral vascular resistance falls during the infusion of 1 - 8 mg etilefrine but begins to rise at higher dosage. Marked falls in pulse rate, cardiac output, stroke volume and peripheral bloodflow, accompanied by rises in mean arterial pressure, occur when etilefrine is infused after administration of intravenous propranolol 2,5 mg. These findings indicate that etilefrine has both β_1 and α adrenergic effects in man.

Key words: Etilefrine, normal man, intravenous, haemodynamic effects, propranolol, phentolamine, α, β_1 stimulation.

Etilefrine, (2-ethylamino-1-(3'-hydroxy-phenyl) ethanol, is a sympathomimetic amine of the 3-hydroxy-phenylethanolamine series used in treating orthostatic hypotension of neurological, cardiovascular, endocrine or metabolic origin. Intravenous infusion of this compound increases cardiac output, stroke volume, venous return and blood pressure in man and experimental animals, suggesting stimulation of both α and β adrenergic receptors, (Nusser, Donath and Russ, 1965; Mellander, 1966; Limbourg, Just and Lang, 1973; Tarnow *et al.*, 1973; Carrera and Aguilera, 1973). However, *in vitro* studies indicate that etilefrine has a much higher affinity for β_1 (cardiac) than for α or β_2 adrenoreceptors, (Offermeier and Dreyer, 1971).

The objects of the study reported here were to record dose responses to etilefrine in man, to determine its mechanism of action, and to initiate investigations leading to the assessment of this preparation as an inotropic stimulant in the treatment of cardiac failure associated with myocardial infarction.

Material and Methods

Nine healthy adults, 1 female and 8 males, volunteered to act as subjects for this investigation. Two were experienced medical technicians and 7 were senior medical students. All had participated in previous experiments of a similar nature either as subjects or observers. A full explanation of the implications of the study was given to each volunteer. All studies were performed with the subjects supine, in a horizontal position, and under the uninterrupted supervision of 3 senior clinicians and a registered nurse. Subjects weighed 55 to 70 kg and were 20 to 35 years old. No clinical evidence of cardiopulmonary disease was present.

Using local analgesia, plastic catheters were inserted percutaneously into the right atrium from an antecubital vein and into the right radial artery. Pressures were measured by Statham strain gauge transducers supported 7,5 cm above the surface of an operating table and recorded continuously on a Philips 3T recorder. Heart rate was measured using a SAN/E1/2D 16 pulsemeter and a finger photocell. Cardiac output was measured by dye dilution using indocyanine green and a Philips XO 1000 combined oximeter/densitometer cuvette. Arterial oxygen saturation and haemoglobin were measured during cardiac output estimation.

At the conclusion of each experiment the cardiac output densitometer was calibrated using the patients own blood and known doses of indocyanine green. Cardiac output was calculated using the method of Williams, Donovan and Wood (1966) to prepare an appropriate programme for an Olivetti Digital Computer. Total peripheral vascular resistance (PVR) was calculated from the formula of Aperia, (1940):

$$\text{PVR}\,(\text{dynes}\cdot\text{sec}\cdot\text{cm}^{-5}) = \frac{\text{mean arterial pressure (mmHg)}}{\text{cardiac output (l/min)}} \times 80$$

Peripheral blood flow in the mid-calf region of the left leg was measured by venous occlusion plethysmography using mercury in silastic strain gauges. Change in resistance of the gauge was measured on a Parks 270 plethysmograph, and record-

ings were made on a Unicorder U 400 potentiometric recorder at paper speed of 15 cm per minute.

Subjects lay quietly for 15 - 20 min following introduction of the catheters after which measurements of pulse, pressures, and cardiac output were made at 5 min intervals until the areas of at least 2 dye dilution curves appeared to agree within 10 percent and the arterial oxygen saturation was steady within 1 percent. Blood pressures and heart rates were noted at this moment and served as control measurements. In 5 experiments etilefrine was infused at the rate of 0,5 mg/min using a Sage 355 constant infusion pump. Further measurements of heart rate, mean arterial pressure, central venous pressure, peripheral blood flow and cardiac output were made when 1, 2, 4, 6, 8 and 10 mg of etilefrine had been infused. These measurements were repeated 20 min after cessation of the infusion.

In 4 experiments propranolol (2 - 5 mg) was injected intravenously at a rate of 1 mg/2 min. Haemodynamic parameters were measured 5 min later and etilefrine 0,5 mg/min then infused to a maximum dose of 10 mg, or until heart rate was 20 - 30% below the control reading. Cardiac output and pressures were measured at this point. Phentolamine 2,5 mg was then administered intravenously over 2 min while pulse and arterial and central venous pressures were monitored. Cardiac output was measured 2 min later.

Tests of statistical significance were applied to the mean differences in measurements made in the control period preceding administration of etilefrine and during its infusion by applying a Student's t-test to the paired comparisons.

Results

A slight increase in pulse rate was noted during etilefrine infusion. This change was significant after 2 and 6 mg etilefrine ($P < 0.025$ and < 0.05), but not at any other stage (Table 1). Mean arterial pressure, central venous pressure, (Table 1), cardiac output and stroke volume, (Table 2), all increased progressively during the infusion of etilefrine and these changes were consistently significant.

Peripheral vascular resistance (Table 3) fell during the infusion of etilefrine 1 - 8 mg, but rose slightly after a 10 mg dose. These changes were significant at 1, 4, 6, and 8 mg. No significant change in peripheral blood flow occurred, although mean flow rose slightly during studies.

There was no significant difference between control readings and those made 20 min after infusions were stopped, except in the case of arterial pressure which remained significantly elevated at 91,0 mmHg compared with a control mean of 79.0 mm Hg ($P < 0.025$). Mean levels for central venous pressure, cardiac output, and stroke volume were greater than those recorded before infusions started, but these differences were not of statistical significance.

Administration of propranolol 2 - 5 mg caused slight falls in pulse rate, central venous pressure, mean arterial pressure and cardiac output, (Table 4). These changes were not significant, however. Total doses of 1,5 - 5 mg (mean 3,5 mg) etilefrine were infused; means of measurements made immediately after stopping the infusion appear in Tables 4 and 5. Pulse rate, cardiac output, stroke volume and peripheral blood-flow fell

Table 1. Effects of etilefrine 0.5 mg/min infused intravenously in man

Dose	0 mg	1 mg	2 mg	4 mg	6 mg	8 mg	10 mg	10 mg + 20 min
Heart Rate (beats/min)	68.6	72.2	74.2[b]	75.2	77.4[a]	75.6	72.6	68.0
Mean								
S.E.M.	3.63	2.99	3.37	3.65	3.74	2.89	2.58	2.68
Arterial Press. (mm Hg)								
Mean	79.0	78.6	81.8[c]	82.0	86.2[b]	89.4[b]	93.4[c]	91.0[b]
S.E.M.	2.00	2.71	2.31	1.70	1.43	0.87	0.60	2.65
C.V.P. (mm Hg)								
Mean	3.40	3.90[b]	4.70[c]	5.60[d]	6.00[d]	6.55[c]	7.40[d]	5.35
S.E.M.	1.06	1.12	0.97	1.03	1.04	1.19	1.07	1.38

Levels of significance [a] $P < 0.050$ [b] $P < 0.025$ [c] $P < 0.005$ [d] $P < 0.001$

markedly during etilefrine infusion and mean arterial pressure, peripheral vascular resistance and central venous pressure rose. All these values returned to control levels after α adrenergic blockade with phentolamine 2,5 mg.

Discussion

The findings reported in this communication are in general agreement with those of other investigators, (Carrera and Aquilera, 1973; Limbourg, Just and Lang, 1973; Tarnow *et al.*, 1973). Intravenous infusion of etilefrine 50 - 200 μg/kg causes marked increases in stroke volume, cardiac output, central venous pressure and mean arterial pressure, accompanied by slight increases in pulse rate and peripheral blood-flow. These changes, and the associated fall in total peripheral resistance may be explained by positive inotropism, mediated through β_1 adrenergic receptors, (Offermeier and Dreyer, 1971) and constriction of capacitance vessels with dilatation of arterioles (Mellander, 1966). Increased venous return, mediated by raised tone in capacitance vessels, probably contributes to the rise in cardiac output which follows etilefrine administration.

Mellander's studies in the cat indicate the etilefrine causes reflex vasodilation in low dosage and α receptor mediated vaso-constriction of peripheral vessels at higher doses. In our studies in man, the dose related fall in peripheral vascular resistance associated with etilefrine infusion stopped when more than 100 μg/kg was ad-

Table 2. Effects of etilefrine 0,5 mg/min infused intravenously in man

Dose	0 mg	1 mg	2 mg	4 mg	6 mg	8 mg	10 mg	10 mg + 20 min
Cardiac Output (l/min)								
Mean	6.52	7.32	8.08[d]	9.34[b]	10.06[c]	10.00	9.34	7.34
S.E.M.	0.77	0.69	0.61	0.68	0.65	0.72	0.75	0.71
Stroke Volume (ml)								
Mean	95.6	103.2	110.2[d]	127.6[a]	132.4[b]	134.0[c]	129.8[c]	109.4
S.E.M.	10.6	13.4	9.90	16.1	14.3	13.6	13.1	13.5

Levels of significance [a] $P < 0.050$ [b] $P < 0.025$ [c] $P < 0.010$ [d] $P < 0.005$ [e] $P < 0.001$

Table 3. Effects of etilefrine 0,5 mg/min infused intravenously in man

Dose	0 mg	1 mg	2 mg	4 mg	6 mg	8 mg	10 mg	10 mg + 20 min
Peripheral Vascular Resistance (dynes·sec·cm^{-5})								
Mean	1010	883[a]	825	715[b]	695[b]	730[b]	825	1023
S.E.M.	94.5	72.1	50.4	43.7	37.0	53.2	78.6	88.0
Peripheral Blood Flow (ml/100 g)								
Mean	2.68	3.01	3.06	3.14	3.27	3.35	3.16	2.92
S.E.M.	0.45	0.64	0.53	0.68	0.81	0.74	0.73	0.74

Levels of significance [a] $P < 0.050$ [b] $P < 0.025$

Table 4. Effects of α and β blockade on effects of etilefrine infusion in man

Dose	0	β Blockade (Propranolol 2-5mg)	Etilefrine (Mean dose 3,5 mg)	α Blockade (Phentolamine 2,5 mg)
Heart Rate (beats/min)				
Mean	61,3	58,0	45,3[b]	64,0
S.E.M.	±6.89	±6,11	±4,18	±7,64
Mean Arterial Pressure (mmHg)				
Mean	76,0	74,0	90,3[b]	73,0
S.E.M.	±3,21	±4,00	±4,91	±2,08
C.V.P. (mmHg)				
Mean	5,42	5,25	9,58[b]	5,67
S.E.M.	±0.82	±0.90	±1.23	±0.67
Cardiac Output (l/min)				
Mean	5,03	4,83	3,43[a]	5,37
S.E.M.	±0.29	±0.12	±0.32	±0,50

Levels of significance [a] P = < 0,05
[b] P = < 0,05

Table 5. Effects of α and β blockade on effects of etilefrine infusion in man

	Control	β Blockade (Propranolol 2-5mg)	Etilefrine (Mean dose 3,5 mg)	α Blockade (Phentolamine 2,5 mg)
Stroke volume (ml)				
Mean	85,3	86,0	78,3[a]	86,7
S.E.M.	±14,9	±11,0	±15,2	±14,6
P.V.R. (dynes sec cm^{-5})				
Mean	1221	1224	2158[b]	1109
S.E.M.	±119	±48,9	±308	±118
Muscle Flow (ml/100 g)				
Mean	2,34	2,34	1,81	2,12
S.E.M.	±0,66	±0,34	±0,08	±0,38

Levels of significance [a] P = < 0,05
[b] P = < 0,025

ministered. The consistent dose-related elevation in central venous pressure suggests that capacitance vessels were constricted by etilefrine and not affected significantly by reflex vasodilation.

The changed responses to etilefrine associated with β and α blockade were impressive, and indicate that etilefrine is a stimulant of α and β adrenergic receptors in man, as in animals (Mellander, 1966; Carrera and Aguile, 1973). When etilefrine was given after β blockade the most striking effects were rises in central venous pressure, mean arterial pressure and peripheral vascular resistance coupled with falls in pulse rate, cardiac output and stroke volume. Since propranolol blocks the β_1 (inotropic) effects of etilefrine without compromising α effects on the peripheral vasculature, the falls in pulse rate, cardiac output and stroke volume might be reflex responses to α mediated change in arterial pressure or represent an unexplained potentiation of propranolol's specific or non-specific effects by etilefrine. The former possibility is favoured by the rapid return of all parameters to control levels after phentolamine administration.

Owing to the complexity of the study, atropine was not administered. α Blockade did not result in further falls in cardiac output, which suggests that an α mediated rise in venous return is less important than β_1 adrenergic stimulation in determining increases in cardiac output following etilefrine administration.

The findings reported here lend support to the contention of Nusser, Donath and Russ, (1965), that etilefrine increases stroke volume and heart contractile force by capacitance vessel constriction and by its positive inotropic effects. Further detailed studies are necessary, however, to clarify its mechanism of action, and to assess whether it has a place in the management of cardiovascular disease.

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PAPER D8

Cardiovascular Effects of Intravenous Indoramin Hydrochloride in Man

The technical expertise required to measure cardiac output was provided by Professor Coleman. Intubation of arteries was a shared procedure as was the analysis of data and preparation of the publication. The protocol followed was a joint venture. Professor A.C. Asmal provided editorial assistance.

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Cardiovascular Effects of Intravenous Indoramin Hydrochloride in Man

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Haemodynamic effects of intravenous indoramin (5–20 mg) were measured in ten healthy volunteers. Slight falls in arterial and central venous blood pressures were noted but no significant changes in heart rate, right atrial pressure, cardiac output or derived values occurred, except for a fall in peripheral vascular resistance in three cases. An increase in skin blood flow to the feet was observed.

An attempt was made to determine the mechanism of these responses and it was concluded that the drug was an alpha adrenoceptor blocking agent which appeared to act preferentially on those receptors controlling blood flow to the skin of extremities.

Introduction

Indoramin, 3-(2-(4-benzamidopiperid-1-yl)ethyl) indole hydrochloride is a relatively new hypotensive agent with interesting pharmacological properties. Preliminary experiments in animals (Alps, Johnson & Wilson 1970) and man indicate that indoramin is antihistaminic, competitively blocks alpha adrenoreceptors and has a sedative effect upon the heart (Royds, Coltart & Lockhart 1972). Sub-hypotensive doses of indoramin increase hand temperature in normal individuals (Royds & Lockhart 1974), and blood flow in the hands and feet of patients with Raynaud's disease and athero-sclerosis with claudication (Fares & Milliken 1974).

In previous studies few direct haemodynamic observations were made concurrent with estimations of peripheral blood flow. It was the purpose of the study reported here to measure blood flow in the lower extremities together with general haemodynamic responses and to assess the effects of indoramin on the cardiovascular responses evoked in man by the alpha and beta agonists, noradrenaline and isoprenaline.

Materials and Methods

Ten healthy males aged 22–28 years and weighing between 55 and 70 kg volunteered to act as subjects for this investigation. All were senior medical students who had participated in previous experiments of a similar nature as subjects or observers. A full explanation of the implications of the study was given to each volunteer. Subjects came to the laboratory

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after an overnight 12-hour fast, and studies were performed with the subjects in a supine horizontal position, with the head resting comfortably on pillows.

Using local analgesia, plastic catheters were inserted percutaneously into the radial artery and into the right atrium from a right antecubital vein. Pressures were measured by Statham gauge transducers supported 7 cm above the surface of the operating table and recorded continuously on a Philips 3T recorder. Pressure gauges were calibrated using mercury manometers. Heart rate was measured using a San-El-2D16 pulsemeter and finger photocell. Lead 1 ECG was displayed on an oscilloscope and recorded continuously. Cardiac output was measured by dye dilution using indocyanine green and a Philips XO-1000 combined oximeter/densitometer cuvette. At the conclusion of each experiment the cardiac output densitometer was calibrated using each subject's own blood and known doses of cardiac green. Cardiac output was calculated from the dye dilution curves using the method of Williams, O'Donovan and Woods (1966) to prepare an appropriate programme for an Olivetti digital computer. Total peripheral vascular resistance (PVR) was calculated from the formula of Aperia (1940):

$$PVR = \frac{\text{Mean arterial pressure (mm Hg)} - 5}{\text{Cardiac output (l/min)}} \times 80 \text{ dynes sec cm}$$

An intravenous infusion of normal saline (3–5 ml/min) was given in the left arm so that drugs could be administered subsequently without disturbing the subjects.

Blood flow in the foot and calf regions of the left leg was measured by venous occlusion plethysmography using mercury in silastic strain gauges placed circumferentially around the instep and mid calf. Sphygmomanometer cuffs were placed just above the left knee so that venous blood outflow could be arrested by sudden application of 60–70 mm Hg pressure to the cuff for a period of 15–20 seconds. Change in resistance of the strain gauges was measured using Parks Plethysmographs (Model 270) and recordings were made on a multi channel potentiometric recorder (Unicorder U-400). Calf strain gauges were attached to the limbs by an adjustable caliper which enabled the gauge to be stretched precisely for calibration purposes, thus permitting calculation of blood flow in absolute terms. Foot gauges could not be calibrated in this way because the shape of the foot did not permit use of a calibration caliper and blood flow to the foot was therefore measured as percentage change from control. Subjects were left undisturbed following the introduction of atrial and arterial catheters until heart rate, blood flow and pressures were steady. Cardiac output estimations were then made at 3 minute intervals until the area under at least two dye dilution curves was the same (within 10%). Blood pressures, heart rate and blood flows were recorded at this time and served as control measurements.

Two subjects were given 20 mg indoramin divided into four equal doses which were infused intravenously over 30 sec at 5 min intervals. Cardiac output and leg blood flow were measured 4½–5 mins after each dose; all other parameters were monitored continuously. In three other subjects cardiovascular responses to isoprenaline were measured before and after indoramin infusion. After the control period increasing doses of isoprenaline hydrochloride were given by rapid intravenous injection; heart rate and blood pressure measurements were made continuously, cardiac output and leg blood flow measurements were made to coincide as closely as possible with peak isoprenaline responses. Heart rate and blood pressures were allowed to return to baseline for a period of at least 3 minutes between isoprenaline injections, and maximum dosage was limited to increase heart rate by 35–45 beats per minute, ranging from 1·5–4 μg. Indoramin (20 mg) was then given by slow intravenous injection over a period of 5 minutes. Cardiac output was measured during infusion of indoramin after 10 mg had been given, and again at completion of the infusion (control period 2). Isoprenaline was then administered again and all parameters measured as before. In a further five subjects, cardiovascular responses to noradrenaline (1–20 μg) were measured before and after the administration of indoramin. The experiments were conducted in an identical manner to the isoprenaline study except that noradrenaline was used in place of the beta agonist and the dosage was

limited to that which increased diastolic blood pressure by 10–25 mm Hg.

Tests of statistical significance were applied to the mean differences in measurements made during the control periods, and following administrations of noradrenaline or isoprenaline by applying Student's t-test to the paired comparisons. Observations in the two control periods (before and after indoramin) were compared similarly.

All subjects were interviewed 4–6 hours after completion of the studies and again the following day. Standing blood pressure was measured at these times using a standard sphygmomanometer cuff and mercury manometer and subjects were asked to report any symptoms relating to the experiments.

Results

Indoramin was well tolerated by the volunteers although all experienced slight drowsiness for a short period after the injections. No other symptoms were reported and there was no change in blood pressure 4–6 and 24 hours after the studies. In the two subjects receiving 5 mg aliquots of indoramin over 30 seconds a transient increase in heart rate of 10–15 beats per minute occurred for about 20 seconds after each injection; thereafter heart rate returned to control levels. The effect of 10 and 20 mg indoramin upon heart rate, blood pressures and cardiac output in all ten volunteers is shown in Figure 1. Systemic blood pressure was reduced by indoramin in a dose-related manner. The fall in blood pressure was not statistically significant after 10 mg but 20 mg caused an average fall in systolic and diastolic pressures of 10 and 6 mm Hg respectively ($p = < 0{\cdot}05$). A similar fall in central venous pressure occurred. Although there was a slight drop in cardiac output after 10 mg indoramin this was not significant and returned to control levels after the dose had been raised to a cumulative 20 mg.

The most striking and consistent action of indoramin was on the skin blood flow of the foot. The compound appeared to have a dose-related effect so that there was an average increase in skin blood flow to this area of 275% after 20 mg indoramin (Figure 2). This increase was sustained at least until termination of the experiment 45 minutes later. In contrast, calf muscle blood flow was only marginally increased, the 20 mg dose giving a mean change of 46%.

Haemodynamic effects of noradrenaline before and after indoramin

Haemodynamic responses to intravenous doses of noradrenaline 6–20 μg before and after administration of indoramin 20 mg are shown in Table 1. The average mean arterial pressure was raised by noradrenaline, and this effect was partially antagonized by indoramin. The mean rise in diastolic pressure following noradrenaline was dose-related for the five subjects investigated, and indoramin caused a parallel shift of the dose/response curve to the right. Linear regression analysis showed that the slopes of the dose/response lines were significantly different from zero ($p = < 0{\cdot}005$) and did not significantly deviate from parallel; the shift in the noradrenaline response due to indoramin (dose ratio 2·3) was statistically significant ($p = 0{\cdot}01$). The depressant effect of noradrenaline on heart rate was diminished by indoramin; and whereas cardiac output fell after noradrenaline a small mean increase was seen after indoramin had been given.

Haemodynamic effects of isoprenaline before and after indoramin administration

Heart rate, blood pressure and cardiac output responses to isoprenaline before and after administration of indoramin 20 mg are shown in Table 2. The upper limit of isoprenaline dosage ranged from 1·5–4·0 μg in the three subjects investigated, and responses tabulated are the average of peak responses of these doses. Considerable increases in heart rate and cardiac output occurred; mean blood pressure fell slightly, central venous pressure and peripheral vascular resistance were more than halved. Indoramin did not modify haemodynamic responses to isoprenaline significantly, although a slight fall in stroke volume was recorded. Skin blood flow, however, was significantly enhanced following indoramin.

Discussion and Conclusions

Of the parameters studied, the increases in cutaneous blood flow were most significant. Changes were seen after doses as small as 5 mg and there was a 2 to 3-fold increase in blood flow following the administration of

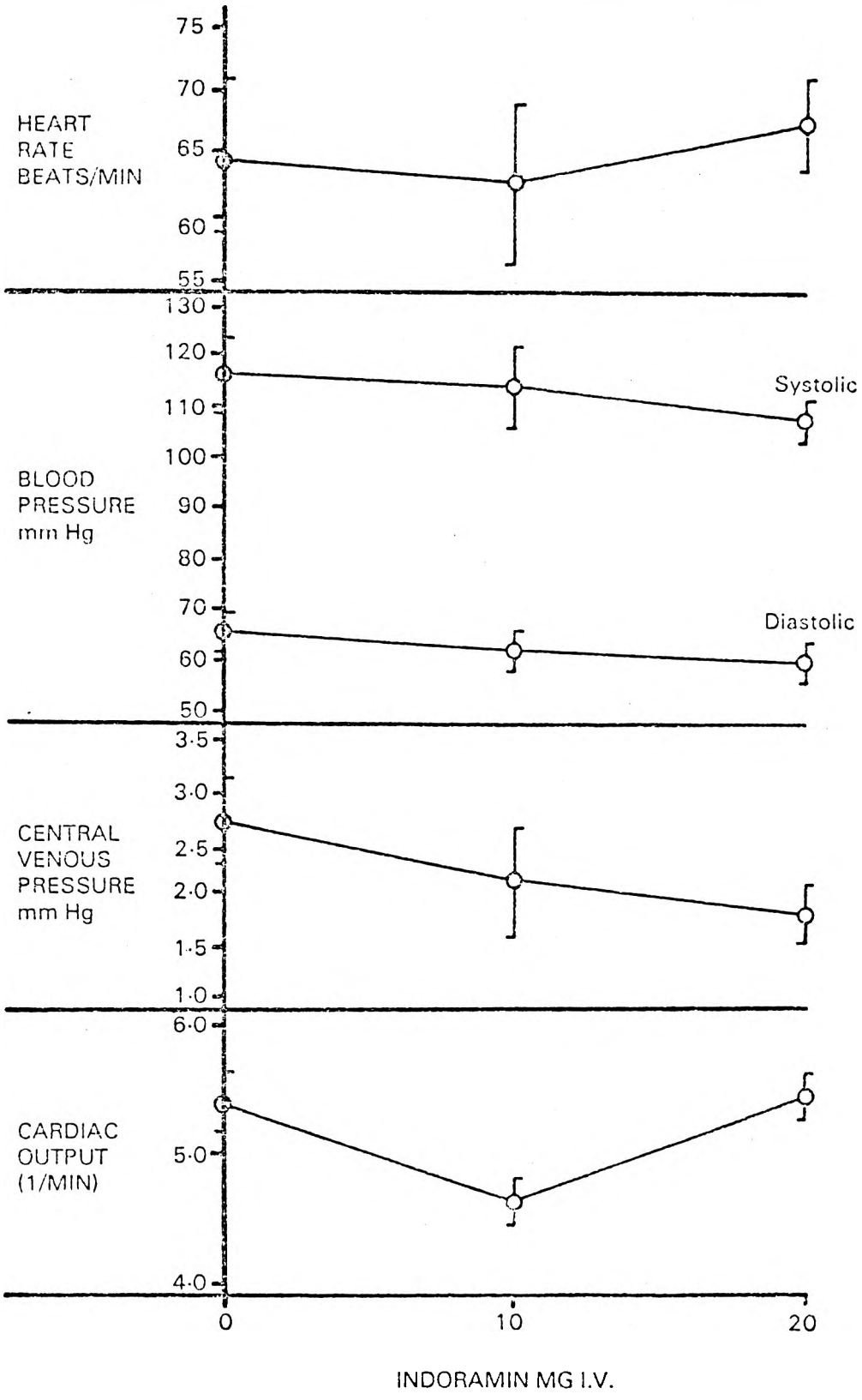

Fig 1 *Effects of intravenous indoramin 10 and 20 mg upon heart rate, blood pressure, central venous pressure and cardiac output. Means of measurements ± SEM have been plotted for ten subjects*

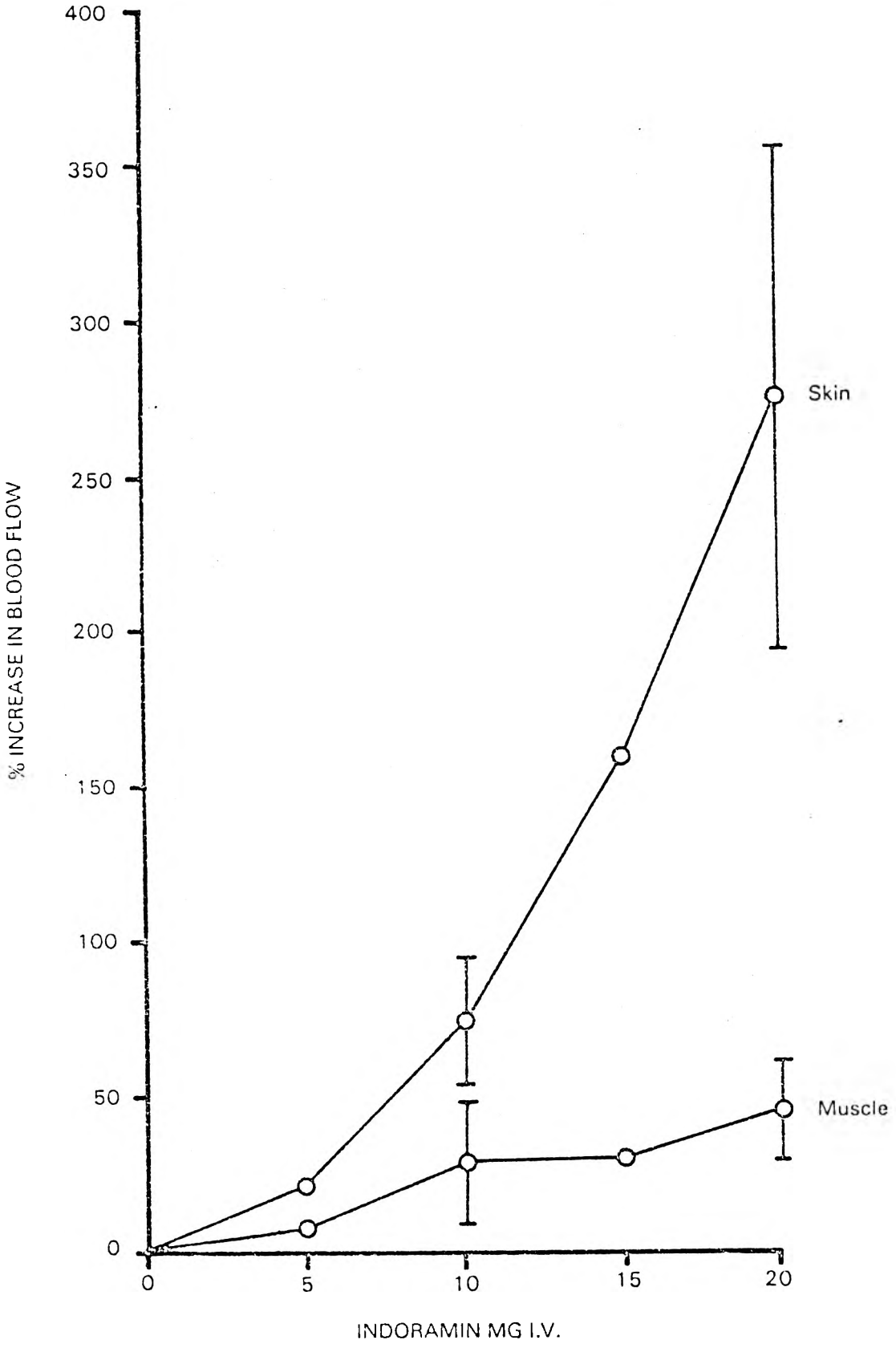

Fig 2 *Effect of intravenous indoramin upon peripheral blood flow in the foot (skin) and calf (muscle). Mean percentage change ± SEM have been plotted for ten subjects*

20 mg indoramin. By comparison, changes in calf muscle blood flow were considerably smaller. These observations were consistent with the findings of Royds and Lockhart (1974) who used skin temperature measurement and plethysmography to show that indoramin (15·5–18·7 mg i.v.) increased forearm muscle volume and skin temperature of the hand. Furthermore, increases in hand and foot skin blood flow have been demonstrated following the administration of indoramin (16–13·5 mg i.v.) to patients suffering from Raynaud's disease and atherosclerosis (Fares & Milliken 1974).

The absence of marked generalized haemodynamic effects in the present experiments suggests that the profound increases in skin blood flow following indoramin were localized phenomena, perhaps confined to the limbs. In this connection, Royds and Lockhart (1974) failed to show any effect of indoramin on chest skin temperature. They argued that vasodilator responses in this area are the result of active vasodilation (Fox, Goldsmith & Kidd 1962) whereas vasodilation of the skin of the hand is the result of release of vasoconstrictor tone (Gaskell 1956). It would seem, therefore, that indoramin acts mainly by releasing vasoconstrictor tone, probably by alpha-adrenoceptor blockade. Following the administration of indoramin there was a transient tachycardia, heart rate returning to normal limits within a few minutes. The absence of marked tachycardia following oral or intravenous administration of indoramin has been reported by others (Royds, Coltart &

Table 1

Effects of indoramin and noradrenaline upon various haemodynamic parameters

Observation		*Control period 1*	*'Peak' response noradrenaline 6–20 µg*	*Control period 2 20 mg indoramin*	*'Peak' response noradrenaline 6–20 µg*
Heart rate (beats/min)	Mean SEM	64·0^{ff} 7·09	52·3^{ff} 6·67	65·3 4·98	56·3 4·81
Mean arterial pressure (mm Hg)	Mean SEM	82·7 7·13	94·0^{ff} 8·08	76·7 4·81	85·0 6·56
Central venous pressure (mm Hg)	Mean SEM	3·33^{f} 0·44	4·83 0·60	1·50^{f} 0·52	2·60 0·60
Cardiac output (litres/min)	Mean SEM	5·1 1·09	4·5 0·83	4·7 0·38	5·3 0·72
Stroke volume (ml)	Mean SEM	78 9·94	84 6·00	73 6·66	93 5·84
Peripheral vascular resistance (dynes sec cm $^{-5}$)	Mean SEM	1395 223	1769 231	1314 141	1307 98
Muscle blood flow (ml/100 g muscle)	Mean SEM	2·10 0·37	2·32 0·62	3·25 0·70	2·19 0·64
Skin blood flow (% change)	Mean SEM		+7 34·7	+485 241	+140 101

Values compared are indicated by ^{f} Levels of significance $^{ff}p < 0{\cdot}025$ $^{f}p < 0{\cdot}05$

Table 2

Effects of indoramin and isoprenaline upon various haemodynamic parameters

Observation		*Control period 1*	*'Peak' response isoprenaline 1·5–4 μg*	*Control period 2 20 mg indoramin*	*'Peak' response isoprenaline 1·5–4 μg*
Heart rate (beats/min)	Mean	56·7●●●●	95·3●●●●	58·0	94·3
	SEM	5·24	3·28	3·06	2·67
Mean arterial pressure (mm Hg)	Mean	83·0	81·3	72·7	70·0
	SEM	3·51	2·40	2·33	6·0
Central venous pressure (mm Hg)	Mean	○ 4·42●●	1·58●●	○ 2·42	0·50
	SEM	0·36	0·65	0·46	0·29
Cardiac output (litres/min)	Mean	5·8●●	11·9●●	6·1	10·3
	SEM	0·17	0·68	0·32	0·58
Stroke volume (ml)	Mean	104	125	106	109
	SEM	8·1	10·4	7·0	7·0
Peripheral vascular resistance (dynes sec cm $^{-5}$)	Mean	○○114·5●●●●	551●●●	○○950	550
	SEM	27·7	23·8	27·4	62·2
Muscle blood flow (ml/100 g muscle)	Mean	2·21	3·94	2·44	3·48
	SEM	0·72	1·55	0·78	1·15
Skin blood flow (% change)	Mean		−35·3	+ 204	+ 283
	SEM		18·2	53·8	87·0

Levels of significance ● $p < 0·050$ ●● $p < 0·025$ ●●● $p < 0·010$ ●●●● $p < 0·005$

Lockhart 1972, Fares & Milliken 1974, Royds & Lockhart 1974) and may indicate that indoramin has a cardio-inhibitory action which obtunds the anticipated reflex response.

Indoramin did not significantly affect cardiac output or central venous pressure but produced a small, though statistically significant, fall in blood pressure. These changes were similar to those observed by Royds, Coltart and Lockhart (1972). The minimal nature of the fall in blood pressure may reflect the low starting pressure of the normal subjects used in both these studies.

Haemodynamic responses to intravenous isoprenaline were not appreciably altered by the prior administration of indoramin, suggesting it to be devoid of significant beta-adrenoceptor blocking activity and confirming the findings of Alps *et al* (1972) in their animal studies. In contrast, the alpha-adrenoceptor blocking properties of indoramin were demonstrated by a parallel shift of the noradrenaline pressor dose response curve to the right in confirmation of the findings of Royds, Coltart and Lockhart (1972).

No adverse reactions to indoramin were encountered. Apart from mild drowsiness, presumably associated with the antihistaminic properties of the compound, no side effects were elicited.

Whereas alpha-blocking agents have an established place in the treatment of peripheral vascular disease (Rose 1967) many of these preparations have unwanted pharmacological effects. Tolazoline, phentolamine and phenoxybenzamine may cause marked

tachycardia, whereas indoramin does not produce this effect. Furthermore, an increase in skin blood flow can be produced with little or no change in blood pressure. Indoramin, therefore, may prove to be a useful agent in the management of peripheral vascular disease. However, confirmation must await the outcome of clinical trials designed to determine the effects of chronic oral administration of indoramin in this condition.

Acknowledgements

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SECTION E : METABOLIC EFFECTS OF MEDICINES USED IN THE TREATMENT OF HIGH BLOOD PRESSURE

These papers describe the ancillary metabolic effects of two medicines which are used primarily in treatment regimens for patients with raised arterial pressures.

PAPER E1

The Effects of Adrenergic B-blockade with Oxprenolol on Peripheral Metabolism

The project followed consultation between the authors. The technique used was developed by Professor A.C. Asmal and formed part of his M.D. Thesis. He was primarily responsible for clinical investigation but this aspect of the work was also shared to some extent by the other authors. Professor Asmal bore the burden of writing a first draft of the paper which was edited by me.

Postgraduate Medical Journal (March 1975) **51**, 173–177.

The effects of adrenergic β-blockade with oxprenolol on peripheral metabolism

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Summary

Peripheral metabolism was studied with the forearm technique in six fasting normal subjects before and after oxprenolol administration. With the forearm technique the product of blood flow and arteriovenous differences is a measure of substrate uptake or release, and, therefore, an index of metabolism. Blood flow was measured with venous occlusion plethysmography, and arterial and venous samples obtained through indwelling catheters in the radial artery and deep forearm vein.

Oxprenolol administration influenced both peripheral flow and the basal pattern of substrate exchange. Before oxprenolol blockade a net uptake of glucose, triglycerides, and FFA, and a net release of glycerol were recorded across the forearm. After oxprenolol blockade there was a marked reduction in triglyceride uptake, with augmentation of glucose uptake and inhibition of lipolysis.

Introduction

Adrenergic β-receptor activity regulates a variety of metabolic events both centrally and peripherally (Havel, 1965; Brodie, Maickel and Stern, 1965; Himms-Hagen, 1970; Imura *et al.*, 1971; Fain, 1973). Such events have been investigated by one of two basic approaches—infusions of catecholamines or β-adrenoreceptor blocking drugs followed by measurements of plasma levels of insulin, growth hormone, FFA, and glucose (Imura *et al.*, 1971; Lundholm, Mohme-Lundholm and Svedmyr, 1968), or by the use of *in vitro* muscle and adipose tissue preparations (Fain, 1973). The *in vivo* techniques measure the sum total of metabolic events, both primary, due to direct action on the β-receptor, and secondary, due to initiation of counter-regulatory mechanisms elsewhere (Brodie *et al.*, 1965). *In vitro* studies have the advantage of providing information on β-receptor activity of a more direct nature, e.g. effects on glucose utilization and lipolysis. A shortcoming of *in vitro* data is that they may not reflect with accuracy events as they occur in the intact organism.

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The human forearm technique has been extensively utilized in the study of peripheral metabolism (Butterfield and Holling, 1959; Rabinowitz and Zierler, 1962; Wahren, 1966; Posefsky, Felig and Tobin, 1969; Zampa *et al.*, 1967; Asmal, 1972). The basis of the technique is the simultaneous measurement of blood flow and arteriovenous differences, the product of which is a measure of peripheral metabolism. It has the virtue of directly measuring metabolic events, albeit only in the periphery, and helps to bridge the gap between data derived from physiological studies in whole man, and those obtained from biochemical studies in the test-tube (Asmal, 1972). Previous studies have reported on the role of insulin in peripheral glucose utilization (Asmal *et al.*, 1971a; Whichelow *et al.*, 1971; Karamanos *et al.*, 1971) as well as on the complex interrelationships of hormones and substrates (Asmal *et al.*, 1971b; Asmal, 1972). More recently the observation of triglyceride release from peripheral tissues into the circulation in diabetes has raised the possibility that this may contribute to the hypertriglyceridaemia of diabetes (Asmal *et al.*, 1973).

This study was designed to investigate the effects of β-receptor blockade on the peripheral metabolism of glucose and lipids. This paper reports our findings in a group of normal volunteers.

Material and methods

Experimental

The nature of the study was explained to all six subjects (five males, and one female), who gave informed consent. The age range was 25–35 years, and weight was within 10% of desirable weight (Scientific Tables, Documenta Geigy, 1962). There was no history of diabetes or other disorder of metabolism.

All investigations were performed in a metabolic laboratory after an overnight fast. Subjects lay comfortably in a horizontal position for the duration of the study. Under aseptic conditions and local analgesia a 'Medicut'® 18 gauge cannula was introduced centripetally into the radial artery of one arm, and a 16 gauge 'Ezi-catheter'® inserted centrifugally into a deep forearm vein of the contralateral arm. A silastic strain gauge was applied around the circumference of the upper third of the latter forearm, and connected to a plethysmograph preamplifier (Parks Electronics Model 270) itself linked to a potentiometric recorder. Cuffs for instantaneous pressure application were placed at the wrist (pressure 200 mmHg) and upper arm (pressure 60 mmHg) and when completed, the subjects were allowed to become 'basal'. The arterial and venous cannulae were kept patent with slow infusions of 0·9% saline containing no heparin.

With the subjects in a basal state, the wrist cuff was inflated to a pressure of 200 mmHg and kept at this level until the completion of the study—about 20 min. Two sets of control flow measurements and blood samples, separated by 5 min, were first taken as follows: several flows were measured over a 2-min period, blood was then taken simultaneously and uniformly from the artery and vein over a 1-min period and concomitant flows measured; after 2 min the second set of control values were taken. After this, therapeutically recommended doses of oxprenolol, ranging from 1 to 4 mg were injected slowly into an indwelling needle. At intervals of 8–10 min after the injection, two further sets of blood flow measurements and blood samples were taken.

For the calculation of uptake, mean blood flows taken immediately before the sampling period were utilized in preference to those obtained concomitantly with the sampling. This was because the sampling procedure tended to produce artifacts in the flow configuration and made attempts to measure flow accurately difficult. Although this may have introduced a systematic error in the measurements, the magnitude of this could not be of much consequence because of the steady state situation prevailing. Other workers have also had recourse to this ploy (Jackson *et al.*, 1973).

Chemical

Plasma glucose was measured by the automated ferricyanide technique (modified from Hoffman, 1937), FFA by Duncombe's method (1964), and glycerol and triglycerides enzymatically (Boehringer method, 1972). Standard statistical methods were used (Bradford-Hill, 1968). The standard deviation (s.d.) of replicate analyses was 3·31, 0·013, and 0·097 for triglycerides, FFA, and glycerol respectively. The formula used was s.d. $= (\Sigma d^2/2n)^{\frac{1}{2}}$, where d = difference between duplicate samples, and n = number of duplicate pairs which were, respectively, 48, 24, and 48 for triglycerides, FFA, and glycerol.

Results

Pulse rate

In the recommended therapeutic dosage range (1–4 mg) no significant change, either in the mean pulse rates or in paired observations (Table 1), was noted.

Plasma flow

Although there was no significant difference in the mean plasma flows before and after blockade (2·69 ml/100 ml/min and 2·36 ml/100 ml/min respectively), comparison of paired values in individual subjects revealed significant changes (Table 1, $P < 0·01$).

Plasma concentration

The plasma arterial and venous concentrations of substrates before and after blockade were as follows. The differences before and after oxprenolol administration are not significant. Blockade is accompanied by an increase in the arterial values of glucose (from 93 to 98 mg/100 ml) and glycerol (from 0·84 to 0·97 mg/100 ml) and decrease in the arterial levels of FFA (from 0·43 to 0·37 mmol/l) and triglycerides (from 117 to 115 mg/100 ml). The effects of blockade on the plasma venous concentrations are less pronounced—the glucose and FFA levels remain more or less unchanged while those of glycerol show a slight fall (from 0·95 to 0·90 mg/100 ml) and those of triglyceride a rise (108 to 114 mg/100 ml).

Arteriovenous differences (Table 1)

In every subject blockade is accompanied by a reduction in the magnitude of the arteriovenous differences of triglycerides ($P < 0·025$), and an increase in the magnitude of the exchange of glucose ($P < 0·01$). The reduction in FFA, and increase in glycerol arteriovenous differences on blockade are not significant changes.

Peripheral exchange (Fig. 1)

In the control period there is net uptake of triglycerides, FFA and glucose, and net release of glycerol. On oxprenolol administration, triglyceride uptake falls from a value of 267 nmol/100 ml/min to 16 nmol/100 ml/min. The fall is significant ($P < 0·025$). There is also an attenuation of FFA uptake (P n.s.). In contrast net glucose uptake is augmented from low basal levels of 510 nmol 100 ml/min, to 990 nmol/100 ml/min on adrenergic blockade. Glycerol release is suppressed by oxyprenolol as shown by the reduction in plasma venous concentration. The stimulation of peripheral glycerol uptake on blockade is an unexpected finding.

TABLE 1. Comparison of parameters in individual subjects before and after blockade

Subjects and dose of oxprenolol		Pulse rate/min		Plasma flow* ml/100 ml forearm/min		Arteriovenous difference μmol/l							
						Triglycerides*		Glycerol†		FFA†		Glucose‡	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	4 mg	51	48	1·8	1·7	110·7	37·3	−1·1	2·3	80	20	165	275
2	2 mg	71	67	3·9	3·3	80·2	5·7	−12·4	−0·6	80	0	−220	220
3	2 mg	54	51	2·1	1·8	85·1	14·7	−46·3	−21·4	−250	−110	110	357
4	2 mg	63	62	2·6	2·3	75·8	66·7	−5·6	−7·9	160	80	825	1100
5	4 mg	65	62	3·0	2·8	76·8	−28·5	−6·8	4·5	110	60	110	303
6	1 mg	63	69	3·0	2·4	162·7	−61·0	0	135	140	−60	–	–

Significance of pre- and post-blockade differences obtained by the 'paired *t* test'.

* $P < 0{\cdot}025$; † P, n.s.; ‡ $P < 0{\cdot}01$.

The minus signs preceding arteriovenous differences designate that venous concentrations are greater than arterial.

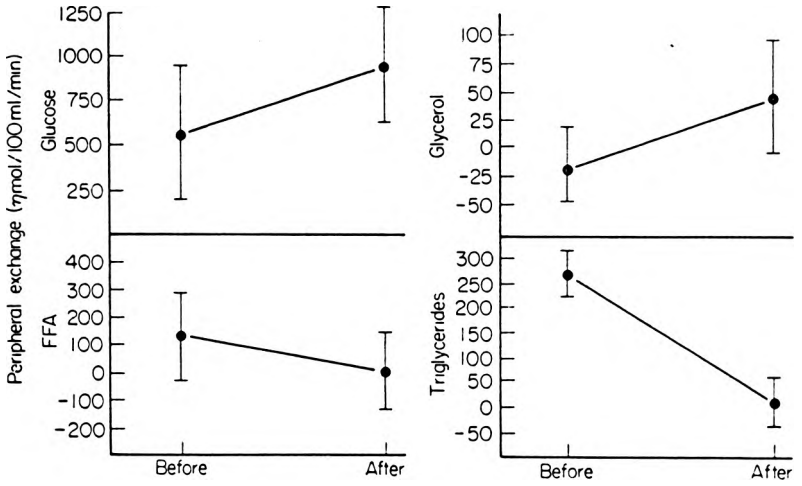

FIG. 1. The effects of oxprenolol blockade on the peripheral exchanges (mean ± s.e.) of glucose (P n.s.), FFA (P n.s.), glycerol (P n.s.) and triglycerides ($P < 0{\cdot}025$).

Discussion

The metabolic events described represent the sum total of biochemical changes in all tissues of the forearm—of which muscle and adipose tissue predominate. A limitation of the forearm technique is that it cannot discriminate *between* metabolic activity in these tissues. Within the scope of the forearm technique the term uptake may denote one of several events:

(1) An adsorption of substrate molecules to the capillary endothelium. This may occur during the uptake of, e.g. triglycerides which are hydrolysed by lipoprotein lipase on the endothelial surface.

(2) A sequestration of substrate in the interstitial space where molecules may be in equilibrium between the plasma and cell, e.g. FFA.

(3) An attachment of the substrate to the cell membrane, e.g. glucose before active transport.

(4) Intracellular metabolism which may represent either oxidation or storage as glycogen or triglycerides.

Both the adipocyte and the blood vessels of fat tissue have a rich sympathetic innervation (Havel, 1965; Brodie *et al.*, 1965; Fain, 1973). Metabolic function of adipose tissue is considered to be under tonic sympathetic control. This is mediated via β_1 receptors (Fain, 1973), which also respond to blood stream-borne catecholamines. β-receptors also modulate metabolic activity in muscle.

The metabolic events occurring in the forearm before blockade show a net uptake of glucose, FFA and triglycerides from the circulation and release of glycerol into it. FFA are preferentially oxidized by muscle in the basal state (Andres, Cader and Zierler, 1956). The distribution of glucose may be shared between adipose tissue where it promotes re-esterification and muscle where it is oxidized. The triglycerides restore depleted stores in both adipose tissue and muscle. Muscle has a higher concentration of the enzyme lipoprotein lipase in the basal state (Robinson, 1970). If uptake of triglycerides were dependent solely on luminal hydrolysis its flux into muscle would be greater than in adipose tissue. Prior hydrolysis may not be a prerequisite for triglyceride uptake.

Glycerol release results from both luminal hydrolysis and the lipolysis of stored intracellular triglycerides. Both the enzymes responsible for lipolysis—lipoprotein lipase, and triglyceride lipase—are controlled by the level of cyclic AMP which has reciprocal effects on the two enzymes (Tepperman and Tepperman, 1970). The level of cyclic AMP activity itself is regulated by β-receptor control.

On oxprenolol blockade FFA and triglyceride uptake is reduced. The reduction in triglyceride uptake may be due either to a direct inhibition of net transfer of unhydrolysed triglycerides, or secondary to a suppression of lipolysis on the capillary endothelium. One effect of this attenuated removal would be the observed elevation in venous plasma. The cessation of peripheral glycerol release is consistent with the suppression of lipolysis both on the endothelium and in the adipocyte. Of greater interest is the finding of net glycerol uptake in the periphery. Evidence for this has been found previously in the periphery (Häggendal *et al.*, 1967), and also across the myocardium (Carlson *et al.*, 1973), and is in keeping with the demonstration of glycerokinase activity in peripheral tissues (Robinson and Newsholme, 1967). The augmentation of peripheral glucose uptake on adrenergic blockade is consistent with the known action of catecholamines in reducing muscle glucose utilization (Lundholm *et al.*, 1968). The rise in arterial glucose concentration is contrary to the

fall expected from direct blockade of hepatic glycogenolysis. It may be due in part to a suppression of insulin secretion by β-blockade (Porte, 1967), thereby removing, perhaps, the more potent inhibitory effect on glycogenolysis. In part it may reflect an absence of direct influence on hepatic glycogenolysis, an action noted with pronetholol (Pilkington, 1962).

Over the limited period of observation β-blockade has been demonstrated to transform the metabolic traffic of substrates. There appears to be a preferential increase in glucose over lipid utilization. Whether these changes are transitory, or contribute significantly to quantitative aspects of energy utilization cannot be assessed on the basis of the present study. These preliminary findings underline the need to examine the situation in different categories of patients, and in greater depth to measure other substrate exchanges. Certainly if β-blockers are found to reduce fat and promote carbohydrate oxidation the implications from a long term point of view may be profound.

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PAPER E2

Immediate Metabolic Effects of Adrenergic Beta-Blockade

The protocol was designed by Professor A.C. Asmal in consultation with me. Clinical work and writing were shared between us. The remaining authors provided technical assistance only and their names have been included as a courtesy.

Immediate metabolic effects of adrenergic beta-blockade

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SUMMARY

Metabolic responses to isoprenaline and beta-blockade were studied in four groups of healthy volunteers. Group A subjects were given isoprenaline i.v. (1, 2 and 3 μg), at 30-minute intervals. Pulse rate was measured and venous blood collected for metabolic analysis before the first injection and 10 and 30 minutes after each isoprenaline dose. Group B subjects were given 20 mg acebutolol i.v. and measurements made before dosage with acebutolol and 30 minutes later, then the same protocol as for Group A was followed. Groups C and D subjects were given 1.0 mg propranolol i.v. or 20 mg practolol i.v. instead of acebutolol. The results showed that isoprenaline induced an increase in pulse rate and plasma glycerol. Lactate was reduced by all 3 beta-blockers and glycerol fell after propranolol. Isoprenaline administration during beta-blockade caused no change of importance. Glycerol rose and lactate fell when isoprenaline was administered to most subjects. Free fatty acids increased transiently after acebutolol and propranolol. The changes recorded, however, were considered to be insignificant biologically.

Key words: Acebutolol – propranolol – practolol – isoprenaline – metabolism

INTRODUCTION

Intravenous injections of 25 mg acebutolol, 2 mg propranolol or 10 mg practolol have negligible effects on the uptake and release by striated muscle of glucose, lipids, insulin or lactate, as measured by the forearm technique during the 10 minutes after medication. This applies whether or not there is prior administration of isoprenaline.[2] The fact that these findings involved only small groups of volunteers stimulated additional studies to collect further information on the effects of isoprenaline upon venous plasma levels of glucose, lipids, insulin and lactate in the presence of beta-blockade.

METHODS AND MATERIALS

Eight senior medical students and medical technologists participated in this study. All were male, aged 20 to 28 years, and were Black African (4), Indian (3) or White (1) in origin. All were in apparent good health and without past history of cardiovascular or major respiratory disease. Particular attention was paid to the exclusion of persons with diabetes mellitus, bradycardia or other cardiac arrhythmia, and obstructive airways disease. All volunteers understood the aims of the study and the procedures to be carried out. Most had participated in similar studies previously and none were taking medication at the time of, or immediately prior to this study.

All investigations were conducted between 0800 and 1100 hrs with subjects fasted for 12 hours and kept supine throughout each study. Volunteers were divided into 4 groups (A to D) in such a way that every subject formed part of Group A (8 subjects)

and at least one other Group (4 subjects each).

The clinical part of each experiment was carried out under the supervision of a Consultant Physician assisted by a Registered Nurse and 2 technologists. Subjective responses to the experimental procedure were monitored throughout these studies. A defibrillator and appropriate antidotes to the test drugs used were kept at the bedside.

A plastic cannula was introduced percutaneously into a suitable forearm vein under local analgesia and kept patent by bolus injections of sterile 0.9% NaCl given through a 2-way tap fitted to it. Recording of pulse rate and rhythm and sampling of venous blood for study began 30 minutes after placement of the cannula. Measurements were then made and 20 ml blood samples collected as follows:

Group A. (i) *Time 0 minutes:* control measurements were followed by slow intravenous injection (2 minutes) of 1 μg isoprenaline. Pulse rate was recorded throughout the injection, (ii) *Times 12 and 32 minutes:* venous blood was drawn for analysis 10 and 30 minutes after the initial isoprenaline injection. The 30 minute measurement was followed by the intravenous injection of 2 μg isoprenaline, over 2 minutes, (iii) *Times 44 and 64 minutes:* measurements were made and 20 ml blood collected 10 and 30 minutes after the second dose of isoprenaline. A further isoprenaline injection (3 μg i.v.) was given over 2 minutes, and (iv) *Times 76 and 96 minutes:* measurements were made and blood samples drawn 10 and 30 minutes after injecting 3 μg isoprenaline.

Group B. These subjects were given 20 mg acebutolol i.v. Pulse rate was measured and venous blood drawn for analysis just before dosage with acebutolol and again 30 minutes later. The protocol for Group A was then followed, beginning with 1 μg isoprenaline i.v. as follows: (i) *Time 0 minutes:* control measurements were followed by slow intravenous injection (5 minutes) of 20 mg acebutolol, (ii) *Time 35 minutes:* a second set of measurements was made 30 minutes after completion of administration followed by 1 μg isoprenaline i.v. given over 2 minutes, (iii) *Times 47 and 67 minutes:* venous blood was collected for analysis 10 and 30 minutes after completion of the first isoprenaline injection. The 30 minute measurement (at Time 67 minutes) was followed by the slow i.v. injection of 2 μg isoprenaline, over 2 minutes, (iv) *Times 79 and 99 minutes:* blood was collected for analysis 10 and 30 minutes after completion of the second isoprenaline injection (2 μg). The 30 minute measurement (at Time 99 minutes) was followed by the slow i.v. injection of 3 μg isoprenaline over 2 minutes, and (v) *Times 111 and 131 minutes:* blood was drawn for analysis 10 and 30 minutes after completion of the third isoprenaline injection (3 μg).

Group C. These subjects received 1.0 mg propranolol i.v. Measurements were made as for Group B before continuing with isoprenaline injections as for Group A.

Group D. The protocol was identical to those of Groups B and C but practolol, the beta-blocker used, was given as 20 mg i.v.

Subjects in all Groups had completed this protocol once blood was collected 30 minutes after 3 μg isoprenaline.

Laboratory analyses

Plasma and serum samples were separated immediately and stored at −20°C before analysis. The following methods were used in the analysis of samples, all of which were examined blind and in triplicate by 2 technologists: (i) Duncombe's method for

free fatty acids,[3] (ii) glycerol and triglyceride were measured enzymatically using the commercially prepared Boehringer (Mannheim) kit,[7] (iii) lactate determination was by the modified Wroblewski-La Due enzymatic procedure,[8] (iv) the UV hexokinase/glucose-6-phosphate dehydrogenase method was used for plasma glucose,[6] and (v) Amersham radio-immunoassay for insulin.

Statistical analyses

All analyses were carried out using programmes for the Hewlett-Packard 67 computer. Means, standard deviations and standard errors were determined for control data and for each set of values recorded after isoprenaline (1, 2 and 3 μg), acebutolol, propranolol or practolol and after isoprenaline given during beta-blockade. The significance of responses to single regimens given to each group of volunteers was determined by Student's 't' test for paired comparisons. Differences between beta-blocking agents were analyzed by Student's 't' test for comparison of sample means.

RESULTS

The results are summarized in Tables 1 to 4.

Isoprenaline increased resting pulse rate by 30% to 50%. This response was inhibited by beta-blockade. Plasma glycerol fell 30 minutes after 1.0 mg propranolol and was increased by isoprenaline doses of 2 and 3 μg. These changes were statistically significant ($p = 0.05$). All three beta-blockers slightly augmented this isoprenaline-induced rise in plasma glycerol level, but changes were not statistically significant in the small groups tested.

Table 1. Effects of intravenous isoprenaline (1 to 3 μg) on venous plasma levels of various metabolites in healthy volunteers: mean (±S.E.M.) values for Group A

Measurement	Triglycerides (mg/100ml)	Free fatty acid (mEq/l)	Glycerol (mg/100ml)	Glucose (mg/100ml)	Lactate (mg/l)
Control (Time 0)	79.0±7.11	0.33±0.03	1.30±0.11	80.4±3.87	98.4±10.96
1 μg isoprenaline i.v.					
10 min after (Time 12 min)	79.2±7.32	0.37±0.03	1.30±0.07	83.5±6.98	96.0±10.29
30 min after (Time 32 min)	80.1±8.61	0.34±0.03	1.29±0.07	77.9±3.25	91.9±9.78
2 μg isoprenaline i.v.					
10 min after (Time 44 min)	82.9±7.59	0.36±0.03	1.44±0.08	72.9±2.72	91.8±9.41
30 min after (Time 64 min)	85.6±8.55	0.33±0.02	1.35±0.10	76.1±2.48	91.2±8.56
3 μg isoprenaline i.v.					
10 min after (Time 76 min)	81.4±7.29	0.39±0.03	1.39±0.09	77.9±3.68	86.8±8.33
30 min after (Time 96 min)	81.0±7.61	0.35±0.03	1.41±0.10	78.5±2.04	83.1±8.67

Table 2. Effects of intravenous isoprenaline (1 to 3 μg) on venous plasma levels of various metabolites in healthy volunteers first given 20 mg acebutolol i.v.: mean (±S.E.M.) values for Group B

Measurement	Triglycerides (mg/100ml)	Free fatty acid (mEq/l)	Glycerol (mg/100ml)	Glucose (mg/100ml)	Lactate (mg/l)	Insulin (μU/ml)
Control (Time 0 min)	77.6±13.42	0.34±0.07	0.92±0.11	75.2±3.52	122.2±21.31	6.5±2.75
20 mg acebutolol i.v.						
30 min after (Time 35 min)	80.0±11.40	0.45±0.06	1.08±0.17	74.7±0.85	81.0±8.69	
1 μg isoprenaline i.v.						
10 min after (Time 47 min)	87.7±8.08	0.45±0.07	1.06±0.25	71.0±6.71	80.4±7.95	12.23±2.26
30 min after (Time 67 min)	89.8±9.36	0.45±0.03	1.05±0.22	76.9±3.12	71.8±5.25	6.98±0.88
2 μg isoprenaline i.v.						
10 min after (Time 79 min)	91.6±9.50	0.40±0.03	1.01±0.21	73.5±2.94	72.0±7.32	9.30±1.44
30 min after (Time 99 min)	88.4±9.50	0.38±0.04	0.97±0.16	80.1±3.67	74.4±8.01	7.73±0.48
3 μg isoprenaline i.v.						
10 min after (Time 111 min)	93.4±11.84	0.39±0.04	0.97±0.21	75.0±3.65	66.0±4.67	7.63±2.61
30 min after (Time 131 min)	88.6±10.85	0.38±0.04	1.97±0.17	77.8±2.69	69.2±0.92	9.73±3.46

Table 3. Effects of intravenous isoprenaline (1 to 3 μg) on venous plasma levels of various metabolites in healthy volunteers first given 1.0 mg propranolol i.v.: mean (±S.E.M.) values for Group C

Measurement	Triglycerides (mg/100ml)	Free fatty acid (mEq/l)	Glycerol (mg/100ml)	Glucose (mg/100ml)	Lactate (mg/l)	Insulin (μU/ml)
Control (Time 0)	77.7±9.49	0.33±0.08	1.12±0.10	82.6±7.49	126.5±15.61	2.43±1.05
1.0 mg propranolol i.v.						
30 min after (Time 35 min)	76.9±6.48	0.32±0.06	0.18±0.08	79.7±7.30	116.7±15.09	
1 μg isoprenaline i.v.						
10 min after (Time 47 min)	77.9±7.07	0.31±0.05	1.15±0.07	81.0±6.15	117.8±18.28	5.63±3.99
30 min after (Time 67 min)	80.5±10.73	0.34±0.08	1.12±0.06	84.9±5.98	81.0±12.18	8.50±3.77
2 μg isoprenaline i.v.						
10 min after (Time 79 min)	70.4±7.80	0.48±0.09	1.37±0.07	76.6±6.34	101.9±21.95	11.75±4.23
30 min after (Time 99 min)	74.1±11.59	0.45±0.08	1.45±0.11	80.8±8.90	124.9±32.18	5.75±2.35
3 μg isoprenaline i.v.						
10 min after (Time 111 min)	76.9±12.40	0.46±0.07	1.50±0.09	85.4±13.21	118.6±17.74	3.00±1.08
30 min after (Time 131 min)	79.7±9.84	0.46±0.12	1.60±0.13	78.7±6.98	117.8±29.50	6.00±4.00

Table 4. Effects of intravenous isoprenaline (1 to 3μg) on venous plasma levels of various metabolites in healthy volunteers first given 20 mg practolol i.v.: mean (±S.E.M. values) for Group D

Measurement	Triglycerides (mg/100 ml)	Free fatty acid (mEq/l)	Glycerol (mg/100 ml)	Glucose (mg/100 ml)	Lactate (mg/l)
Control (Time 0)	77.2±13.40	0.33±0.09	1.27±0.13	78.2±4.48	131.3±18.67
20 mg practolol i.v.					
30 min after (Time 35 min)	72.6±6.46	0.28±0.06	1.41±0.25	72.2±3.67	88.3±16.34
1 μg isoprenaline i.v.					
10 min after (Time 47 min)	85.2±13.47	0.26±0.07	1.24±0.29	72.5±6.53	86.6±13.96
30 min after (Time 67 min)	71.8±13.20	0.31±0.07	1.32±0.07	66.9±3.47	64.2±3.11
2 μg isoprenaline i.v.					
10 min after (Time 79 min)	74.6±9.06	0.27±0.05	1.20±0.34	72.3±1.76	68.8±13.05
30 min after (Time 99 min)	76.9±10.54	0.29±0.05	1.41±0.30	77.5±4.02	67.2±9.73
3 μg isoprenaline i.v.					
10 min after (Time 111 min)	75.1±12.45	0.33±0.05	1.33±0.16	78.4±2.69	73.7±8.75
30 min after (Time 131 min)	70.8±10.05	0.33±0.06	1.54±0.21	73.2±5.09	65.1±4.14

Venous lactate fell slightly after isoprenaline, acebutolol, propranolol or practolol, and was further reduced when isoprenaline was administered during beta-blockade. Changes were significant ($p = 0.05$), 10 and 30 minutes after each isoprenaline dose given following acebutolol, propranolol or practolol.

Significant rises in plasma free fatty acid were noted 10 minutes after 2 and 3 μg isoprenaline in subjects given propranolol and also 10 and 30 minutes after 1 μg isoprenaline in those given acebutolol. No side-effects occurred with any of the drugs.

DISCUSSION

These findings were consistent with those of an earlier study,[2] which indicated that single intravenous therapeutic doses of acebutolol, propranolol and practolol have no clinically important acute metabolic effects in healthy individuals. Forearm metabolism was not measured in the studies reported here, but the increases in plasma glycerol and free fatty acid levels recorded when isoprenaline was given during beta-blockade are consistent with the increased release and venous levels of these substances noted previously. Changes in lactate levels were qualitatively the same as those seen during earlier experiments, though quantitatively greater.

These results should be compared to those of other research groups, remembering, however, that only the acute responses to limited doses of 4 compounds were studied. Gibbons *et al.*,[4] who studied lipolysis in isolated rat epididymal fat and in human

volunteers given beta-blockers by mouth, reported free fatty acid release from fat cells and a rise in human plasma free fatty acid after stimulation by isoprenaline. This response was inhibited by beta-blockade. As reported here, isoprenaline caused no change in free fatty acid, although acute administration of beta-blockers with isoprenaline was associated with a slight rise in plasma free fatty acid levels. However, changes consistent with the Gibbons *et al.*[4] findings in venous plasma were recorded during earlier studies using slightly larger doses of acebutolol and propranolol. Interactions at beta-receptors would presumably explain these differences but, in view of their degree, their relevance to clinical medicine and toxicology appears obscure.

Asmal[1] reported changes in forearm metabolism after oxprenolol administration, but did not publish all the venous plasma levels of the parameters measured. Nevertheless, examination of the arteriovenous differences published indicates that glycerol levels fell in venous plasma after oxprenolol administration. This was reproduced in the present study by 1.0 mg propranolol.

Newman[5] reported a fall in free fatty acid after acebutolol or propranolol given orally for 48 hours. This result was not confirmed by the acute studies reported here. The small groups studied by all observers, the varied protocols followed, and variations in endogenous catacholamine release by different volunteers during experiments might all contribute to the results obtained by different investigators.

In conclusion, the results of this study support other evidence that 12.5 to 25 mg acebutolol, 0.5 mg propranolol, and 10 to 20 mg practolol have no important acute metabolic effects in healthy individuals. It should be stressed, however, that the slight changes observed in plasma lactate, glycerol and free fatty acid levels possibly merit investigation in parallel with studies involving administration of acebutolol for a period exceeding 6 to 12 months.

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SECTION F : REVIEWS

Papers included in this section review aspects of the action and uses of diuretics. These medicines are commonly prescribed in the management of hypertension. Some papers deal largely or solely with magnesium depletion which may be induced or aggravated by diuretics in some cases; this deficiency may be associated with alterations in cardiac rhythm and vascular tone. Most of the work included in publications F1 -11 is presented in a single review, F12.

PAPER F1

Diuretics

This review was shared by the authors.

Reprinted from the "SA Medical Journal". Vol. 59, 3 January 1981, pp. 9 - 13.

GP Review

Diuretics

W. P LEARY, A. J. REYES

Summary

Diuretics of current interest are classified according to their potency or site of action. The pharmacokinetics of furosemide, xipamide, hydrochlorothiazide and triamterene are briefly described, and important adverse reactions and drug interactions involving diuretics are reviewed. The clinical indications for diuresis are presented.

S. Afr. med.J. 59, 9 (1981).

Diuretics are compounds which increase urinary volume and renal excretion of sodium and chloride. Since they are of major importance in the management of hypertension, cardiac insufficiency and oedema of any origin, and no ideal diuretic exists at present, the main characteristics of currently available preparations should be understood and taken into consideration when prescribing a diuretic in clinical practice.[1,2]

Classification

Diuretics are usually classified according to their potency or site of action, two characteristics which largely determine clinical responses to these medications. A simple classification, taking both criteria into consideration and including various compounds presently in use or under clinical investigation, is given in Table I.

Since the response to diuretics is affected by numerous factors related to water and electrolyte homeostasis in health and disease, potency is best defined under standard conditions in normal healthy individuals taking 1 - 2 g dietary sodium per 24 hours. In oral therapeutic doses, high-potency diuretics may induce an excretion of up to 30% of the filtered sodium load, thereby provoking marked diuresis. About 10% of filtered sodium is excreted in response to medium-potency diuretics, whereas low-potency compounds are associated with an output of approximately 5%.[3] In practical terms, at usual chronic therapeutic doses, high-potency diuretics provoke a response similar to that elicited by 40 mg furosemide, whereas medium-potency diuretics are comparable in effect to hydrochlorothiazide 50 mg.

The principal anatomical sites of diuretic action are represented in Fig. 1. Loop diuretics have certain properties in common such as high potency, rapid onset and short duration of activity, ototoxicity and relative lack of antihypertensive effect. No real differences in clinical effects have been demonstrated between members of this group of diuretics, all of which are powerful inhibitors of chloride reabsorption in the ascending limb of Henle's loop.[3,4]

In physiological terms, the distal tubule is not a homogenous entity, but has a number of biochemical functions. Differences in composition of urine excreted after the administration of diuretics acting upon the distal convoluted tubule (Table I) may reflect diverse sites of action within the tubule and imply differences in the underlying biochemical mechanisms involved.

Diuretics such as the benzothiadiazides, chlorthalidone and xipamide all inhibit sodium and chloride reabsorption within the distal tubule, although differences in their potencies and diuretic time courses have been demonstrated.[4-6] Triamterene and amiloride, which are related in structure and activity, alter potassium exchange for

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Based on a plenary lecture delivered at the 3rd Regional Latin American Congress of Pharmacology, Montevideo, 2 - 5 March 1980.

TABLE I. CLASSIFICATION OF DIURETICS

Renal site of action	High	Medium	Low
Ascending limb of Henle's loop; biochemical site A	Furosemide, bumetanide, ethacrynic acid and piretanide*		
Distal convoluted tubule; biochemical site			
B	Xipamide*	Benzothiadiazides, chlorthalidone, chlorexolone and metolazone	
C			Triamterene, amiloride
D			Spironolactone
E		Tienilic acid*	

* Compounds not commercially available in South Africa, 1 May 1980.

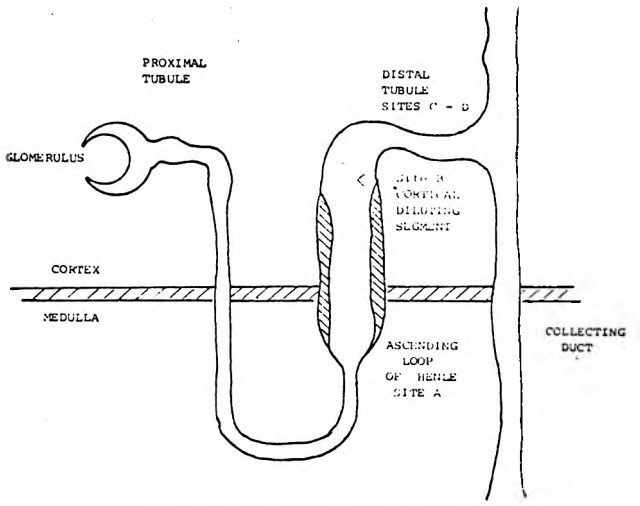

Fig. 1. Sites of action of diuretics.

sodium in the distal tubule through imperfectly understood mechanisms. Spironolactone has a similar effect, mediated through the competitive inhibition of aldosterone at the distal tubule.[4] Tienilic acid differs from other diuretics by its unique uricosuric action.[7] The vascular effects of these diuretics cannot be fully explained by their renal actions, although they may be mediated by events within the kidney.[8,9]

Pharmacokinetics

In the absence of overt disease the diuretics listed in Table I are effectively absorbed from the human gastrointestinal tract. However, variations in lipid solubility, protein-binding, hepatic metabolism or clearance, and renal excretion exist. The pharmacokinetics of furosemide, hydrochlorothiazide, xipamide and triamterene are described below as examples of diuretics in classes A, B and C, with slightly different characteristics.

Furosemide absorption is complete within 30 - 60 minutes of oral administration, maximal at pH 5, and unaffected by the presence of food in the gut. Furosemide, which is slightly soluble in aqueous solutions, is distributed throughout the body after absorption, even though about 80% is bound to plasma albumin. It is eliminated within 24 hours in urine and faeces, unchanged or conjugated with glucuronic acid.[4] In common with other diuretics, furosemide is filtered at the glomerulus and actively secreted in the proximal tubule, and exerts its diuretic action from within the tubular lumen before excretion.[10] Administration of furosemide in divided doses has little effect on its kinetics; there is a simple temporal additive effect.[11]

Xipamide has an absorption half-time of 18 - 27 minutes after oral administration, with resultant peak plasma levels achieved in 1,2 - 2,0 hours. This compound is almost completely protein-bound in human plasma initially, but is subsequently distributed throughout the body. More than 80% is excreted by the kidney within 24 hours of administration, approximately 25% conjugated with glucuronic acid, and the remainder as free xipamide.[12]

About 60 - 70% of hydrochlorothiazide, a representative benzothiadiazide of medium potency, is absorbed after oral administration. Absorption is linear in the dose range 5 - 75 mg and is increased slightly by food or medicines which delay gastric emptying.[13,14] Maximum plasma levels are achieved within 1,5 - 3,0 hours and urinary excretion of the absorbed dose is complete within 24 hours. Unlike many other benzothiadiazides, hydrochlorothiazide is not metabolized by the liver.[4,13]

The time courses of diuretic activity after different doses of furosemide, xipamide and hydrochlorothiazide have been extensively studied in healthy individuals.[5,6] The 24-hour urine volumes after the administration of furosemide 40 mg or xipamide 40 mg are similar, despite differences in time course of diuretic activity. When the three diuretics are compared (Fig. 2), time-to-peak activity varies after administration of the drugs. Furosemide rapidly achieves peak activity (2 h), whereas xipamide and hydrochlorothiazide resemble each other (4,5 h).[15] The effects of all three diuretics are complete within 24 hours. Thus, in healthy subjects 84% of the 24-hour urine volume is excreted within 14 hours of ingesting furosemide 40 mg, 74% after xipamide 40 mg, and 76% after hydrochlorothiazide 100 mg.[5,6]

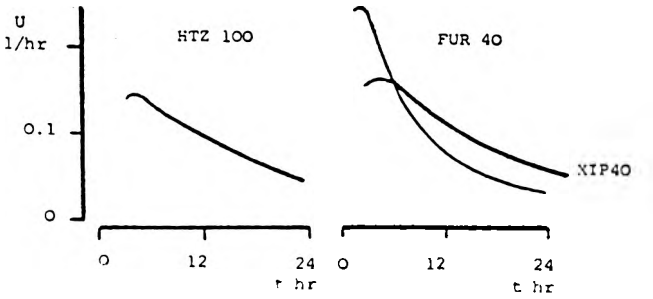

Fig. 2. Time courses of diuretic action in healthy volunteers (HTZ 100 = hydrochlorothiazide 100 mg, FUR 40 = furosemide 40 mg, XIP 40 = xipamide 40 mg, and U = urine output).

Triamterene[4] is rapidly but irregularly absorbed after oral administration and 10 - 80% excreted by glomerular filtration and tubular secretion within 24 hours. About 60 - 70% of triamterene is bound to plasma protein, and the weak diuretic action of the drug is generally complete within 24 hours. The dosage must not be increased above 300 mg because of the danger of significant hyperkalaemia.[4]

The pharmacokinetics of diuretics are altered to a different extent in diseases for which these compounds are indicated. In cardiac insufficiency the absorption of diuretics may be delayed or diminished owing to oedema of the intestinal mucosa. These changes vary in degree among patients with heart failure and fluctuate from time to time in individuals.[16-18] If diuretics are given by mouth during the initial treatment of cardiac oedema, double the standard dose should be used to ensure maximal diuresis.[19] In toxaemia of pregnancy there is little alteration in the pharmacokinetics of furosemide, even though the compound crosses the placenta and despite changes in body fluid compartments and renal function.[20]

Adverse reactions

Numerous adverse reactions to diuretics have been reported and are listed in Table II. Relatively few, however, are of clinical importance in terms of severity or frequency of occurrence.

TABLE II. POTENTIAL UNTOWARD EFFECTS OF DIURETICS

Hypokalaemia (A, B)	Azotaemia
Hyponatraemia	Ototoxicity
Hypochloraemia	Pseudo-Bartter syndrome
Hypomagnesaemia	Acute pancreatitis
Changes in serum calcium	Hepatic damage
	Allergy
Hyperglycaemia	Blood dyscrasias
Hyperuricaemia	Hyperkalaemia (C, D)

Hypokalaemia and hypomagnesaemia. Urinary potassium and magnesium excretion is increased by all the diuretics listed in Table I, except amiloride, triamterene and spironolactone, which are potassium-sparing diuretics.[4] Reduction in serum potassium levels below 3,0 mmol/l, whether iatrogenic or associated with primary disease, is undesirable and may be associated with serious reactions, including potentially lethal cardiac arrhythmias. Potassium loss may be diminished by limiting the sodium intake to approximately 1 g of sodium daily, thereby reducing both the pre-urine flow and the sodium load presented at the distal convoluted tubule for exchange with hydrogen and potassium ions. Alternatively, potassium supplements may be used, but since absorption is erratic these are often ineffective and potassium-sparing diuretics may be preferred, particularly if alkalosis is present.[4,21] In severe potassium depletion associated with cardiac arrhythmias, intracellular potassium repletion may be achieved by administering magnesium chloride intravenously or by mouth (Fig. 3).[22]

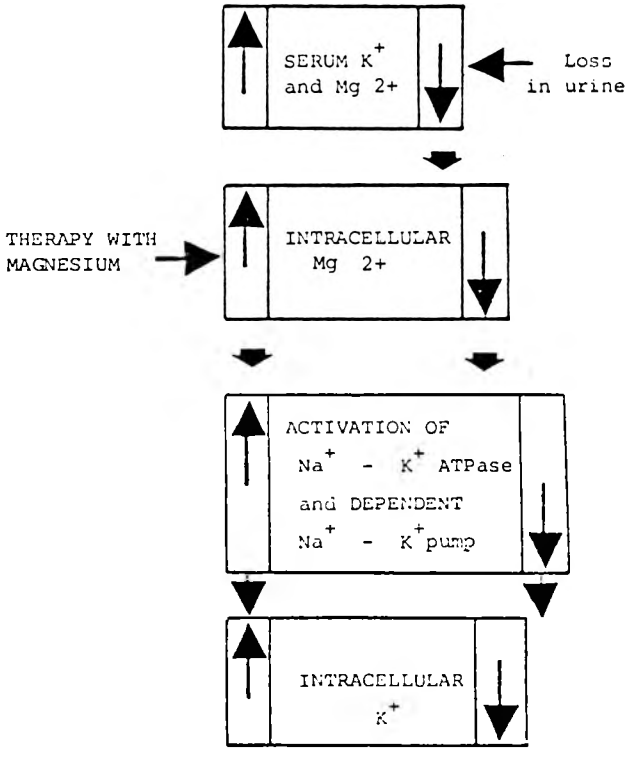

Fig. 3. Schematic representation of relationship between K^+ and Mg^{++} loss in diuresis and subsequent intracellular K^+ depletion. Therapy with magnesium may be used to activate the Na^+, K^+ pump, with consequent repletion of intracellular K^+.

Hyponatraemia. Hyponatraemia associated with inappropriate secretion of antidiuretic hormone (ADH) may develop during intensive treatment with high doses of diuretics. This can be distinguished from hyponatraemia due to other causes by the presence of hypokalaemia, and is best treated by temporary withdrawal of diuretics and fluid restriction.[23]

Metabolic changes. Diuretic therapy can potentially aggravate cardiovascular risk factors, since it induces hyper-reninaemia, hypercholesterolaemia, hypertriglyceridaemia and increased blood viscosity. The ultimate significance of these alterations in long-term treatment remains to be elucidated through appropriate trials, however.[24] Hyperglycaemia and hyperuricaemia may complicate therapy with diuretics in groups A and B, but are not usually of major clinical importance except in patients with diabetes mellitus or gout.[4] In such cases the indications for using a diuretic should be reassessed, and appropriate adjustments made to the therapeutic regimen if indicated.

Deafness and equilibrium disturbances. Deafness and equilibrium disturbances occur occasionally when high doses of loop diuretics are administered, cochlear damage possibly arising from inhibition of chloride transport and competitive blockade of the crossed olivocochlear hair cell synapse.[4,25]

Pseudo-Bartter syndrome. Chronic overdosage with diuretics of groups A and B may produce the pseudo-Bartter syndrome, consisting of hypokalaemic alkalosis and hyponatraemia associated with increased renin secretion.[26]

Drug interactions

Diuretics may interact with a variety of other medicines (Table III), although clinically serious consequences seldom occur.[27] Hypokalaemia, induced by diuretics, carbenoxolone, laxative abuse, or other means, predisposes to potentially lethal digitalis toxicity and may also potentiate the effect of non-depolarizing muscle relaxants. Synergism between diuretics and hypotensive agents may result in severe postural hypotension, especially in elderly patients. Loop diuretics and cephalosporins are potentially nephrotoxic in combination and renal function should be carefully monitored when they are given conjointly. Loop diuretics should be avoided in patients on aminoglycosides, since both groups are ototoxic.

The natriuretic response to diuretics is decreased by the concomitant administration of mineralocorticoids or broad-spectrum anti-inflammatory agents, which possibly act through inhibiting synthesis of natriuretic renal prostaglandins.[28,29] Appropriate adjustments in dosage

TABLE III. DRUGS WITH WHICH DIURETICS MAY INTERACT

Carbenoxolone	Indomethacin
Laxatives	Clofibrate
Cardiac glycosides	Amitriptyline
Antihypertensive agents	Carbamazepine
Cephalosporins	Xanthines
Aminoglycosides	Quinidine
Corticosteroids	Lithium
Oestrogens	Neuromuscular blockers
Phenylbutazone	Sulphonylureas

should be made. Inappropriate ADH secretion, which may complicate diuretic therapy, may be associated with several other medicines, including clofibrate, amitriptyline, carbamazepine and haloperidol. These medications should not be given together as a rule.[27]

Xanthine derivatives such as theophylline have a weak diuretic action and potentiate the effects of other diuretics.[2] Benzothiadiazide diuretics may enhance tubular reabsorption of quinidine or increase lithium excretion, thereby affecting plasma levels of these compounds.

Clinical uses

The main indication for using diuretics is in the treatment of oedema associated with raised venous pressure (cardiac insufficiency), reduced plasma colloid osmotic pressure (cirrhosis, nephrotic syndrome), renal sodium retention, or abnormal capillary permeability.[4]

Cardiac insufficiency. The treatment of cardiac failure includes a number of important measures of which diuretic therapy is only one. In heart failure, sodium retention is increased, renal cortical blood flow is decreased, and medullary flow and inner cortical flow are increased, notably in the region of the ascending limb of Henle's loop. Hepatic congestion, associated with cardiac failure, may decrease metabolic clearance of aldosterone and renin, thereby aggravating the situation. Diuresis mobilizes sodium and water in congestive cardiac failure, which may be accompanied by secondary hyperaldosteronism, with resultant increased potassium excretion. In this event aldosterone antagonists may be rationally combined with thiazide diuretics. In left ventricular failure important haemodynamic changes occur in response to diuretics, which probably reflect vascular mechanisms. Intravenous furosemide promotes a fall in left ventricular filling pressure and in pulmonary arterial pressure, with changes in the venous capacitance of leg veins, before the onset of diuresis.[30] It should be noted that in some patients with pulmonary oedema acute diuresis may induce shock through severe volume depletion; a central venous line should be kept *in situ* to monitor changes. Pulmonary oedema should never be treated with diuretics only; classic therapeutic measures should always be taken.[3,4]

Cirrhosis of the liver. Ascites associated with cirrhosis should probably not be treated unless it causes distress, since diuretic therapy will not increase life expectancy and may cause severe adverse reactions. Secondary hyperaldosteronism is commonly present, so that potent diuretic agents may cause serious potassium loss leading to electrolyte imbalance and coma. Carefully controlled diuresis associated with restricted sodium and fluid intake is preferred.[4] Spironolactone, triamterene or amiloride should be used as a starting regimen and furosemide may be added later if weight loss is unsatisfactory.[31] Dilutional hyponatraemia may occur in cirrhotic patients and should be treated, when diagnosed, by fluid restriction.

Hypertension. Arterial pressure can be reduced by restricting sodium intake or increasing sodium excretion, although exchangeable sodium and potassium are normal in essential hypertension. It is by no means certain that the hypotensive effect of diuretics is mediated through changes in sodium balance. In hypertension associated with hyperaldosteronism, spironolactone or amiloride may lower arterial pressure and return electrolyte levels to normal, whereas furosemide or a thiazide can be expected to aggravate the potassium depletion found in this condition.[3,4,22]

The mechanisms whereby diuretics lower raised blood pressure are largely unknown. Benzothiadiazides, xipamide, chlorthalidone, metolazone and other related diuretics may be expected to decrease erect, supine and exertional arterial pressure within 7 days when administered alone in patients with mild to moderate hypertension.[33-36] It has been shown that xipamide lowers blood pressure without altering circadian variations (D. I. Melville and E. B. Raftery — personal communication); other diuretics have yet to be evaluated in this respect, although a similar response can be expected. Tolerance does not develop and the effective antihypertensive dose of these compounds may be less than a standard diuretic dose[37] Loop diuretics are especially effective in hypertensive crises or as adjunctive therapy when established treatment with compounds such as β-adrenergic receptor blockers, reserpine, clonidine or α-methyldopa has been inadequate. Triamterene, amiloride and spironolactone have little effect upon blood pressure when given alone, unless they are given with a thiazide or hyperaldosteronism is present[3,4]

Renal disease. The benzothiadiazides are often as effective as high-potency diuretics in the maintenance therapy of patients with the nephrotic syndrome. In patients with advanced chronic renal failure benzothiadiazide diuretics are no longer effective, but high-potency compounds such as furosemide may be administered. Furosemide in doses of 1,2 - 3,0 g daily may provoke diuresis, even in patients with glomerular filtration rates below 4 ml per minute. This form of therapy may be regarded as an adjunct but not as a substitute for haemodialysis in renal failure.[3,31] Since adverse reactions to such high doses of loop diuretics may occur, therapy should be initiated in hospital. Potassium-sparing drugs are contraindicated, since their use may lead to fatal hyperkalaemia.

Pituitary and renal diabetes insipidus will both respond to diuretics.[37] The mechanism of action is uncertain, but a transitory decrease in plasma osmolality occurs with diminished thirst. It is possible that increased plasma concentrations of angiotensin also reduce urine volume in these patients.

Conclusions

Diuretics are used to increase urinary sodium and water excretion in a variety of clinical situations. The incidence of untoward side-effects may be decreased by selecting the most suitable preparation for a particular clinical situation and by avoiding the use of excessive dosages or polypharmacy.

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PIONEER

PAPER F2

Diuretics, Magnesium, Potassium and Sodium

This review was shared by the authors.

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Diuretics, magnesium, potassium and sodium

W. P. LEARY, A. J. REYES

Summary

Diuretics increase renal magnesium excretion and, when chronically administered, decrease intracellular magnesium levels. This deficiency reduces active transport of potassium into the cell and intracellular potassium decreases irrespective of serum potassium levels. Potassium supplementation or the co-administration of potassium-sparing diuretics cannot maintain intracellular potassium at normal levels when intracellular magnesium is reduced. The effects of chronic magnesium supplementation have not been adequately studied. Maintenance of the diuretic dose at an effective minimum and curtailing sodium intake when diuretics are chronically prescribed are the simplest and safest manoeuvres which can be undertaken in order to prevent the occurrence of events usually ascribed to hypokalaemia and in fact principally due to low intracellular potassium which is associated with magnesium deficiency.

S. Afr. med. J., 61, 279 (1982).

The magnesium-potassium relationship

Magnesium, an important cofactor for various enzymatic reactions,[1] is a critical determinant of Na^+ - K^+-ATPase activity in several tissues, including ventricular myocardium, skeletal muscle and the nephron.[2,3] This enzyme is necessary to release energy for the sodium-potassium pump that actively incorporates potassium into the cells of electrically excitable tissues, thus maintaining the intracellular concentration of potassium within normal limits. Decreases in tissue magnesium levels may therefore result in reduced intracellular potassium concentrations, provided other determinants of potassium equilibrium potential remain constant. If, in addition, the serum potassium level is reduced, intracellular potassium concentration will be further decreased. Such changes may be associated with serious cardiac arrhythmias which are usually ascribed to hypokalaemia but are in fact basically provoked by a decrease in intracellular potassium due to magnesium depletion.[2]

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Date received: 20 May 1981.

Diuretics and magnesium

The most familiar clinical situations associated with magnesium depletion are prolonged diarrhoea and vomiting and the chronic administration of diuretics; magnesium excretion is enhanced by most modern compounds including the thiazides,[4,5] furosemide[6,7] and ethacrynic acid.[6,7] The mechanism whereby diuretics increase renal magnesium excretion is incompletely described, although it is known that the kidney is the principal organ involved in magnesium homeostasis and that, normally, most filtered magnesium is reabsorbed at the proximal tubule and at the thick ascending limb of the loop of Henle.[8] The chronic urinary losses of magnesium provoked by diuretics eventually decrease serum and intracellular concentrations of magnesium.[2]

Studies in progress support the view that biologically important magnesium losses may result from chronic administration of diuretics. Administration of piretanide 12 mg/d to 9 patients with uncomplicated essential hypertension resulted in a statistically significant fall in serum magnesium after 12 weeks (Fig. 1). Other loop diuretics might be expected to have similar effects.[9-11] When 15 hypertensive patients received a combination of hydrochlorothiazide 100 mg and amiloride 10 mg daily, serum magnesium also decreased significantly from a mean pretreatment value of 0,861 mmol/l to a mean value of 0,797 mmol/l after 12 weeks of treatment ($P < 0,02$). Such changes could be associated with a decrease in intracellular magnesium, leading in turn to a diminution in intracellular potassium (Fig. 2).

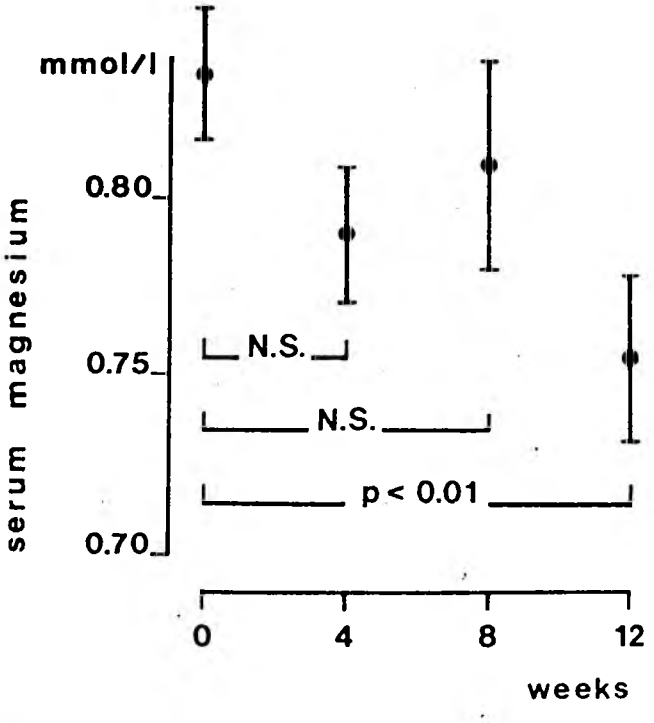

Fig 1. Changes in serum magnesium values in 9 hypertensive patients treated with piretanide 12 mg/d (mean values ± SEM).

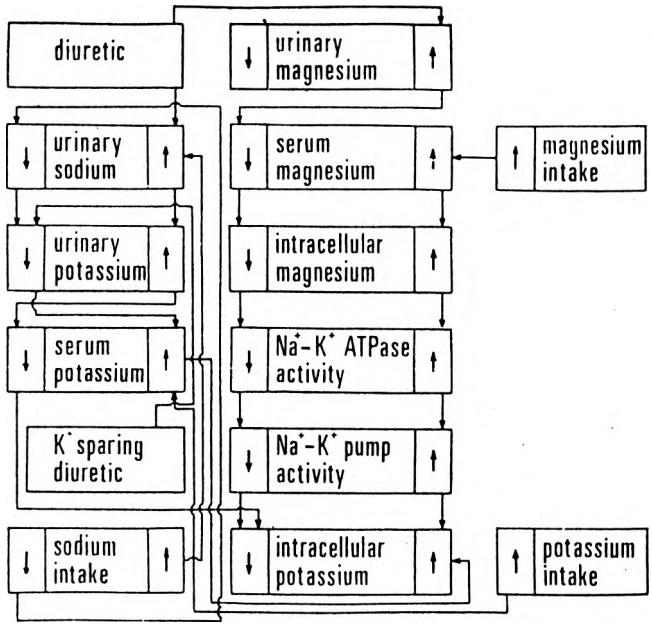

Fig. 2. Schematic representation of the mechanism whereby (common) diuretics decrease intracellular magnesium and potassium. Potassium supplementation and potassium-sparing diuretics increase serum potassium but intracellular potassium remains low if intracellular magnesium is decreased. Magnesium supplementation would increase intracellular potassium. Sodium dietary restriction has an overall potassium-sparing effect.

Practical handling of patients receiving chronic diuretic treatment

Hypokalaemia secondary to the administration of diuretics may be to some extent prevented or corrected by appropriate potassium supplementation or by the co-administration of potassium-sparing diuretics. Nevertheless, intracellular potassium levels may remain low in the presence of intracellular magnesium depletion.[2] The effects of magnesium supplementation on intracellular potassium levels during chronic diuretic therapy have not been adequately described, although such therapy would be theoretically correct (Fig. 2), and it has been demonstrated that, acutely, magnesium infusions increase intracellular potassium.[2]

Potassium and magnesium losses can be minimized by simple clinical procedures. In hypertension, most thiazide-type diuretics will effectively reduce high blood pressure when prescribed at less than the standard diuretic doses, which may cause electrolyte depletion. Thus no enhancement of the antihypertensive effects of chlorthalidone[12] and hydrochlorothiazide[13] has been found at doses greater than 12,5 mg/d. In addition to the direct effect on renal potassium excretion of diminishing the diuretic dose to a minimum, potassium losses associated with hyperaldosteronism secondary to diuretic administration are also reduced.

Restriction of dietary sodium will reduce filtered sodium, pre-urine flows and the absolute amount of sodium available for interchange with potassium at the distal convoluted tubule, thus further decreasing the potassium loss.[9,14] Reducing sodium intake may result in an increased aldosterone secretion, in turn provoking enhanced renal potassium excretion. This may balance the potassium-sparing effect of reducing the amount of sodium available at the nephron to some extent.

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PIONEER

PAPER F3

Diuretics and Zinc

Mr. C.J. Lockett and Dr. L. Alcocer provided some references upon which this review was based. The article was written by me and Professor A.J. Reyes.

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Diuretics and zinc

A. J. REYES, W. P. LEARY, C. J. LOCKETT, L. ALCOCER

Summary

Distal tubule diuretics (DTDs), including chlorothiazide, hydrochlorothiazide, bendroflumethiazide, chlorthalidone and xipamide, have been found to increase urinary zinc output through a poorly understood mechanism which could involve both direct and hormone-mediated processes.

Significant zinc depletion may occur during long-term administration of DTDs, principally in conditions associated with diminished total body zinc levels such as hepatic cirrhosis, diabetes mellitus, gastro-intestinal disorders and several renal diseases.

Attention to the early symptoms of zinc deficiency such as hypogeusia, hyposmia, abnormal dark adaptation and impotence and the monitoring of serum zinc levels are advisable during long-term treatment with common DTDs.

S Afr Med J 1982; 62: 373-375.

Zinc, a constituent of all cells, is one of the most abundant of the essential trace elements in the human body. The major functions of zinc seem to be related to enzymes. More than 70 metalloenzymes, including carboxypeptidases, thermolysin, alcohol dehydrogenases and nucleotide polymerases,[1] are known to require zinc for their activities.

The so-called converting enzyme, which intervenes in the metabolic paths of vasoconstricting and vasodilating peptides, is a carboxypeptidase containing a firmly bound zinc ion essential for the hydrolytic reactions it catalyses. Because the role played by the vaso-active peptides such as angiotensin II and bradykinin in the pathogenesis of hypertension is a highly controversial issue, a possible role for zinc in this disease is only speculative at present.

Participation of zinc in the metabolism of nucleic acids, through its binding to enzymes such as thymidine kinase, DNA polymerase, RNA polymerase and reverse transcriptase, affects biochemical and ultimately morphological events in the cell. RNA synthesis, protein synthesis and increase in cell size depend on corresponding zinc-related reactions or entities: uridine incorporation into RNA, DNA-dependent RNA polymerase and RNA stabilization, respectively. DNA synthesis depends on zinc-mediated processes such as thymidine incorporation into DNA and DNA stabilization. Mitosis provides another area for the effects of zinc on the cell cycle; some morphological characteristics of the nucleoli, the spindle apparatus and the chromosomes have been clearly identified as zinc-dependent.[2]

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Zinc metabolism

Only 5-15% of zinc ingested in standard foodstuffs is absorbed in the small intestine. Zinc seems to enter the mucosal cells across a concentration gradient through specific binding to a transport albumin. Daily zinc requirements determine the amount of the substance that is actually incorporated into the body from the cells of the intestinal mucosa.[3] The mechanism whereby zinc absorption is regulated is largely unknown; positive involvement of 1,25-dihydroxycholecalciferol has been identified.[4] Ingested zinc-binding substances such as phytate and fibre reduce the amount of free zinc liable to be absorbed within the intestinal lumen.

Some 34% of blood zinc is firmly bound to protein, the remainder being only loosely bound. The principal carrier of zinc in plasma is an α_2-globulin which accounts for 30-40% of serum zinc. Transferrin and some enzymes also bind zinc tightly. Loosely bound zinc exists mainly as a macromolecular complex with albumin; a small amount of the trace element (1-4% of all serum zinc) forms micromolecular complexes with histidine, cysteine, threonine, glycine, asparagine and other amino acids. When assayed by atomic absorption, the plasma zinc level is 100 μg/dl, with a standard deviation of 10 μg/dl. In spite of being quantitatively negligible within plasma, microligated zinc seems to be the major determinant of zinciuria because of the high filterability of these compounds.

The total body zinc level in a fat-free 70 kg adult ranges between 1,4 g and 2,3 g. High concentrations exist in the liver, voluntary muscle, myocardium, bone, hair, skin, pancreas, prostate gland, erythrocytes and leucocytes. Whole blood has a higher concentration of zinc than serum or plasma.[3]

Zinc is principally eliminated through the gut, and the zinc finally eliminated by this route includes both endogenous and ingested zinc. Faecal excretion of zinc is approximately twice that via the kidneys.

In normal adults mean daily urinary zinc excretion is 0,1 - 0,7 mg. The mechanisms which participate in determining this output remain obscure, although it has been established clearly that zinc reaches the nephronal lumen through filtration of plasma microligands and that it undergoes a process of reabsorption while traversing the nephron. Zinc is also eliminated in sweat and through the hair, nails and the skin itself.

Zinc deficiency

Clinical and laboratory findings

Zinc deficiency is probably more frequent than clinicians suspect, especially in its early and subtle expressions which include hypogeusia, hyposmia, abnormal dark adaptation, impaired wound healing, dermatoses and impotence.[1-3] Severe chronic zinc depletion gives rise to retarded growth and hypogonadism. Existing evidence is insufficient to characterize

zinc deficiency fully in clinical terms since abnormally low serum zinc levels have been found to coexist with negative taste and smell tests, and, on the other hand, oral replacement of zinc in patients with essential oligospermia or azoospermia and normal serum zinc values has resulted in increases in the number of normal mobile spermatozoa, ultimately leading to pregnancy.[3] These contradictory observations preclude any definite guidelines for depicting zinc deficiency at the clinical level. Serum or plasma zinc values remain the most reliable criterion for the diagnosis of zinc deficiency, however, although the relation to total body zinc is not known and decreased serum zinc values may exist in entirely asymptomatic individuals.

The diagnosis of zinc deficiency during diuretic treatment should be established either when serum zinc values are significantly low (< 80 μg/dl), irrespective of the presence of symptoms or signs, or when at least two definite symptoms or signs develop, even in the presence of normal serum zinc values.

Aetiology and pathogenesis[1,2]

A severe, although not unequivocally pure zinc deficiency syndrome has been described in malnourished children. Impediments to a normal zinc supply from the gut include a low zinc content in the diet, as determined by geological conditions, large amounts of phytate or fibre in the diet, and exaggerated losses of zinc due to gastro-intestinal disorders.

Genetic disorders such as sickle cell disease and acrodermatitis enteropathica are related to zinc deficiency, though in different ways. Sickle cell disease possibly causes zinc deficiency through hyperzinciuria. Whether this increased renal zinc output is due to increased zinc filtration provoked by continued haemolysis or to an impairment of the nephronal reabsorption of the ion is not known. In acrodermatitis enteropathica there is a congenital defect in the synthesis of a protein related to zinc absorption.

Alcoholism provokes hyperzinciuria, possibly through a direct effect of alcohol on the renal tubular epithelium. A decrease in serum zinc concentration follows excessive renal losses in chronic alcoholism, and clinical evidence of zinc deficiency has been found in this condition. Abnormal zinc metabolism occurs in hepatic cirrhosis in which decreased serum and hepatic zinc and hyperzinciuria coexist. Whether this syndrome is independently related to cirrhosis or is a consequence of alcoholism has not been determined.

Burns cause extensive zinc losses in exudates, and the losses of skin mass which may occur in psoriasis afford a circumstance in which zinc stores cannot be maintained. Some diabetic patients exhibit hyperzinciuria of unknown origin. Hypozincaemia has been found during pregnancy, possibly because of the uptake of the element by the products of conception. Zinc deficiency usually occurs in uraemia before haemodialysis is initiated,[5] and patients with renal diseases in which albumin and amino acids are lost in the urine may also present with zinc depletion.

Zinc and diuretics

Diuretics whose main site of renal activity lies at the cortical diluting segment (roughly, at the early portion of the distal convoluted tubule) such as chlorothiazide, hydrochlorothiazide, bendroflumethiazide, chlorthalidone and xipamide have been found to provoke hyperzinciuria both when administered as a single dose and when given on a long-term basis to normal subjects.[6,7] Conversely, the loop diuretics furosemide and bumetanide and the potassium-sparing distal tubule diuretic (DTD) triamterene have been found not to affect urinary zinc excretion.[7]

Hyperzinciuria following administration of common DTDs could be determined not only by direct blockade of zinc reabsorption. In a recent experimental series in which excretion of several urinary electrolytes following administration of various diuretic formulations to normal volunteers was described continuously through a mathematical model, it was found that the time-courses of water, sodium, chloride, potassium, magnesium and zinc urinary flows coincide after the administration of placebo.[8-11] However, zinc excretion after hyperzinciuric diuretics was dephased with regard to the excretion of water, sodium, chloride and potassium, which coincided in time-course (Fig. 1). Urinary peak excretion of zinc succeeded the peak excretion of water and of the monovalent ions by 3-5 hours, suggesting that the process involved in the hyperzinciuric response to diuretics involves indirect mechanisms which take time to act, as would be the case if hormone-mediated phenomena were implicated. A similarly delayed response due to hormonal intervention is observed in the time-course of hypermagnesiuria elicited by diuretics.[9-11] The mechanism whereby metabolism of the divalent ions calcium, magnesium and zinc could interact in order to produce this delayed response to diuretics is unclear.

Impotence is not all that rare a symptom during long-term diuretic administration and we have observed dysgeusia and dysosmia, principally in the form of suboptimal function, in patients receiving distal DTDs on a long-term basis for the treatment of raised blood pressure.

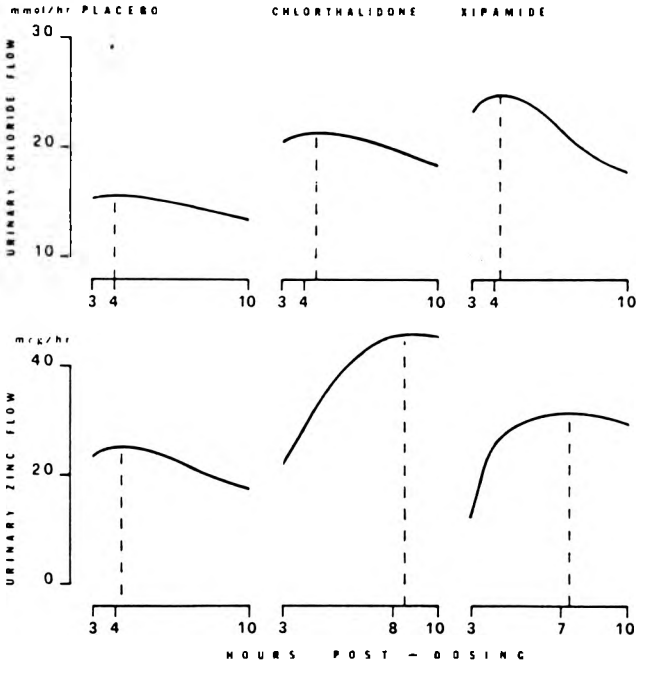

Fig. 1. Ten healthy subjects received placebo (Reyes and Leary — unpublished data), chlorthalidone 100 mg[9] and xipamide 10 mg (Reyes and Leary — unpublished data) on different trial days. Urine was collected at different intervals after dosing and mean chloride and zinc urinary flows expressed as functions of time.[8] The chloride and zinc flows after medication are shown during intervals which comprise all peak flows, thus allowing easy comparison of the time-courses of their excretion after each medication.

Prophylaxis and treatment of zinc deficiency during diuretic treatment

Long-term treatment with common DTDs should be conducted with minimal dosages in order to prevent depletion of potassium, magnesium and zinc; although the dose-response relationship for the hyperzinciuric effect of diuretics has not been studied, it is worth maintaining minimal dosages as a simple primary prophylactic approach. Treatment of hypertension with one-third of the standard daily doses of DTDs permits blood

pressure control to the same degree as that achieved with standard dosages.[12]

When zinc deficiency is diagnosed, measures should be taken to optimize zinc absorption and to cope with any mechanism actually or potentially contributing to the pathogenesis of the syndrome.[3,13] Oral zinc supplementation should be provided as zinc sulphate administered at a dose of 50-200 mg/d, which is generally well tolerated.[3,1]

In patients undergoing long-term haemodialysis, intestinal absorption of zinc is often altered, apparently because of abnormal metabolism of vitamin D,[1] which impedes satisfactory replenishment of bodily stores; parenteral zinc supplementation is indicated in such cases and the dosage regimen should be individually titrated.

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PAPER F4

Magnesium Deficiency Provoked by Diuretics

This review was shared by the authors.

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Review Article

Magnesium deficiency provoked by diuretics

A. J. REYES, W. P. LEARY

Summary

Many diuretics cause hypermagnesiuria which may lead to magnesium deficiency, presenting as hypomagnesaemia, cardiac arrhythmias and tetany.

Loop diuretics cause hypermagnesiuria mainly through direct blockade of magnesium reabsorption at the loop of Henle. Distal tubular diuretics block magnesium reabsorption at the distal convoluted tubule and also reduce magnesium reabsorption at the loop of Henle by an indirect mechanism.

S Afr Med J 1983; **63**: 410-412.

Commonly used diuretics such as the thiazides, chlorthalidone and furosemide increase urinary magnesium losses. Deficiency of the ion develops after prolonged diuretic treatment, notably when other factors contributing to magnesium depletion are also present.[1-3] Significant magnesium losses in association with diuresis may pass unrecognized because the signs and symptoms of magnesium depletion are usually attributed to potassium deficiency, which may also be present.[2]

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Date received: 19 October 1982.

Hypermagnesiuria provoked by diuretics

Urinary magnesium excretion is significantly increased in healthy adults by single therapeutic doses of diuretics which act principally in the loop of Henle (furosemide, piretanide) or in the first portion of the distal convoluted tubule (hydrochlorothiazide, xipamide, chlorthalidone).[1-7] Magnesium depletion may complicate prolonged treatment with either type of diuretic.

Pathophysiology of magnesium deficiency provoked by loop diuretics

Approximately 70% of plasma magnesium is diffusible and therefore subject to filtration at the glomerulus. About half the filtered magnesium is reabsorbed in the thick limb of the loop of Henle,[8] where diuretics such as furosemide, ethacrynic acid, bumetanide and piretanide[9] appear to have a common receptor and exert their principal biochemical effects within the kidney. Loop diuretics inhibit magnesium reabsorption by a mechanism unrelated to the interference with chloride transport in the loop of Henle which primarily determines their natriuretic and diuretic effects.[8,10,11]

Direct blockade of magnesium reabsorption within the loop of Henle might account for the hypermagnesiuria induced by loop diuretics, were it not for evidence that other processes are also involved. Mathematically derived curves describing sodium and magnesium flows in urine of healthy individuals given placebo show that the time courses of both excretions are parallel (Fig. 1). When the same individuals are given single therapeutic doses of furosemide, urinary magnesium flow is delayed with respect to sodium flow (Fig. 2).[5] This strongly suggests that slow mechanisms, possibly endocrine in nature, may act in conjunction with

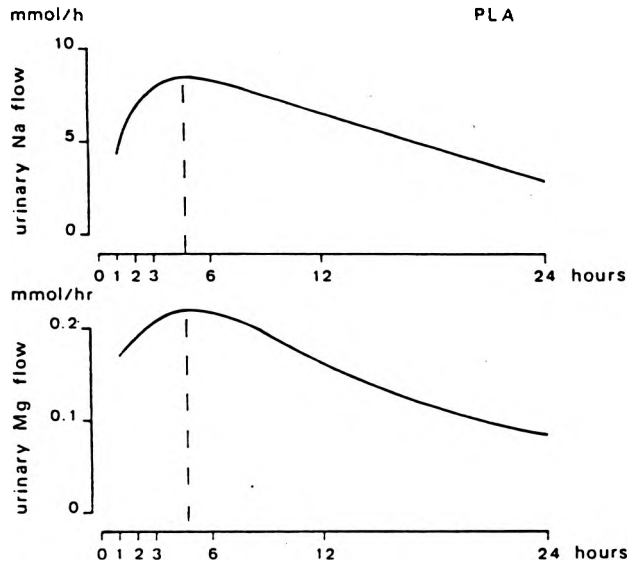

Fig. 1. Urinary sodium and magnesium flows after administration of placebo to 9 healthy volunteers at time 0 (08h00). Both curves exhibit similar time-courses and their peaks thus practically coincide. (From Reyes and Leary[5] by courtesy of *Current Therapeutic Research*).

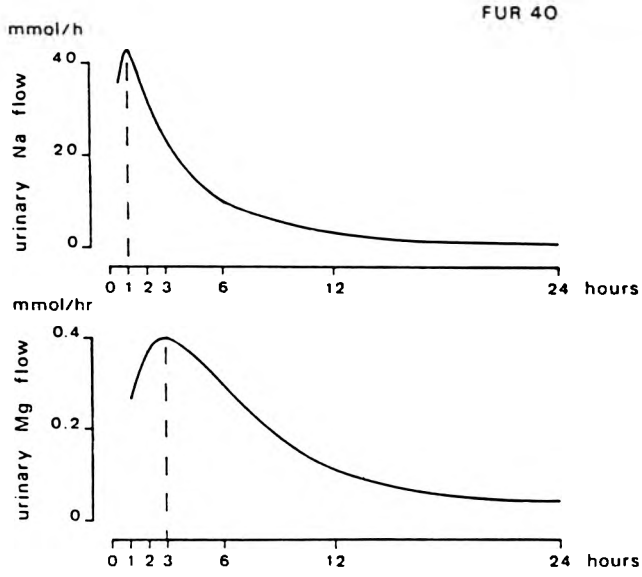

Fig. 2. Urinary sodium and magnesium flows after oral administration of furosemide 40 mg to 9 healthy volunteers at time 0 (08h00). Magnesium flow is retarded with respect to sodium flow and the peaks of the corresponding curves therefore occur at different times. (From Reyes and Leary,[5] by courtesy of *Current Therapeutic Research.*)

direct blockade of magnesium reabsorption at the loop of Henle in determining hypermagnesiuria.

Loop diuretics cause hypercalciuria,[1,11] a negative gradient in serum calcium level and an increase in the serum parathyroid hormone (PTH) value.[12] PTH tends to mobilize calcium and magnesium from bone,[13] thereby re-establishing normal plasma calcium and magnesium values. This may explain why hypomagnesaemia only manifests itself after 8-12 weeks of treatment.[14,15] The transfer of magnesium from bone stores to plasma, under PTH control, increases the amount filtered at the glomerulus and ultimately excreted. However, magnesium reabsorption at the loop of Henle and possibly in the distal convoluted tubule is positively controlled by PTH,[8,10] which would tend to diminish the urinary excretion of magnesium.

When magnesium excretion exceeds intake an increasing mobilization of bone magnesium takes place, the magnesium balance becomes negative and deficiency may develop. This can be reflected by an early fall in the plasma magnesium level, or no change may occur before there has been a major decrease in the intracellular concentration of the ion affecting bone and other tissues.

Depletion of tissue magnesium inhibits the release of PTH, and, in addition, resistance to the action of this hormone develops limiting mobilization of bone calcium and magnesium and reduces the nephronal reabsorption of these ions.[16,17] Hypocalcaemia develops as a result of these changes and tends to decrease magnesiuria slightly in a direct manner independent from PTH.[16,18] The renal effect of PTH is not only diminished because sensitivity to the hormone is decreased but also, in the case of furosemide, because the diuretic blocks it directly.[10] Loop diuretics dilute pre-urinary magnesium, and its reabsorption is thus further reduced.

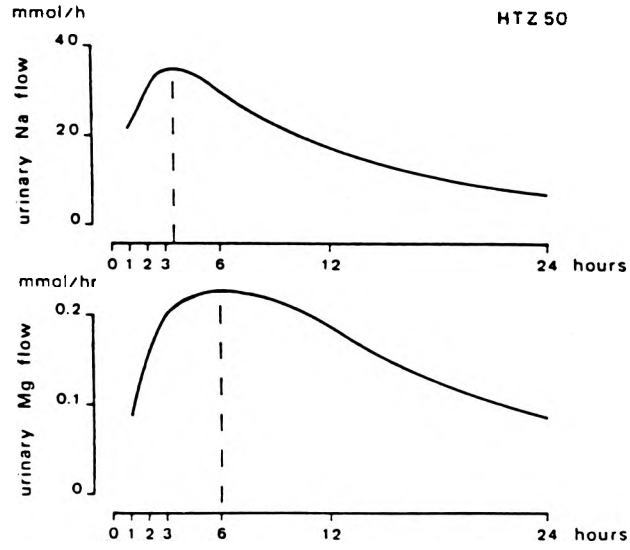

Fig. 3. Urinary sodium and magnesium flows after oral administration of hydrochlorothiazide 50 mg to 9 healthy volunteers at time 0 (08h00). Magnesium flow is retarded with respect to sodium flow and the peaks of the corresponding curves therefore occur at different times. (From Leary and Reyes,[7] by courtesy of *Current Therapeutic Research.*)

Pathophysiology of magnesium deficiency provoked by distal tubular diuretics

Only 1-5% of filtered magnesium is normally reabsorbed from the distal tubule. The hypermagnesiuria provoked by distal tubule diuretics therefore cannot be explained solely on the basis of a direct blockade of magnesium transport across the nephronal wall at that level. This view is supported by experiments similar to those described for furosemide, in which it was found that an important dephasing exists between the curves expressing time-courses of excretion of sodium and magnesium after the administration of distal tubular diuretics such as hydrochlorothiazide,[7] chlorthalidone[4] and chlorexolone (Fig. 3).[6] This dephasing is

more pronounced than that which appears after the administration of loop diuretics and suggests that the hypermagnesiuria caused by distal tubule diuretics involves indirect mechanisms.

Some distal tubule diuretics, such as hydrochlorothiazide[19] and xipamide (A. J. Reyes and W. P. Leary — unpublished data), provoke hypercalciuria for 24 hours following their initial administration but hypocalciuria thereafter if treatment is prolonged, whereas distal tubule diuretics, such as chlorthalidone and indapamide, cause hypocalciuria from the onset of treatment of healthy volunteers or patients with uncomplicated essential hypertension. Diuretics which provoke hypercalciuria initially cause less dephasing between the time-courses than those which reduce urinary calcium excretion from the onset of treatment. Hypercalciuric distal tubule diuretics may behave as loop diuretics initially, at least with respect to hypermagnesiuria during the first 24 hours of treatment, thereafter sharing indirect mechanisms with the other distal tubule diuretics. Hypocalciuria results in relative hypercalcaemia and decreased PTH release with diminished magnesium reabsorption at the loop of Henle. This process *per se* may explain the magnitude of the hypermagnesiuria caused by distal tubular diuretics. Other mechanisms are similar to those associated with the hypermagnesiuria of loop diuretics.

The potassium-sparing diuretic amiloride,[1-3,16] which acts at the last portion of the distal convoluted tubule, does not provoke hypermagnesiuria (W. P. Leary, A. J. Reyes and K. van der Byl —unpublished data) because magnesium in the pre-urine is not transferred to the extracellular fluid at this site and because this diuretic does not affect the renal excretion of calcium to any significant degree.

Magnesium deficiency

Circumstances other than treatment with diuretics may cause magnesium deficiency (Table I).[16] Some of these may also provoke potassium deficiency and may adversely affect patients on diuretic regimens.

Magnesium deficiency seldom occurs as an isolated entity, although its consequences — notably its cardiac effects — may be fatal. Ventricular arrhythmias including ventricular fibrillation[1,2,16] have been attributed to magnesium depletion, and deficiency of the ion increases the myocardial hyper-excitability induced by digitalis.

Other clinical manifestations of magnesium deficiency include the classic signs of tetany, which are usually attributed to calcium deficiency, dysphagia and haemolytic anaemia.[16]

TABLE I. CONDITIONS WHICH MAY PRIMARILY CAUSE MAGNESIUM DEFICIENCY OR ACT AS PRECIPITATING OR AGGRAVATING FACTORS IN ITS DEVELOPMENT DURING DIURETIC TREATMENT

- **Factors decreasing supply of magnesium to the internal environment**
  - **Dietary insufficiency**
    - **Low magnesium content in available foods**
    - **Poor selection of foods**
    - **Global intake of food inadequate to supply magnesium needs**
  - **Malabsorption syndromes**
  - **Extensive gut resection**
  - **Enteral and biliary fistulas**
- **Factors which principally increase magnesium loss**
  - **Gastro-intestinal**
    - **Vomiting**
    - **Diarrhoea**
  - **Renal**
    - **Renal insufficiency with hypermagnesiuria**
    - **Osmotic diuresis (glucose, mannitol, urea)**
    - **Alcohol**
    - **Medications inducing hypermagnesiuria**
      - **Cis-platin**
      - **Gentamicin**
      - **Cardiac glycosides**
    - **Long-term parenteral fluid infusions**
- **Multifactorial conditions**
  - **Hungry bone syndrome**
  - **Hypoparathyroidism**
  - **Hypothyroidism**
  - **Hyperthyroidism**
  - **Excessive lactation**
  - **Malnutrition**
  - **Conventionally treated diabetic keto-acidosis**
  - **Phosphate deficiency**
  - **Protein malnutrition**

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PAPER F5

Prophylaxis and Treatment of Magnesium Depletion

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Prophylaxis and treatment of magnesium depletion

W. P. LEARY, A. J. REYES

Summary

Magnesium deficiency may develop during diuretic treatment, causing serious cardiac arrhythmias hitherto ascribed to potassium depletion. Intravenous magnesium replacement therapy replenishes intracellular magnesium and potassium and inhibits cardiac arrhythmias which may occur during diuretic treatment; oral magnesium supplementation is also effective. The co-administration of amiloride with a distal-tubule diuretic prevents increased renal excretion of magnesium. Doses of diuretics should be restricted to the minimum compatible with the achievement of clinical objectives.

S Afr Med J 1983; **64**: 281-282.

Recent publications have indicated the importance of magnesium depletion as a complication of prolonged treatment with loop or distal-tubule diuretics (LDs, DTDs), laxative abuse and various pathological conditions. This article describes measures which should be taken to limit magnesium losses associated with diuretic therapy and to replenish depleted magnesium stores.

Limitation of diuretic dose

Diuretics are commonly prescribed for patients with oedema or hypertension and increase urinary magnesium losses in most cases. Oedema should be treated initially by appropriate attempts at reversal of the primary cause, optimization of dietary salt intake and administration of diuretics at standard doses. The standard, recommended dose usually amounts to one tablet daily, assuming a natriuretic potency equal to furosemide 40 mg for LDs or hydrochlorothiazide 50 mg for DTDs. Higher doses will be required if malabsorption is present. When prolonged diuresis is necessary, the initial dose should be reduced to the minimum compatible with therapeutic objectives, thus limiting urinary potassium and magnesium losses. This maintenance dose must obviously be individually titrated but usually lies between one-third and one-half of the dose initially required to control oedema.[1-3]

Unnecessarily high doses of diuretics are commonly prescribed in the treatment of essential hypertension. This should be avoided by applying simple rules to the choice of a suitable therapeutic regimen. LDs such as furosemide and ethacrynic acid have an important role but are of limited value in the treatment of uncomplicated essential hypertension and do not satisfy all the criteria for an antihypertensive diuretic. DTDs of the power type, such as a combination of hydrochlorothiazide and amiloride or xipamide, reduce blood pressure rapidly, with stabilization from a clinical point of view occurring after 12 - 14 weeks of treatment.[4-6] Linear antihypertensive diuretics, such as hydrochlorothiazide, decrease blood pressure smoothly, allowing adjustments in regional blood flow and reducing the likelihood of vascular accidents mediated through the mechanism of critical closing pressure.[4] The minimal effective antihypertensive dose of both types of DTD is approximately one-quarter of the conventional diuretic dose. Doses as small as 10-15 mg xipamide and 12,5 mg hydrochlorothiazide or chlorthalidone per day should therefore be prescribed in most cases of uncomplicated essential hypertension.[7] At these doses hypermagnesiuria and hyperkaliuria are minimized, while the antihypertensive effect is similar to that achieved with standard diuretic dosages.

Magnesium administration

Arrhythmias developing during treatment with diuretics respond to acute magnesium replacement.[8-10] The intravenous administration of magnesium sulphate normalizes intracellular concentrations of both potassium and magnesium in skeletal muscle of patients with hypomagnesaemia and hypokalaemia

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Date received: 19 October 1982.

secondary to prolonged diuresis. Simple potassium replacement does not restore the intracellular levels of either cation to normal.[8-9] Despite a relatively poor correlation between the intracellular magnesium concentrations of skeletal and cardiac muscles, it is likely that these tissues respond similarly to magnesium repletion and that the suppression of arrhythmias by magnesium is due to a reactivation of Na^+,K^+-ATPase.

Decreases in myocardial magnesium and potassium concentrations are not only caused by the administration of diuretics; digitalis inhibits myocardial Na^+,K^+-ATPase and myocardial ischaemia has a similar effect on intracellular electrolytes which might contribute to the development of the arrhythmias commonly associated with myocardial infarction.[11,12] In these circumstances magnesium infusion has an anti-arrhythmic effect; animal studies have shown that this measure results in repletion of intracellular potassium and magnesium ions in the infarcted area.[12]

Intravenous magnesium sulphate can be administered as an immediate measure in the treatment of ventricular arrhythmias associated with diuresis, infarction or digitalis therapy.[13] The solution can be given as a bolus of 2,5 g, which usually suppresses arrhythmias within 20 - 30 seconds, or at a dose of 40 mg/min in a saline infusion. The latter dose is safer in patients with respiratory or cardiac insufficiency and can be continued indefinitely without any detectable rise in the serum magnesium level. Alternative parenteral dosage regimens may be used.[9,12,14]

During prolonged treatment with diuretics, magnesium should be taken orally as a prophylactic measure at a dose of 10-15 mmol/d (20 - 30 mEq). This dose should be doubled if evidence of magnesium depletion appears. The magnesium formulations available for therapy (chloride, aspartate) are equally well absorbed and should be taken in divided doses at mealtimes.[15,16]

The only absolute contraindication to magnesium administration is an excess of the ion which sometimes accumulates in patients with acute or chronic renal insufficiency with a glomerular filtration rate below 30 ml/min, hypothyroidism, viral hepatitis, or Addison's disease and in patients on lithium therapy. Hypermagnesaemia is diagnosed when the plasma level exceeds 1,20 mmol/l but clinical evidence of magnesium overload seldom appears at levels below 2 mmol/l.[17]

Initially, nausea and vomiting, rashes, somnolence and reduction of deep tendon reflexes develop. The ECG displays prolongation of the P-R interval and disturbances of intraventricular conduction. When the plasma magnesium level reaches 5 mmol/l, skeletal muscles, including respiratory muscles, may become paralysed, with cardiac arrest in diastole likely if the plasma magnesium concentration rises to 7,5 mmol/l. Accordingly, plasma levels should be monitored during treatment with magnesium supplements. Therapy should be withdrawn and temporary treatment with an LD instituted if mild hypermagnesaemia develops; if important cardiac complications due to magnesium excess are diagnosed, calcium (as calcium gluconate) 100-200 mg should be infused intravenously over 5-10 minutes.

Magnesium-sparing diuretics

Whereas LDs and DTDs cause significant urinary magnesium losses, the potassium-sparing diuretic amiloride has no such effect when single doses of 5 or 10 mg are given. Preliminary studies, carried out in healthy volunteers, suggest that amiloride has a definite magnesium-sparing effect when given in conjunction with standard diuretic doses of hydrochlorothiazide and that the use of a DTD with a magnesium potassium-sparing diuretic might limit intracellular depletion of magnesium and potassium ions. This is supported by other studies in man which have shown that the intracellular magnesium content of skeletal muscle is unaffected by protracted treatment with a combination of amiloride and hydrochlorothiazide.

Replacement of potassium losses

One of the main objectives of attempts to maintain magnesium homeostasis is the restoration of Na^+,K^+-ATPase function to normal so that intracellular potassium levels are replenished. However, a normal plasma potassium level is an essential component in the dynamic equilibrium of Nernst. This may be achieved by measures which have been reviewed elsewhere,[17] including the prescription of a sodium-restricted diet, administration of potassium supplements or the correct use of potassium-sparing diuretics.

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PIONEER

PAPER F6

Magnesium and Sudden Death

This review was shared by the authors.

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Magnesium and sudden death

W. P. LEARY, A. J. REYES

Summary

Magnesium deficiency may result from reduced dietary intake of the ion or increased losses in sweat, urine or faeces. Stress potentiates magnesium deficiency, and an increased incidence of sudden death associated with ischaemic heart disease is found in some areas in which soil and drinking water lack magnesium. Furthermore, it has been demonstrated experimentally that reduction of the plasma magnesium level is associated with arterial spasm. Careful studies are required to assess the clinical importance of magnesium and the benefits of magnesium supplementation in man.

S Afr Med J 1983; 64: 697-698.

Ischaemic heart disease (IHD), seldom diagnosed before World War I, is a common cause of ill-health and death in the developed world. In South Africa, the death rate from IHD is disturbingly high among young adults of European or Asian descent and the incidence of IHD appears to be gradually increasing among urban Blacks.[1] Despite the identification of risk factors related to the occurrence of myocardial ischaemia or infarction (such as hypertension, diabetes mellitus, smoking and hyperlipidaemia), little is known about the causes of sudden death in patients with relatively normal hearts.[2,3] It follows, therefore, that such events cannot be avoided by the application of rationally based preventive measures.

The incidence of sudden death due to cardiovascular disease varies in certain geographical areas and has been ascribed to differences in their geological characteristics. In Canada,[4,5] Finland,[6] Britain[7] and the USA[8] sudden death is commonest in soft-water areas, suggesting that inhabitants of these regions are more susceptible to serious cardiac arrhythmias. Since water, soil and plants are magnesium-deficient in soft-water areas[6] and loss of myocardial magnesium and potassium predispose to major ventricular arrhythmias, it appears reasonable to investigate the possibility that some sudden deaths are due to magnesium deficiency rather than to IHD *per se*. Certainly the magnesium content of the myocardium and coronary arteries is reduced in individuals from soft-water areas.[9] Magnesium depletion decreases the activity of Na^+, K^+-ATPase and Ca^{2+}-ATPase in the sarcolemma, with resultant increases in myocardial sodium and cytosolic calcium levels and falls in intracellular concentrations of magnesium and potassium. This imbalance electrically destabilizes the myocardial sarcolemma, making the occurrence of ventricular arrhythmias likely. Other apparent relationships between magnesium balance and cardiac disease exist.[10]

Magnesium and stress

Individuals with so-called type A personalities commonly develop IHD.[11] They exhibit an intense drive towards the achievement of personal objectives, competitiveness, a persistent need for recognition, commitment to numerous projects subject to deadlines, an accelerated work rate, and inability to relax. Stress associated with a type A personality or with measurable exogenous factors such as noise[11-13] provokes hypermagnesiuria, an increase in overall sympathetic drive and in plasma catecholamine and lipid levels and, ultimately, magnesium deficiency.[8,14] This combination of changes might predispose to lethal arrhythmias and contribute to the overall incidence of sudden cardiac death, particularly when magnesium intake is reduced or accelerated. Magnesium losses can also be due to the injudicious use of alcohol, cathartics or diuretics.[9,12,14]

Magnesium and vascular reactivity

Under experimental conditions, tone in both coronary and cerebral blood vessels is increased by the acute or chronic reduction of the extracellular magnesium concentration. Conversely, hypermagnesaemia or the correction of chronic magnesium deficiency promotes vasodilation.[13-15] Vasospasm is potentiated by calcium ions which replace magnesium at sites on some of the proteins linked with contractility, such as troponin C. In addition, magnesium deficiency increases vascular responses to angiotensin, catecholamines, acetylcholine, serotonin and potassium ions.

Reduced magnesium concentrations in infarcted myocardial tissues and in the immediately adjacent areas were first reported by Condorelli,[16] who studied human autopsy material and the hearts of several animal species subjected to coronary ligation. This finding has been confirmed in man[17,18] and, while it represents a response to oxygen deficiency,[19-22] should be considered as a cause of serious cardiac arrhythmias associated with infarction.[22] Theoretically, the severity of this local imbalance might be aggravated by the presence of chronic magnesium depletion with attendant coronary spasm.[23]

Prophylaxis and therapy

An adequate dietary intake of magnesium should be the rule, particularly in individuals at high risk for myocardial infarction or sudden cardiac death. A diet rich in fresh vegetables and fruit is recommended; high intakes of fat, sugar and alcohol increase magnesium requirements and susceptibility to magnesium deficiency.[22,24-26] Supplementary magnesium should be added to the diet in those areas in which content of the ion in soil and water is low.

The dosage of supplementary magnesium should ultimately be based on accurate studies, similar to those which were used to determine rational iodine supplementation. In the mean time it would be reasonable to prescribe a magnesium salt, taken orally at a dose of 7,5 mmol of the cation (180 mg) daily, for all adults in whom plasma magnesium levels below 0,85 mmol/l are found on

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three separate occasions. This is recommended whether or not signs or symptoms of magnesium deficiency are present.

In acute myocardial infarction magnesium sulphate 40 mg/h should be administered intravenously during the initial 72 hours in hospital, whether or not arrhythmias occur. This innocuous manoeuvre may improve contractility in the perinecrotic area and infarct size may possibly decrease as a result of the protective effects of magnesium on function of the coronary vasculature and the structure of myocardial cells.[27-30]

The possible importance of magnesium deficiency as an aetiological factor in sudden cardiac deaths in South Africa is purely speculative at present. Indeed, it should be stressed that the role played by magnesium ions in all such deaths, although critical in theory, remains unproven, despite a wealth of circumstantial evidence supporting the central hypothesis that magnesium deficiency is a potentially lethal condition.

Within South Africa's borders the drinking water supply has a very low magnesium content, ranging from 1 to 5 mg/l in several areas. In Durban, plasma magnesium levels and urinary excretion of the ion, a rough measure of daily intake, are almost uniformly low in otherwise healthy individuals.[31] Magnesium losses increase when there is profuse sweating or if alcohol, laxatives or common diuretics are taken, further depleting inadequate mineral stores. Arrhythmias are a possible consequence, particularly in patients treated with potentially arrhythmogenic compounds such as sympathomimetics and digitalis or in the presence of potassium depletion or myocardial ischaemia.

Careful prospective epidemiological and clinical studies involving multifactorial analyses are required before the exact role played by hypomagnesaemia in cardiac deaths is determined. In the interim, supplementation of the diet with magnesium salts should be considered in all individuals at risk.

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PIONEER

PAPER F7

Magnesium and Deaths Ascribed to Ischaemic Heart Disease in South Africa

Dr. D.D. Arbuckle, Messrs. C.J. Lockett and K. van der Byl provided data and statistics upon which this work was based. The paper was written by me and edited by Professor A.J. Reyes. The related editorial provides an overview of the subject.

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Magnesium and deaths ascribed to ischaemic heart disease in South Africa

A preliminary report

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Summary

The incidence of death from ischaemic heart disease (IHD) and acute cardiac arrhythmias is increased in some regions where magnesium levels are reduced in soil and water.

Magnesium levels in the drinking water of twelve South African magisterial districts have been evaluated together with corrected statistics for deaths apparently due to IHD in White males from the same districts. A significant negative correlation was found between the incidence of deaths ascribed to IHD and the magnesium content of drinking water. Future, prospective, multivariate studies are required to elucidate whether magnesium scarcity in a geological environment is a major coronary risk factor.

S Afr Med J 1983; **64**: 775 - 776.

In South Africa statistics based on the examination of death certificates indicate that there is a high death rate from ischaemic heart disease (IHD) among Whites, Indians and Coloureds,[1] although a significant number of deaths ascribed to IHD by practitioners who sign death certificates are certainly due to acute cardiac arrhythmias unrelated to IHD or, in some cases, to catastrophic cerebral haemorrhage. Diagnostic errors of this kind are commonly made in all countries, particularly in rural areas where pathology services are often limited.

Data from various sources suggest that the incidences of death from IHD and acute cardiac arrhythmias may be influenced unfavourably by regional deficiencies in dietary magnesium content.[2-4] This view is supported by clinical and pathophysiological evidence that serious cardiac arrhythmias occur in magnesium deficiency of any origin.[5,6]

Magnesium concentrations in soil, water and locally grown fruit and vegetables vary in accordance with regional geological characteristics.[3,4,7] When domestic water is supplied from natural sources its magnesium content is similar to that of the local soil and farm produce and, in the absence of significant supplementation from other sources, the level of magnesium in drinking water provides an accepted indication of magnesium intake in any given area.[2-4] Utilizing this principle a preliminary examination of official data[8,9] has been carried out to determine whether any apparent relationship exists between deaths ascribed to IHD and magnesium levels in the drinking water of several selected South African towns.

Methods

Data on the average magnesium content of drinking water in various districts during 1982 were provided by the Department of Environmental Affairs, Pretoria.[9] These data were based on a sampling procedure whereby water from regional dams and rivers supplying them was repeatedly examined by atomic absorption spectrophotometry.

A preliminary survey of the data allowed identification of a set of localities where the magnesium content of water varied from

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0,04 to 1,85 mmol/l (1-45 mg/l), allowing a wide range of possible magnesium intakes. The districts studied were those of Bloemfontein, Bothaville, Duiwelskloof, Durban, Hennenman, Mafeking, Pietersburg, Potgietersrust, Postmasburg, Venterstad, Virginia and Welkom. This selection preceded the acquisition of any data on regional death rates from IHD.

Corrected statistics[10] for deaths in White males, apparently due to IHD and derived from death certificates issued during 1978, were supplied by the Department of Statistics, Pretoria.[8]

Spearman's (non-parametric) correlation coefficient (rho) was calculated to determine whether any correlation existed between deaths attributed to IHD and magnesium content in drinking water. The test was two-tailed and $P = 0,05$ was considered the limit of significance.

Results

A significant negative correlation was found between the incidence of death apparently due to IHD and the magnesium content of drinking water (Fig. 1).

Discussion

The view that magnesium deficiency may be of pathogenic importance in precipitating deaths from ischaemic heart disease is currently supported by experimental[11] and epidemiological findings.[2-4] It may also be postulated that magnesium deficiency is an aetiological factor in IHD since it decreases glucose tolerance[12] and decreases the high-density/low-density lipoprotein ratio.[13]

The death rates for IHD used in the present study are undoubtedly biased by the inclusion of patients who did not suffer from IHD but died as a result of unrelated acute cardiac arrhythmias. However, since magnesium deficiency is a proven determinant of cardiac arrhythmias[6] this bias is of minor importance.

It is possible that a small percentage of sudden deaths attributed to IHD were caused by acute cerebrovascular events. In this regard it is noteworthy that experimental evidence has been published indicating that magnesium depletion causes spasm of the cerebral arteries.[14]

Future prospective multivariate studies are required in which somatic magnesium status is related to the prevalence of IHD and occurrence of deaths due to acute myocardial infarction, independent arrhythmias and cerebrovascular accidents. The incidence of all other known risk factors for IHD should also be evaluated. Such studies should be multicentric and be carried out by specially trained medical practitioners in small environmentally and biologically homogeneous communities where patients can be followed up.

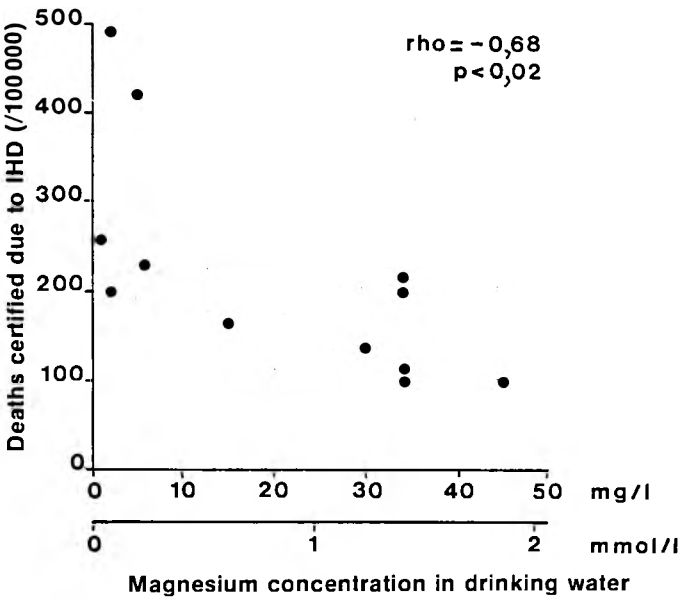

Fig. 1. Relationship between deaths of White males certified as due to IHD and the magnesium content of drinking water. Each point corresponds to a magisterial district in the RSA. There is a significant negative correlation between the variables.

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PIONEER

Magnesium deficiency — a cardiac disease risk factor?

In recent years interest in the development of magnesium-related research has given rise to the foundation of the International Magnesium Society and a journal devoted to the mineral, *Magnesium Bulletin.* Magnesium depletion in man usually results from inadequate dietary intake of magnesium salts or increased excretion of the ion in urine or faeces. Clinical evidence of deficiency may include tetany, dysphagia, anaemia and cardiac arrhythmias.[1]

Ionic magnesium is a co-factor for the function of many enzymes, of which the sodium and adenosine triphosphatase responsible for maintaining electrolyte balance across myocardial cell membranes is of major interest. Evidence suggests that simple potassium replacement regimens during periods of depletion due to disease or drugs may be insufficient to restore intracellular potassium levels to normal when magnesium deficiency is also present.[2] An argument can therefore be made for dietary magnesium supplementation in certain patients.

The incidence of magnesium deficiency in South Africa is unknown, although the low magnesium content of drinking water in some areas and the widespread use of preparations such as laxatives and diuretics, which increase magnesium losses, suggest that this question deserves attention.

An interesting parallel exists between some areas of the RSA and parts of Finland, in which water supplies are relatively magnesium-deficient and sudden death, apparently from cardiac causes, is common.[3] Whereas Finnish studies have been fairly detailed, and the concept of magnesium deficiency as a major cardiovascular risk factor is gaining ground on pathophysiological and epidemiological evidence, no satisfactory studies have been carried out in South Africa. Careful epidemiological and clinical studies are needed to determine whether any link exists between magnesium deficiency and sudden death in South Africa and, if it exists, to determine why such deaths show an apparent racial bias. In addition, it might be wise to include magnesium and other elements in all future basic investigations of cardiac arrhythmias and the essentials of myocardial metabolism.

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PAPER F8

Urinary Zinc Excretion, Diuretics, Zinc Deficiency and Some Side-Effects of Diuretics

This review was shared by the authors. Dr. J.V. Olhaberry provided many of the biochemically orientated references and also his expertise in that aspect of the work.

Review Article

Urinary zinc excretion, diuretics, zinc deficiency and some side-effects of diuretics

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Summary

Diuretics which principally act in the first portion of the distal convoluted tubule, such as the thiazides and chlorthalidone, significantly increase the urinary excretion of zinc. The potassium-retaining diuretic amiloride reduced urinary zinc excretion significantly.

The hyperzincuria provoked by common distal tubular diuretics can cause zinc deficiency, especially when other aetiological factors such as alcoholism, renal insufficiency and pregnancy are also present. Zinc deficiency may be expressed clinically by hypogeusia, hyposmia, sexual impotence or delayed healing; the latter might be an important factor in cases of myocardial infarction.

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Urinary excretion of zinc

Urinary zinc excretion accounts for 30-50% of daily zinc output and is relatively constant in healthy adults, amounting to approximately 0,5 mg/24 h irrespective of intake. Excretion increases in cases of alcoholism, renal insufficiency and during therapy with certain diuretics.[1]

The manner in which the kidney handles zinc is not fully understood. Studies carried out in dogs artificially overloaded with amino acids, and based on the fact that ultrafiltrable zinc is

bound to them, suggest that the urinary concentrations of certain amino acids (such as cystine and histidine) are major determinants of zinc excretion and that significant reabsorption of the cation takes place in the distal convoluted tubule.[1-7] However, the validity of these findings is questionable since the studies were unphysiological and there are differences between dogs and man as regards both plasma protein patterns and nephronal function.

Effects of diuretics on urinary zinc excretion

The effects that different types of diuretics have on urinary zinc excretion provide some insight into the handling of zinc in the kidney in both healthy subjects and patients with uncomplicated essential hypertension.[8-11] Urinary zinc excretion is unaffected by the loop diuretics furosemide and bumetanide, or the potassium-retaining preparation triamterene.[11] Amiloride, another potassium-retaining diuretic, has no effect at a dose of 5 mg, but significantly decreases urinary zinc excretion at 10 mg.[9] 'Distal tubule' diuretics such as the thiazides, chlorthalidone and xipamide increase urinary zinc losses significantly,[10-12] although the simultaneous administration of hydrochlorothiazide 50 mg and amiloride 5 mg appears to maintain urinary zinc excretion within control ranges (W. P. Leary, A. J. Reyes and K. van der Byl — unpublished data).

When the mathematical model of Reyes and Leary[13] is fitted to the time-courses of urinary solute excretions, urinary flows of volume, chloride, sodium, potassium, zinc and magnesium are shown to coincide in time after the administration of a placebo (Fig. 1). However, after chlorthalidone administration potassium, magnesium and zinc flows are delayed in comparison with volume, sodium and chloride (Figs 2 and 3).[10] The time-courses of volume, sodium and potassium excretions in response to amiloride are similar and precede the corresponding flows after placebo by 1-2 hours (Figs 4-6); the flow curves of urinary zinc excretion coincide whether placebo or amiloride is given (Fig. 7).[9]

Since loop diuretics have not been shown to alter urinary zinc excretion in individuals with normal renal function, it may be inferred that zinc is neither excreted nor reabsorbed at the thick portion of the ascending loop of Henle. However, diuretics such as thiazides and chlorthalidone, the principal site of renal action of which is at the cortical diluting segment of the nephron, significantly increase the amount of zinc excreted in 24 hours, suggesting that the ion is normally reabsorbed from pre-urine in the first portion of the distal convoluted tubule. The dephasing that occurs between the zinc flow curves and those of sodium, chloride and volume indicates that processes other than direct blockade of reabsorption from the tubular lumen may be involved in the mechanism that ultimately gives rise to the hyperzincuric effect of distal tubule diuretics. Since it has been postulated that an endocrine mechanism is partially responsible for the hypermagnesiuria associated with the administration of diuretics,[14] a similarly slow mechanism might also account for hyperzincuria — however, its nature is obscure since little information on the endocrine regulation of zinc metabolism exists[1,15] and various relevant biochemical and endocrine effects of diuretics are poorly documented at present.[16-20]

The effect of amiloride on urinary zinc excretion is conceptually and clinically interesting; the compound can be described as a zinc-retaining diuretic. This effect becomes apparent if amiloride is administered in a 10 mg dose alone or in a 5 mg dose combined with 50 mg of hydrochlorothiazide. It is uncertain whether zinc is normally excreted at the most distal portion of the convoluted tubule and amiloride reverses this process, or whether amiloride induces reabsorption of zinc when administered in high doses or when the amount of zinc in pre-urine is raised by the effects of a thiazide diuretic at relatively proximal renal sites.

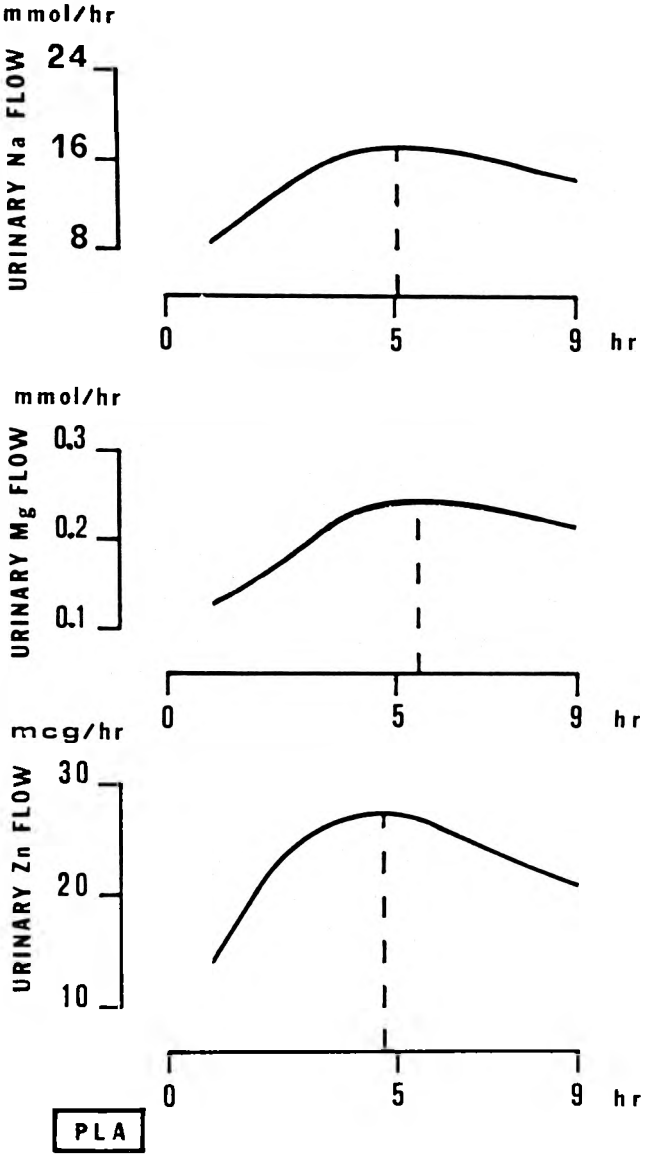

Fig. 1. Mean urinary flows of sodium, magnesium and zinc after oral administration of placebo to 9 healthy volunteers at time 0 of the experiment (08h00). All three curves have similar time-courses and then their peaks coincide in time. By courtesy of *Current Therapeutic Research*.[10]

Implications of diuretic-induced hyperzincuria in cardiology

Those diuretics that cause hyperzincuria (and therefore zinc deficiency in some cases) act mainly at the first portion of the distal convoluted tubule. Coincidentally, these diuretics, which include the thiazides and chlorthalidone,[21] lower blood pressure to a clinically satisfactory level when administered alone in about 70% of patients with essential hypertension.[22]

Hypertensive patients treated with a thiazide or chlorthalidone may become impotent.[23] So far this adverse effect has not been explained, although it could well be due to zinc deficiency secondary to diuretic treatment. Similarly, possible links between zinc and various other unexplained side-effects of diuretics[24-29] merit further investigation. As a general measure plasma zinc concentration should be monitored during prolonged therapy with thiazides or chlorthalidone, especially in patients in whom

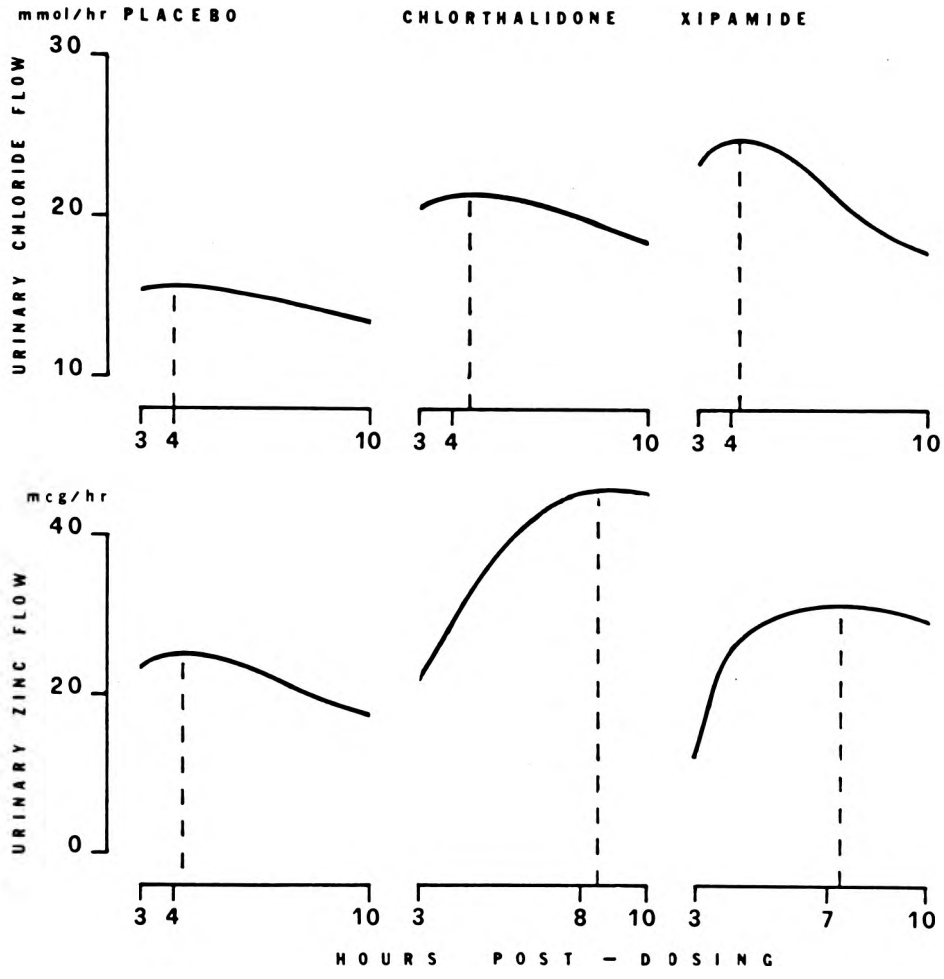

Fig. 2. Mean urinary flows of chloride and zinc after oral administration of placebo, chlorthalidone 100 mg and xipamide 40 mg on separate days to 10 healthy volunteers at time 0 (08h00). The urinary zinc flow coincides in time with the chloride flow after placebo but is delayed with respect to the urinary chloride flow, and a time lapse therefore exists between the peaks of both curves.[21]

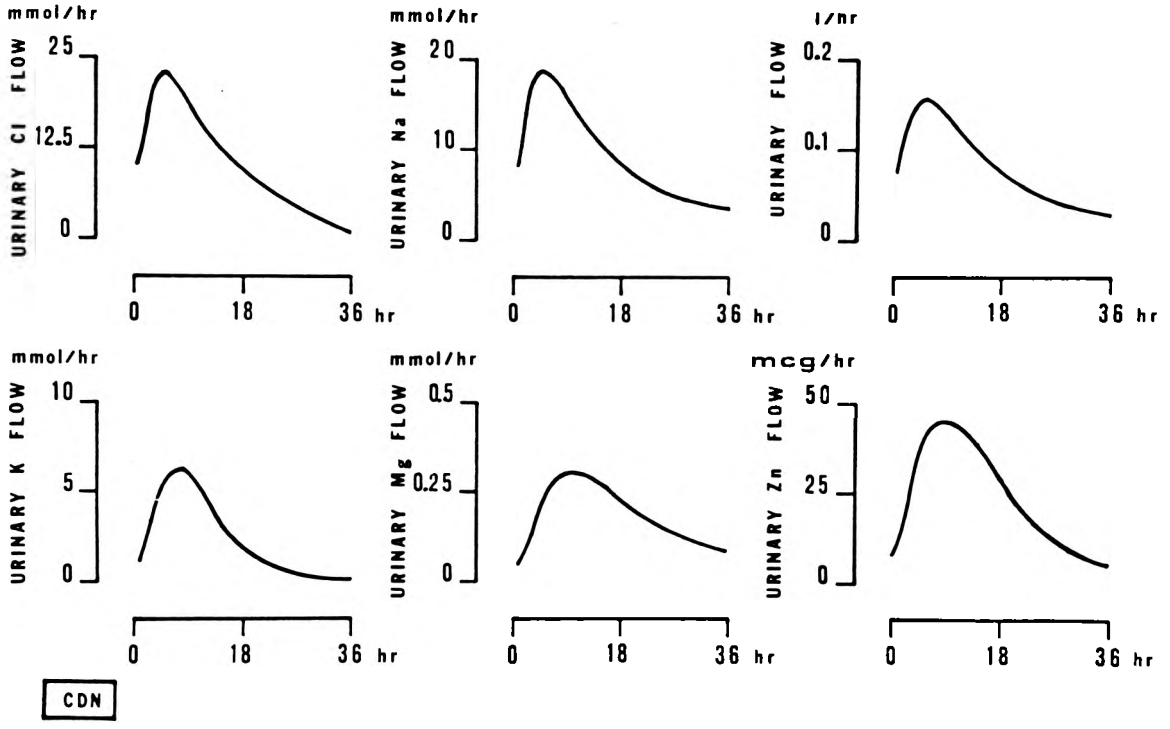

Fig. 3. Mean urinary flows of chloride, sodium, volume, potassium, magnesium and zinc after oral administration of chlorthalidone 100 mg to 10 healthy volunteers (9 for magnesium) at time 0 (08h00). The chloride, sodium and urine flow curves have similar time-courses and then their peaks coincide in time. The potassium, zinc and magnesium urinary flow curves show progressive delays with respect to the first three curves. By courtesy of *Current Therapeutic Research*.[10]

additional factors which could lead to the development of zinc deficiency are present. Simultaneous administration of amiloride with a thiazide or with chlorthalidone may prove to be a desirable precautionary measure as regards the maintenance of 'zinc balance'.

A low plasma zinc level has been described in cases of acute myocardial infarction, possibly having been determined by the hyperzincuria associated with tissue necrosis. How diuretic therapy before or after myocardial infarction may contribute towards provoking zinc deficiency needs to be studied, since zinc depletion might retard the healing of the infarct (this also has to be evaluated).

Zinc deficiency

Zinc deficiency is a poorly understood condition, but there have been some excellent reviews of the subject recently.[1,30]

Pathogenesis

The supply of zinc to the body may be diminished when the total food intake is reduced or when the diet contains an insufficient quantity of the cation.[2,31] In addition, nonspecific circumstances that result in a decrease in the absorption of nutrients, such as malabsorption syndromes, extensive enteral resection and enterobiliary fistulas, reduce zinc absorption. Many dietary substances, including phytates and fibre, decrease zinc absorption and may contribute to the development of zinc deficiency. Inherited enteropathic acrodermatitis is apparently caused by zinc malabsorption, and parenteral feeding without adequate zinc replacement may also give rise to zinc deficiency.[32]

Vomiting and diarrhoea may both reduce zinc absorption and increase zinc losses to the extent that zinc deficiency develops, particularly when these occur recurrently or in subjects prone to zinc deficiency for other reasons. Chronic blood loss from the gut, whether due to haemorrhoids, parasites, drugs or other causes, also promotes zinc deficiency since erythrocytes are very rich in the element.

Zinc deficiency may also follow increased losses of zinc through the skin in cases of extensive burns and psoriasis or increased zinc excretion secondary to disease or medication. In renal diseases hyperzincuria occurs in association with hyperproteinuria or because of alterations in the renal handling of zinc.[33-36] When tissue necrosis is present increased mobilization of tissue zinc occurs via an endogenous leucocyte mediator with a resultant increase in renal zinc excretion. Significant hyperzincuria always exists in cases of chronic alcoholism, presumably as a result of damage to the renal tubular epithelium.

The prolonged administration of common diuretics which act mainly at the distal convoluted tubule, such as the thiazides and chlorthalidone, may provoke zinc deficiency by promoting urinary zinc excretion.

Pathophysiology and clinical picture

The most frequently seen symptoms of zinc deficiency are hypogeusia, hyposmia, sexual impotence, abnormal adaptation to darkness, retardation in the rate of healing and dermatoses.[1] Severe zinc deficiency retards growth and causes hypogonadism in children and adolescents by altering protein synthesis.[37] In zinc-deficient adults oligospermia, azoospermia and consequently infertility occur. In these cases replacement therapy corrects the disturbance and increases the plasma levels of testosterone and dehydrotestosterone. In pregnant women zinc deficiency is associated with malformations of the newborn.[38]

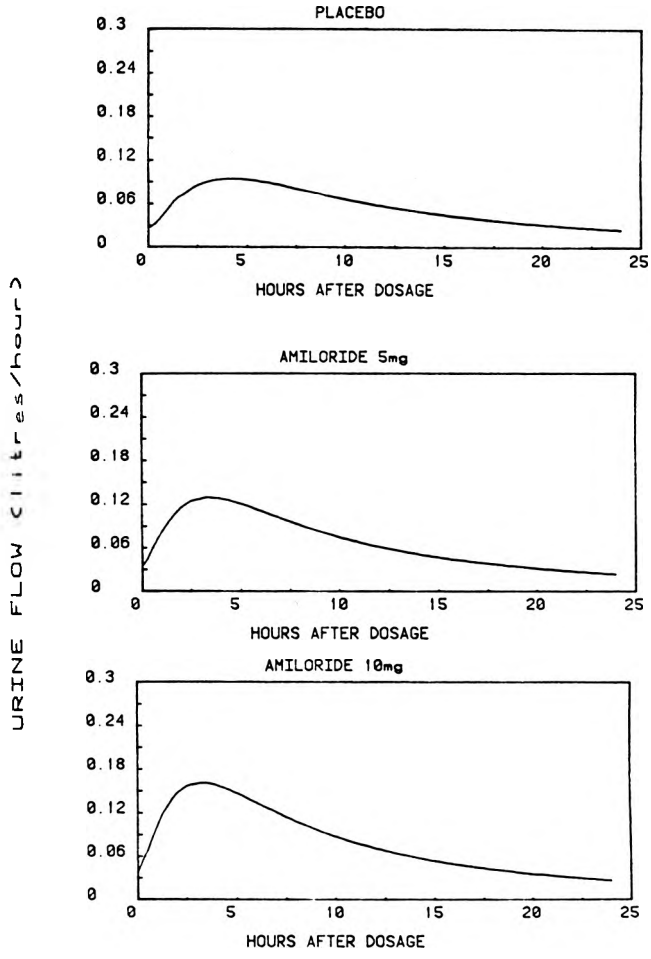

Fig. 4. Mean urine flows after oral administration on separate days of placebo, amiloride 5 mg and amiloride 10 mg to 15 healthy volunteers at time 0 (08h00). The post-amiloride curves have accelerated time-courses with respect to the post-placebo curve, and then their peaks precede the peak of the latter. By courtesy of *Current Therapeutic Research*.[9]

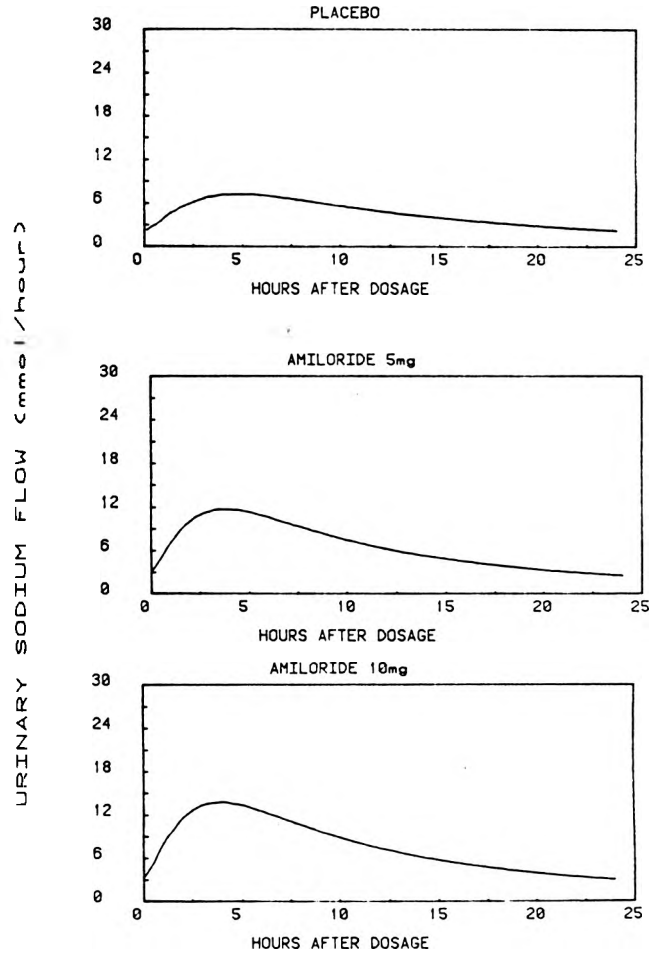

Fig. 5. Mean urinary sodium flows after oral administration on separate days of placebo, amiloride 5 mg and amiloride 10 mg to the same 15 healthy volunteers at time 0 (08h00). The post-amiloride curves have accelerated time-courses with respect to the post-placebo curve. By courtesy of *Current Therapeutic Research*.[9]

Diagnosis

Zinc deficiency is diagnosed when concentrations fall below 65 μg/dl in plasma. The concentration of zinc in biological specimens is usually measured by atomic absorption spectrophotometry, although strict precautions are necessary to avoid biased results.[39] Contamination of samples with tap-water, which generally has a zinc content the same as that of urine, or haemolysis of red cells, which contain zinc at ten times the concentration of plasma, may generate incorrect results.

Treatment

Zinc deficiency is easily treated in adults by the oral administration of 220 mg zinc sulphate three times a day. This may disturb the digestive tract and even cause enteral bleeding; tolerance is improved by taking zinc sulphate capsules after meals.

The Committee of Nutrition of the American Academy of Pediatrics recommends the use of zinc acetate trihydrate solution in children. The preparation should be given in three equally divided doses daily to provide 1 mg of zinc/kg body weight.

The normal upper level of plasma zinc concentrations in healthy adults is 250 μg/dl. Patients with zinc intoxication present with dehydration, electrolytic alterations, abdominal pain, nausea, vomiting, lethargy, dizziness and muscular incoordination, and death as a result of the ingestion of 45 g of zinc sulphate has been described.[1]

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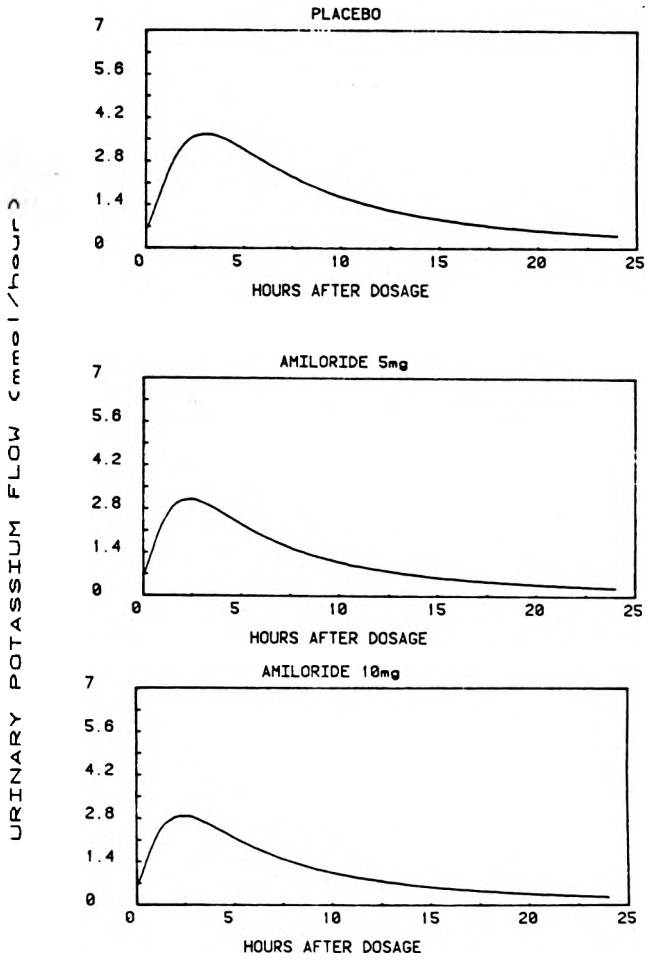

Fig. 6. Mean urinary potassium flows after oral administration on separate days of placebo, amiloride 5 mg and amiloride 10 mg to 15 healthy volunteers at time 0 (08h00). The post-amiloride curves have accelerated time-courses with respect to the post-placebo curve and their peaks therefore precede the peak of the latter. By courtesy of *Current Therapeutic Research*.[9]

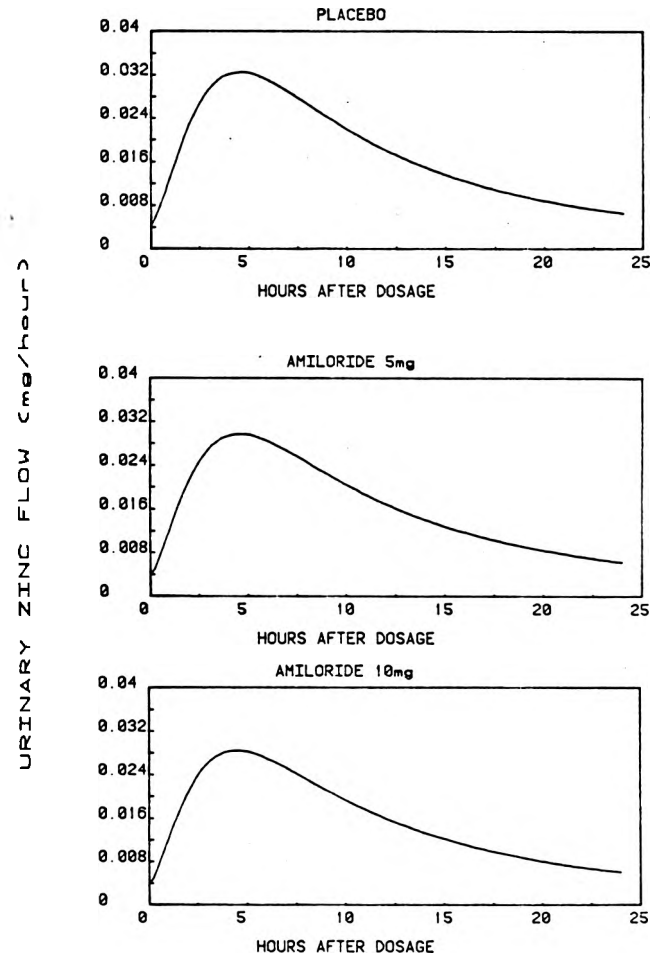

Fig. 7. Mean urinary zinc flows after oral administration on separate days of placebo, amiloride 5 mg and amiloride 10 mg to 15 healthy volunteers at time 0 (08h00). All three curves have similar time-courses and their peaks therefore coincide in time. By courtesy of *Current Therapeutic Research*.[9]

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PAPER F9

Drug Interactions with Diuretics

This review was shared by the authors.

Drug interactions with diuretics

W. P. LEARY, A. J. REYES

Summary

Interactions between diuretics and other substances may have beneficial or adverse consequences. Co-prescription of diuretics with antihypertensive agents, potassium, magnesium or acid salts, probenecid, quinidine, anticoagulants, lithium, cardiac glycosides or other diuretics can result in both beneficial and adverse interactions. Laxatives, oral antidiabetic agents, non-steroidal anti-inflammatory drugs, adenylate cyclase activators, mineralocorticoids, hypolipidaemic agents, neuromuscular blockers, chloral hydrate, carbenoxolone, drugs likely to produce the syndrome of inappropriate secretion of antidiuretic hormone and some antibiotics may be involved in adverse interactions with diuretics.

S Afr Med J 1984; 65: 455-461.

Clinically important interactions with either beneficial or adverse consequences may take place when diuretics are prescribed together with other compounds. Interactions involving diuretics may occur at different anatomical and biochemical sites and have various mechanisms. The interactions described here have been reported clinically or can be postulated on the basis of well-established pharmacological principles. Some responses to treatment might be regarded as coincidental pharmacological effects rather than true drug interactions, but their inclusion here is justified by the fact that they occur in clinical practice when diuretics are prescribed with other medicines.

Classifications

Various criteria may be used in describing drug interactions involving diuretics. One approach is based upon the separation of these interactions in accordance with their clinical effects. However, a given interaction may have either beneficial or untoward effects upon the same variable, depending upon the circumstances. Thus, for example, adverse clinical effects may occur when interactions involve displacement of drugs from the plasma protein-binding sites, but once appropriate adjustments in dosages are made the interaction may become beneficial since the dosage of one or both drugs is reduced without loss of therapeutic effect. Interactions may also have both adverse and desirable effects when more than one biological variable is considered.

A further model for the classification of interactions is presented in Table I. This approach is slightly more quantitative and provides some insight into directional variations in the responses that may occur during the co-administration of diuretics and other medicines. This classification is open to criticism on the grounds that some interactions are sufficiently complicated to fall under more than one subheading.

In the description which follows interactions are dealt with as far as possible in order of overall practical clinical importance taking the frequency and magnitude of the interaction into consideration.

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Antihypertensive agents

Beneficial interactions with antihypertensive drugs

Antihypertensive effects

Antihypertensive diuretics are the first choice in the treatment of hypertension, and since other drugs are prescribed with diuretics to lower blood pressure further, the most important interactions between these agents are beneficial. For example, when the antihypertensive diuretics hydrochlorothiazide[1] and chlorthalidone, which reduce blood pressure linearly, are combined with reserpine[2] and atenolol respectively (W. P. Leary, K. van der Byl and A. J. Reyes — unpublished data), the gradual linear hypotensive response to diuretics alone is replaced by a decreasing power response[3,4] and the blood pressure falls rapidly. The mechanisms of beneficial hypotensive interactions are very variable and usually obscure, since the antihypertensive mechanisms of interacting substances differ and are often unknown.

Sodium retention

Antihypertensive drugs other than diuretics and angiotensin I-converting enzyme inhibitors cause sodium retention. Diuretics reduce this effect and are particularly useful if ß-blockers or vasodilators are used.

Since sodium retention reduces aldosterone release and consequently decreases renal potassium excretion, administration of other antihypertensive agents with diuretics would, theoretically, tend to counterbalance the potassium-losing effects of the diuretic. Studies aimed at measuring the overall result of this interaction have not as yet disclosed any clinical benefit.

Increased blood viscosity secondary to haemoconcentration induced by diuretics has been hypothetically incriminated as a cardiovascular risk factor. This may be balanced by the properties of other antihypertensive drugs, such as ß-adrenergic blockers and vasodilators, which provoke sodium retention and therefore restore the intravascular volume.

Guanethidine and glucose tolerance

Guanethidine has been shown to improve glucose tolerance and to reduce the need for hypoglycaemic agents and insulin in diabetics. Common diuretics have the opposite effect, which may be counterbalanced by giving guanethidine.

Plasma renin activity

Arguments in favour of pivotal aetiological and/or pathophysiological roles for peripheral renin activity in hypertension

TABLE I. DRUGS INTERACTING WITH DIURETICS

Drugs enhancing the effects of diuretics	**Drugs enhancing their therapeutic effects when interacting**
Antihypertensive agents	Antihypertensive agents
Digitalis	Digitalis
Diuretics	Potassium salts
Laxatives	Magnesium salts
Probenecid	Laxatives
Parasympatholytic agents	Quinidine
Acid salts	Anticoagulants
	Neuromuscular blockers
	Lithium
Drugs inhibiting the effects of diuretics	**Drugs inhibiting their therapeutic effects when interacting**
Vasodilators	Potassium salts
Cephalosporins	Magnesium salts
NSAIDs	Oral antidiabetics
Phenylbutazone	Anti-uricaemic agents
Mineralocorticoids	**Drugs enhancing their adverse effects when interacting**
Hypolipidaemic agents	Antihypertensive agents
Carbenoxolone	Digitalis
Colestipol	Potassium salts
	Diuretics
	Cephalosporins
Drugs enhancing adverse effects of diuretics	Aminoglycosides
Digitalis	Tetracycline
Potassium salts	Laxatives
Magnesium salts	NSAIDs
Cephalosporins	Phenylbutazone
Aminoglycosides	Mineralocorticoids
Oral antidiabetics	Adenylate cyclase activators
Laxatives	Hypolipidaemic agents
NSAIDs	Quinidine
Phenylbutazone	Anticoagulants
Anti-uricaemic agents	Drugs that are likely to produce SIADH
Adenylate cyclase activators	Neuromuscular blockers
Parasympatholytic agents	Chloral hydrate
Drugs that are likely to produce SIADH	Lithium
Acid salts	Carbenoxolone

NSAIDs = non-steroidal anti-inflammatory drugs, SIADH = syndrome of inappropriate antidiuretic hormone secretion.

have recently been compromised to some extent by carefully conducted clinical research and the emergence of the kallikrein-kinin system as a possible balancing mechanism.[5] The extent by which interaction between diuretics and other drugs affecting the renin-angiotensin-aldosterone axis might be relevant is not clear, except in a few situations in which secondary hypertension is definitely due to hyper-reninaemia or possibly in a small proportion of primary cases with definitely higher peripheral renin activity values at an early stage.

If the elevation of plasma renin activity caused by diuretics were of importance in determining the evolution of hypertensive disease, other antihypertensive agents which diminish plasma renin activity, such as most ß-adrenergic blockers, could interact beneficially with diuretics in this respect while those increasing plasma renin activity, such as vasodilators, would interact adversely.

Adverse interactions with antihypertensive agents

Hypotension

The most frequent adverse result of interaction between diuretics and antihypertensive drugs is an exaggerated hypotensive effect of combined administration, regardless of the biochemical processes involved. Clinical complications include postural hypotension and syndromes associated with diminished perfusion of the myocardium, brain, intestine and kidneys. Any change in treatment involving such a combination should therefore be carefully supervised.

Diazoxide

When diazoxide is administered intravenously for the treatment of hypertensive crises in patients on diuretics its effects upon blood glucose levels may be exaggerated with development of clinically significant hyperglycaemia.[6]

Clonidine

Clonidine and other central α_2-agonists reduce salivary secretion. Administration of these agents with diuretics further compromises salivary flow if dehydration occurs.

Renal blood flow

In common with most antihypertensives (other than hydrallazine and the converting enzyme inhibitors) antihypertensive diuretics[1,3,4,6-8] diminish renal blood flow. In patients with impaired renal function the co-administration of loop diuretics which increase renal blood flow must be considered, despite their relatively meagre effect upon arterial blood pressure.

Guanethidine

Thiazides are known to impede the uptake of guanethidine by the neuronal terminals and thus decrease or prevent its antihypertensive effect. This can be overcome by adjusting the dose of guanethidine and avoided by prescribing non-thiazide diuretics.

Digitalis

The benefits derived from a combination of digitalis and diuretics in chronic or acute cardiac insufficiency are well

known. Administration of digitalis with common potassium-losing diuretics usually leads to a higher incidence of arrhythmias than occurs when either drug is administered alone.[9,10] This probably results from the combined effects of potassium and magnesium depletion provoked by diuretics and the direct inhibition of Mg^{2+}-dependent myocardial Na^+,K^+-ATPase induced by cardiac glycosides.[6,11,12]

Although glomerular filtration accounts for most of the renal excretion of digoxin it is also excreted by the distal convoluted tubule, where it shares intracellular transport mechanisms with spironolactone and triamterene.[13] These diuretics may therefore increase the plasma concentration of digoxin and its half-life and enhance all its untoward effects, including arrhythmogenicity. Plasma levels of digoxin should therefore be carefully monitored when it is given with spironolactone or triamterene. No clinical evidence of a similar interaction with amiloride has yet been presented, and the possibility that furosemide could alter the renal excretion of digoxin has been ruled out.[13]

Potassium salts

The degree of hyperkalaemia attained when diuretic therapy is supplemented with potassium salts may be such that the concentration gradient across electrically excitable membranes (such as myocardium and skeletal muscle) overcomes the reduced activity of Na^+,K^+-ATPase resulting from concomitant diuretic-induced magnesium depletion. In rare instances the intracellular potassium content increases and causes hyperkalaemic paralysis or death due to cardiac arrest.[14,15] These circumstances usually arise only after chronic administration of potassium, but the syndrome may develop relatively soon after the initiation of therapy if renal insufficiency is present.

Diabetes

The administration of potassium-sparing diuretics or potassium salts to insulin-dependent diabetics with associated hypoaldosteronism can have lethal effects[16,17] and should therefore only take place under supervision.

Magnesium salts

Urinary magnesium losses caused by diuretics lead to intracellular potassium depletion because Na^+,K^+-ATPase, a critical energy source for the potassium pump, depends upon intracellular magnesium as a co-factor. There is definite evidence that severe ventricular arrhythmias occurring during chronic diuretic administration respond better to intravenous therapy with magnesium than to that with potassium; the intramyocardial content of potassium increases more after magnesium administration than after potassium supplementation in this circumstance.[6,11,12,18] There is therefore a sound theoretical basis for chronic long-term oral administration of magnesium salts for the avoidance of the deleterious effects of diuretics on intracellular potassium. It remains to be seen whether combining potassium-sparing diuretics and magnesium salts results in an exaggerated increase in potassium entry to the cell with potentially toxic effects.

Diuretics

Beneficial interactions between diuretics

Beneficial interactions between diuretics exist which can be exploited clinically even when untoward interactions coexist as side-effects.

Diuretic potency

To increase diuretic potency the additive effects of diuretics acting at separately located or biochemically distinct principal renal acceptor sites may be used. When established diuretic therapy is reinforced by the addition of a second diuretic, the safest procedure for increasing diuretic potency while using the smallest possible doses is based on the fact that there is an increased tubular reabsorption of sodium at sites distal to those at which any given diuretic blocks sodium absorption. Therefore, a diuretic blocking sodium reabsorption in the distal tubule, such as a thiazide, should be added when the response to a high-ceiling diuretic acting at the loop of Henle, such as furosemide, is regarded as being clinically inadequate.

Giving two loop diuretics together does not increase diuresis once maximal doses of either compound have been used[6,7] since loop diuretics share a single renal acceptor.

Beneficial interactions between diuretics are also exploited in conditions such as anasarca which require rapid induction of sustained diuresis. In such circumstances the use of loop diuretics such as furosemide or bumetanide may be usefully complemented by adding a medium- (chlorthalidone) or high-potency (xipamide) diuretic[6] with a longer time-course of action, although this may increase the incidence of side-effects.

Potassium turnover

A beneficial effect upon potassium turnover is achieved by the co-administration of potassium-losing and potassium-sparing diuretics, a practice which has achieved wide clinical acceptance and is supported by research data.[6,8,11,12,19]

Beneficial antihypertensive interactions

Favourable interactions between diuretics are also useful in the treatment of raised arterial pressure in certain circumstances.

Hypertensive crises

The intravenous administration of a loop diuretic during hypertensive crises in patients receiving treatment with antihypertensive diuretics lowers the blood pressure satisfactorily. This can be explained principally by the acute vasodilating properties of intravenously administered loop diuretics, although temporary acute changes in plasma volume also occur.[7]

Resistant hypertension

Despite the fact that loop diuretics given alone generally have little effect on blood pressure, their oral administration sometimes lowers blood pressure in cases of resistant hypertension treated with distal tubular antihypertensive diuretics. The mechanism involved depends upon the additive sodium depletion brought about by administration of the loop diuretic.[7] The addition of an antihypertensive diuretic to a therapeutic regimen which already includes a loop diuretic also further decreases blood pressure, as has been shown by the addition of xipamide or metolazone to inadequate previous treatment regimens.[7]

Power antihypertensive effects

Giving a potassium-sparing diuretic with hydrochlorothiazide as initial treatment has been shown to change the gradual linear antihypertensive effect of the latter[1] to a more rapid power response.[3] Since power antihypertensive activity is sought in the treatment of most hypertensives this preliminary finding merits investigation.

Adverse interactions between diuretics

Diuretic interactions may not be simply additive, since diuretics such as furosemide and the thiazides have, for example, opposite effects upon renal blood flow and the glomerular filtration rate. The co-administration of loop and thiazide diuretics might cause greater potassium losses than would be expected from the sum of their individual effects; the raised glomerular filtration rate stimulated by loop diuretics increases the sodium load presented for exchange with potassium.

Allergy

Most diuretics in current use are structurally related to sulphonamides and cross-sensitivity reactions occasionally occur; the frequency of such reactions increases when two diuretics are given together.

Nephrotoxicity

An infusion of mannitol and furosemide is commonly prescribed in order to normalize dilutional hyponatraemia, but may cause acute renal insufficiency through a toxic effect upon the proximal convoluted tubular epithelium.[20] Since the benefits of this combination are limited, it should not be prescribed.

Hyperkalaemia

Potassium-sparing diuretics, particularly amiloride and triamterene, may provoke hyperkalaemia when prescribed alone or in combination with potassium-losing diuretics. In the latter case hyperkalaemia is likely to occur after chronic administration of the combination when relative tolerance to the hyperkaliuric effect of the potassium-losing diuretic develops and sodium depletion causes reduced sodium filtration and tubular interchange for potassium.

Antibiotics

Cephalosporins

It is well established that certain cephalosporins, such as cephaloridine, cephalothin and cephalexin, sometimes induce renal damage. This may be aggravated by the co-administration of common loop or distal tubule diuretics which share renal excretory mechanisms with the cephalosporins at the proximal tubule[7] thus leading to prolongation of the serum half-life and a rise in the renal concentration of these cephalosporins to toxic levels.

Aminoglycosides

Aminoglycosides such as kanamycin, streptomycin and gentamicin can impair auditory and cochlear functions, particularly in patients with renal disease; loop diuretics such as furosemide, bumetanide and ethacrynic acid may also adversely affect hearing and equilibrium. Aminoglycosides and loop diuretics have additive toxic effects on the hair cells of the inner ear, especially when these compounds are highly concentrated within the endolymphatic system.[21] Experimental evidence from animal studies shows that kanamycin and ethacrynic acid exert a synergistic ototoxic action, and the existence of such synergism can also be inferred from case reports of fixed or permanent deafness following the intravenous injection of ethacrynic acid in patients taking low doses of aminoglycosides.[22,23] Deafness is likely to develop in the presence of renal insufficiency or when the drugs are prescribed at high doses or are administered rapidly.[23]

Aminoglycosides are nephrotoxic, and since diuretics may increase local concentrations of any nephrotoxic antibiotic within the kidney, renal function must be appropriately monitored during co-administration of these.

Oral antidiabetic drugs

Common diuretics impair glucose tolerance in healthy and diabetic individuals, sometimes raising the blood glucose concentration.[6,7] This effect has not been fully explained, but should not be regarded as being equivalent to a diabetic syndrome since long-term studies in humans to determine details of the general metabolic changes involved have not been carried out. However, hyperglycaemia — especially if sustained — may provoke serious systemic metabolic imbalance. In diabetics treated with oral hypoglycaemic agents blood glucose levels may rise significantly when diuretics are added, this necessitating an adjustment in the dosage of the antidiabetic agent. This reaction occurs irrespective of the mechanism of action of the antidiabetic agent (sulphonylureas or biguanides), but is more likely when sulphonylureas are being prescribed.[7]

Drugs which tend to increase the blood glucose concentration, such as diazoxide, phenylbutazone or hormones activating adenylate cyclase, have additive effects with diuretics in impairing glucose tolerance and increasing blood glucose levels.

Laxatives

Whatever their mechanisms of action, dosage or indications for usage, laxatives are likely to cause significant losses of fluid and electrolytes, including sodium, potassium, magnesium and zinc. Since water and these electrolytes are excreted freely in response to diuretics, laxatives may marginally contribute to the beneficial effects of diuretics, but they are more likely to cause dehydration and significant depletion of sodium, potassium and magnesium stores.

Whenever a diuretic is prescribed patients should be warned about the possible consequences of concurrent laxative abuse. Ideally, laxatives should only be prescribed when absolutely necessary and while diuretic therapy is temporarily discontinued. Since most laxatives are purchased without prescription the situation is difficult to control.

Non-steroidal anti-inflammatory agents

Non-steroidal anti-inflammatory drugs (NSAIDs) exert their pharmacological activity mainly by inhibiting the synthesis of various prostaglandins. Preparations that inhibit cyclo-oxygenase (e.g. aspirin, indomethacin), or selectively inhibit further specific steps leading to synthesis of the prostaglandin E group, such as phenylbutazone, interact with those diuretics (including loop diuretics and thiazides) that increase the synthesis of PGE_2 within the kidney.[24-30] For this reason, the renal excretory effects of diuretics are reduced by adding NSAIDs to the regimen.[31-34]

A second mechanism exists whereby NSAIDs and diuretics interact; all these are organic acids and share their renal excretory pathway at the proximal convoluted tubule. For example, indomethacin causes prolonged elevation of plasma furosemide levels, decreasing its plasma and renal clearance.[7] This interaction should increase the renal effects of furosemide; however, the overall result of interaction between diuretics and NSAIDs when both are repeatedly prescribed at standard doses

is a diminution in the renal effects of diuretics, possibly because of interaction at the prostaglandin level.[7]

The extent to which diuretics counteract the effect of NSAIDs in cases where PGE_2 is deeply involved in an inflammatory focus has not been studied.

Phenylbutazone

Phenylbutazone and its analogues provoke increased sodium and water retention, decreased tolerance to glucose, hyperglycaemia and increased hydrochloric acid secretion in the stomach. All these adverse reactions principally derive from the same anti-inflammatory mechanism, the inhibition of prostaglandin E_2 synthesis. Conversely, common diuretics increase the synthesis of PGE_2.[28,32,33] Interaction of these drugs at the biochemical level is followed clinically by partial blunting of the natriuretic and diuretic effects of diuretics by phenylbutazone. In addition, both phenylbutazone and diuretics tend to raise blood sugar levels.

Phenylbutazone is formally contraindicated whenever diuretics are prescribed. If the existence of inflammation makes it necessary to prescribe medication, anti-inflammatory agents such as aspirin and indomethacin should be used.

Drugs affecting urate excretion

The effect of drugs used for lowering serum urate levels may be impaired by the administration of sulphamoyl diuretics which enhance the reabsorption and inhibit the normal excretion of uric acid in the renal tubule, thereby tending to raise serum urate level.[6,7] This change does not necessarily imply any basic derangement in purine metabolism equivalent to that present in gout; however, elevated serum uric acid levels provoke further metabolic changes that are best avoided.

Tienilic acid

Tienilic acid is an uricosuric diuretic[7] which may be administered together with those diuretics which cause urate retention. This combination increases diuretic potency with no change or reduction in the serum urate concentration. The interaction between tienilic acid and conventional diuretics is therefore beneficial, and it may be expected that other uricosuric diuretics under development will also be used in this manner.[6,7]

Probenecid

Probenecid has a definite uricosuric effect and is also used in laboratory and clinical situations to alter the pharmacokinetics of other drugs, including diuretics.[35-38]

Diuretics share their excretory mechanism with other organic acids excreted in the proximal tubule; probenecid is a weak organic acid, interaction of which with diuretics in the kidney has a biphasic effect in man.[7] When furosemide is administered to patients pretreated with probenecid, the natriuretic and diuretic effect of the former is at once diminished because of decreased secretion of the diuretic through the proximal tubule. This primary response is followed by a marked increase in diuresis and natriuresis because the amount of furosemide available at renal acceptor sites is increased once probenecid has been cleared in the urine. Probenecid prolongs both the renal and hepatic clearances of furosemide, and because of delayed excretion elevates the serum furosemide level. Thiazide diuretics interact with probenecid in a similar manner, although to a lesser extent, this mainly being dependent upon the time lapse between doses. The biphasic nature of this effect is not apparent experimentally unless urine collections after the co-administration of probenecid and a diuretic are made when the second phase has emerged. This has led to apparent contradictions in the literature referring to interactions between diuretics and probenecid. The uricosuric effects of probenecid are not seriously impaired by diuretics.[39]

Drugs with mineralocorticoid activity

Drugs with mineralocorticoid activity include compounds such as desoxycorticosterone acetate, the oestrogens, progestogens, glucocorticoids and androgens. All these substances may cause sodium and water retention and potassium loss; overt oedema may develop as a result of their activity or as a consequence of the underlying pathophysiological conditions which originally prompted the use of a diuretic. In these circumstances the diuretic and natriuretic effects of diuretics will be diminished and potassium losses will be enhanced by the addition of mineralocorticoids.

Drugs that activate adenylate cyclase

Naturally occurring substances, mainly hormones like glucocorticoids, adrenocorticotrophic hormone, thyroxine, adrenaline, glucagon, androgens, somatotrophic hormone, thyrotrophic hormone and many of their metabolites and related synthetic compounds, activate adenylate cyclase, thereby increasing the rate of glycogenolysis. Consequently, hyperglycaemia and an associated chain of metabolic events develop. Since diuretics also have hyperglycaemic properties, the blood glucose level should be closely monitored when these preparations are prescribed together, especially in the case of diabetics or prediabetics and patients considered to have a fragile acid-base equilibrium.

Hypolipidaemic agents

Clofibrate

Clofibrate, which is still widely used as a hypolipidaemic agent despite controversy regarding its safety, interacts specifically with furosemide by competing for common plasma albumin binding sites. This raises the serum concentration of free clofibrate, leading to a syndrome of malaise, muscle pain, stiffness, weakness and elevated creatine kinase levels.[40] Particular care must therefore be exercised when furosemide and clofibrate are prescribed together, especially with patients who have reduced plasma binding sites due to hypo-albuminaemia associated with conditions such as the nephrotic syndrome or cirrhosis, or who receive phenprocoumon.[41]

Cholestyramine

Cholestyramine decreases the intestinal absorption of many substances, including lipids. Absorption of some diuretics is delayed by this compound, but this interaction causes no important decrease in the percentage absorption of diuretics and no change in their renal excretory effects.[42]

Colestipol

Colestipol, a bile-acid-sequestering polymer, appears to bind chlorothiazide within the intestinal lumen; in consequence, the absorption and pharmacological effects of this diuretic are diminished by large doses of colestipol.

Quinidine

Quinidine is partly catabolized by liver enzymes whose activity may be increased by spironolactone.[43] Diuretics which inhibit carbonic anhydrase (such as the sulphamoyl diuretics) tend to increase the pH value of the pre-urine within the nephron. This results in an increased proportion of molecular non-ionized quinidine, which is readily reabsorbed, with resultant significant elevation of plasma quinidine levels. Measures tending to counteract this potentially dangerous interaction should include a diet which does not alkalinize the urine, the avoidance of sodium bicarbonate or other systemic alkalinizing agents and the prescription of loop diuretics such as furosemide or bumetanide which have little carbonic anhydrase-inhibiting capacity.

Parasympatholytic drugs that reduce gastro-intestinal motility

Medicines that display antimuscarinic actions or that block the parasympathetic ganglia serving the upper gastro-intestinal tract where diuretics are absorbed (e.g. atropine, propantheline, chlorpromazine) slow the transit of diuretics through the gut and increase the percentage absorption of those diuretics which are usually totally absorbed[7] (such as hydrochlorothiazide).

Drugs likely to produce the syndrome of inappropriate secretion of antidiuretic hormone

Giving diuretics with other drugs such as clofibrate, carbamazepine, cyclophosphamide, fluphenazine, haloperidol, thioridazine, vinca alkaloids, amitriptyline and chlorpropamide is likely to produce a syndrome of inappropriate secretion of antidiuretic hormone.[6,7] Treatment of these combinations must be carefully monitored in order to avoid this syndrome of water intoxication at an early stage. Clinical symptoms such as drowsiness, headache, anorexia, nausea, vomiting, depression and confusion suggest inappropriate antidiuretic hormone secretion.

Neuromuscular blockers

Preparations used together with general anaesthetics in order to promote skeletal muscle relaxation interfere with ionic exchange across the neuromuscular postsynaptic membrane, causing a net shift in potassium and calcium from the muscle cells to the extracellular compartments. The losses of potassium and magnesium that diuretics provoke may interact at this level, increasing the intensity and duration of neuromuscular blockade. It appears immaterial whether the neuromuscular blocker used is a depolarizing or non-depolarizing agent. This interaction is another reason for exercising care in the administration of diuretics to patients undergoing surgery with general anaesthesia.[6]

Chloral hydrate

The main metabolite of chloral hydrate is trichloro-acetic acid, which is highly bound to the plasma albumin sites that transport furosemide. It has been reported that the rapid administration of furosemide as an intravenous bolus may provoke a displacement interaction whereby trichloro-acetic acid is released from binding sites with a resultant toxic syndrome comprising copious diaphoresis, flushing and tachycardia.[44] In consequence, chloral hydrate should be avoided in patients likely to be given furosemide.

Lithium

Thiazide diuretics increase sodium reabsorption at the proximal tubule and a secondary enhancement of lithium reabsorption follows, resulting in decreased lithium clearance.[45,46] Serum lithium levels may then rise to overtly toxic concentrations (2 - 3 mmol/l).[47] Depending upon serum lithium levels, the toxic syndrome may range from drowsiness, muscle twitching, vomiting and diarrhoea to oliguria, supraventricular arrhythmias, hypokalaemia and ataxia;[48,49] diabetes insipidus has been described as a complication associated with prescribing lithium with chlorothiazide.[31]

Whenever diuretics are given with lithium, serum lithium levels should be carefully monitored, principally if it is administered in long-acting formulations. However, there is no conclusive evidence that the potassium-sparing diuretics have similar effects to the thiazides with respect to lithium, and preliminary evidence suggests that the loop diuretics do not interact with it.[45]

Carbenoxolone

Carbenoxolone exhibits a powerful mineralocorticoid-like effect — sodium and water are retained and potassium is lost in the urine. The diuretic and natriuretic effects of diuretics are therefore counteracted, while their kaliuretic action is enhanced. This effect of carbenoxolone may be sufficient to cause oedema requiring diuretic treatment, resulting in a significant reduction in total body potassium content. Supplementary potassium and magnesium salts, amiloride or triamterene should then be prescribed. The co-prescription of spironolactone is inadvisable since it has some glucocorticoid activity which may counteract the therapeutic effects of carbenoxolone.[50]

Acidifying salts

Ammonium chloride may be given with diuretics for acidifying the urine, thereby increasing their natriuretic effects. Administration of ammonium chloride with a potassium-sparing diuretic has provoked metabolic acidosis in 1 case.[51]

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PAPER F10

The Antihypertensive Effect of Diuretics

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The antihypertensive effect of diuretics

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The high incidence of arterial hypertension constitutes an important epidemiological problem, particularly since the risks of sudden cardiac death, myocardial infarction, cardiac insufficiency, cerebrovascular disease, obstructive arteriopathy of the lower limbs and chronic renal insufficiency all increase linearly with arterial blood pressure (BP) values. Accordingly, it is commonly accepted that diastolic BPs above 90 mmHg and systolic BPs above 150 mmHg should be treated, provided the peripheral circulation is not compromised.[1] Initially, if values are not too high, pressure may be reduced by non-pharmacological measures, including the restriction of dietary sodium intake.[2-4] When this proves unsuccessful, pharmacological treatment is instituted according to established guidelines.

Chlorothiazide became available 3 decades ago and, since then, diuretics have been used as first choice antihypertensive agents by many clinicians.[2,5] This approach is justified because a regimen of sodium restriction and diuresis controls BP effectively in about 65% of all cases of essential hypertension.[6] Despite their widespread clinical use, a number of fundamental questions concerning diuretics remain to be answered. The mechanism of their antihypertensive action and many pathophysiological effects of diuretics are still poorly understood. In recent years academic interest in diuretics has revived and this review deals with developments in the use of diuretics as sole medication in the treatment of essential hypertension.

Definition of an antihypertensive diuretic

Not all diuretics are effectively antihypertensive when administered as monotherapy for essential hypertension. An antihypertensive diuretic is defined as a substance which consistently lowers the basal supine diastolic BP below 90 mmHg in at least 55% of patients in representative unstratified samples of the total population of essential hypertensive subjects treated for 16 weeks. In addition, antihypertensive diuretics maintain this reduction when treatment is prolonged for at least 1 year.

Those diuretics whose principal site of renal action is a common acceptor situated at the thick ascending portion of the loop of Henle (loop diuretics) are not antihypertensive diuretics in terms of the above definition. Furosemide, ethacrynic acid, bumetanide and piretanide are included in this category.[6,7] Loop diuretics presented in slow-acting formulations do not exhibit antihypertensive efficacy either.

Those diuretics acting principally on specific acceptors in the first portion of the distal convoluted tubule, including thiazides, chlorthalidone, indapamide and xipamide, are efficacious antihypertensive substances. Throughout this review these substances will be referred to as antihypertensive diuretics or simply as diuretics.

The relationship between antihypertensive efficacy and location of the principal site of renal action of diuretics does not imply that the mechanism of antihypertensive action of diuretics may be totally or partially accounted for by their renal actions.

Loop diuretics in the treatment of hypertension

Even though loop diuretics are not effective as monotherapy in the prolonged management of hypertension, they are useful in the treatment of hypertensive crises, resistant hypertension and hypertension complicated by advanced chronic renal failure.

In hypertensive crises, the intravenous administration of furosemide (40-120 mg as a bolus or up to 400 mg during a 24-hour continuous infusion) contributes to a rapid fall in BP.[6] The mechanism whereby this occurs is still controversial; vasodilation and the natriuretic action of loop diuretics are possibly involved. The rapid intravenous administration of loop diuretics is ototoxic and sometimes gives rise to irreversible deafness.[6] Consequently, intravenous doses of these diuretics should not exceed 120 mg as a bolus or 400 mg/d.

Resistant hypertension is present when it is impossible to control BP by the coadministration of a diuretic, a ß-adrenergic blocker, a vasodilator and a sympathoplegic drug such as guanethidine.[8] In these circum-

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stances the addition of a loop diuretic sometimes achieves BP control.[6,9-11] This effect of loop diuretics may result from the significant natriuresis induced by these substances, since retention of water and sodium is provoked by antihypertensive drugs such as ß-adrenergic blockers, vasodilators, α-adrenergic blockers, reserpine, clonidine, debrisoquine and guanethidine in many patients with resistant hypertension.[8]

In patients with hypertension and significant chronic renal insufficiency (serum creatinine levels above 220 μmol/l) loop diuretics should be prescribed because retention of sodium and water usually contributes to the maintenance of elevated pressure in these patients; furthermore, they are preferable to antihypertensive diuretics which may decrease renal blood flow and glomerular filtration to a clinically significant degree.[12]

Intensity of antihypertensive effect of diuretics

The intensity of the antihypertensive effect of diuretics should be evaluated only when a maximal effect on basal supine diastolic BP has been attained. This happens after about 7-16 weeks of continuous monotherapy with any given antihypertensive diuretic and consequently the introduction of an additional drug to the therapeutic regimen should not be considered before the completion of 16 weeks of treatment.

Diuretics reduce both supine and erect arterial pressures at rest, although erect diastolic BP is less responsive to treatment than the other variables. Antihypertensive diuretics also reduce the BP increases associated with isometric and isotonic exercise, but do not affect the rate at which these changes occur. Diuretics control BP without altering its reflex regulation as is revealed by the lack of effect which diuretics have on changes in arterial BP induced by the Valsalva manoeuvre (A. J. Reyes, unpublished observations).

Antihypertensive diuretics reduce BP for 24 hours when they are given as a single daily dose,[13] and this improves patient compliance with treatment.[14]

Time course of the antihypertensive effect

No experimental data recording the hourly level of BP immediately after onset of treatment with a diuretic are available; and only a few studies describe daily changes in BP. Most published evaluations have been carried out 1 week after the initiation of treatment and at fortnightly or monthly intervals thereafter. In current experimental designs there is a tendency to decrease the frequency of clinical evaluations as treatment progresses, since further mean decreases in BP are unlikely to occur after the 4th month of treatment.

The description which follows refers to the mean changes in BP of various groups of patients treated with a diuretic as monotherapy.

Usually, BP decreases from the 1st or 2nd day of treatment. During the 1st week there is a reduction in pressure which generally has statistical significance and has a clinical magnitude which depends on the drug administered.

During the 7-16 weeks that follow after completion of the 1st week of treatment there is a further fall in BP. This period is succeeded by one of stability which lasts while treatment is maintained.[15]

Some diuretics (power diuretics) cause a clinically significant fall in BP during the 1st week of treatment and a rapid reduction thereafter.

Diuretics of another group (linear diuretics) cause a less marked reduction of pressure than that induced by power diuretics during the 1st week of treatment; thereafter BP falls gradually in a linear manner.

Indapamide, xipamide and a combination of hydrochlorothiazide with amiloride are power diuretics. Cyclothiazide, hydrochlorothiazide and tizolemide are linear diuretics. Other diuretics have not yet been classified (Table I).

Fig. 1 depicts the main patterns of BP decrease induced by power and linear diuretics and shows the parameters involved in defining the function graphically. Fig. 2 shows an actual example of the action of power diuretics. The fact the power model applies to long-term treatment is exemplified in Fig. 3, where the results of a 2-year treatment study with xipamide are shown.

TABLE I. CLASSIFICATION OF DIURETICS IN ACCORDANCE WITH THE CHARACTERISTICS OF THEIR ANTIHYPERTENSIVE EFFECT WHEN USED MONOTHERAPEUTICALLY IN ESSENTIAL HYPERTENSION

- **Antihypertensive diuretics**
  - **Power diuretics**
    - **Indapamide**[16]
    - **Xipamide**[17-19]
    - **Combination of hydrochlorothiazide + amiloride**[20]
  - **Linear diuretics**
    - **Hydrochlorothiazide**[21]
    - **Cyclothiazide**[22]
    - **Tizolemide**[23]
  - **Unclassified**
    - **Bendroflumethiazide**
    - **Benzthiazide**
    - **Buthiazide**
    - **Chlorothiazide**
    - **Chlorthalidone**
    - **Clopamide**
    - **Clorexolone**
    - **Cyclopenthiazide**
    - **Diasulphamide**
    - **Epithiazide**
    - **Ethiazide**
    - **Hydroflumethiazide**
    - **Indacrinone**
    - **Mebutizide**
    - **Mefruside**
    - **Methyclothiazide**
    - **Metolazone**
    - **Polythiazide**
    - **Quinethazone**
    - **Tienilic acid**
    - **Trichlomethiazide**
  - **Non-antihypertensive diuretics**
    - **Bumetanide**
    - **Ethacrynic acid**
    - **Furosemide**
    - **Piretanide**

The rate at which BP falls after the 1st week of treatment with a power diuretic is graphically represented in Fig. 4.

An actual example of the effect of linear diuretics is given in Fig. 5. The rate of fall in BP during treatment with linear diuretics is constant. The eventual stabilization level of BP cannot be differentiated from that which is obtained when the diuretic employed is a power substance.

Evolution of blood pressure after withdrawal of therapy

Many antihypertensive substances such as clonidine, ß-adrenergic

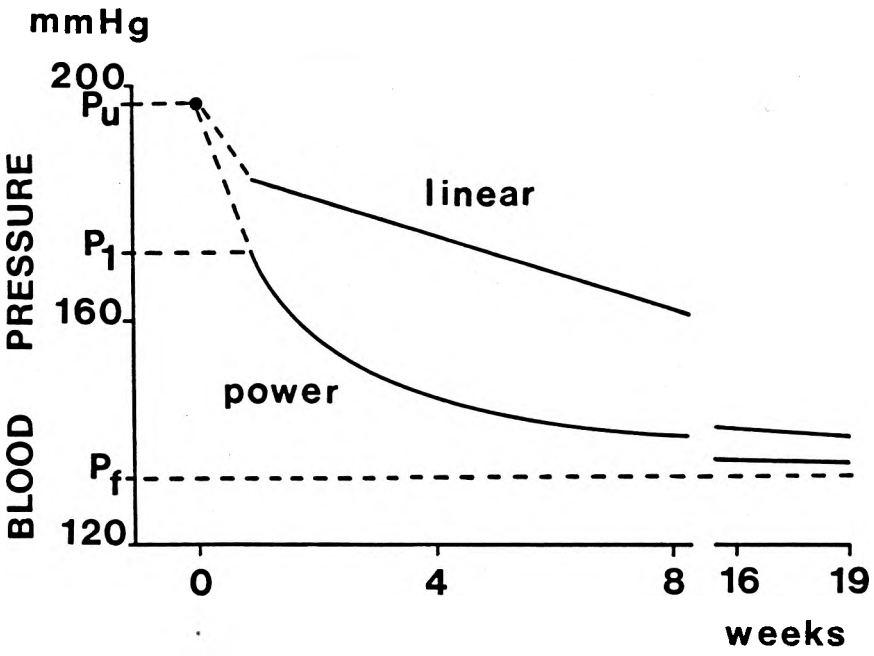

Fig. 1. Schematic representation of the evolution of elevated blood pressure during monotherapy with a power or linear diuretic (P_u = arterial pressure before treatment; P_1 = arterial pressure at end of 1st week of treatment with a power diuretic; P_f = limit value to which arterial pressure tends when treatment with a power or linear diuretic is prolonged indefinitely).

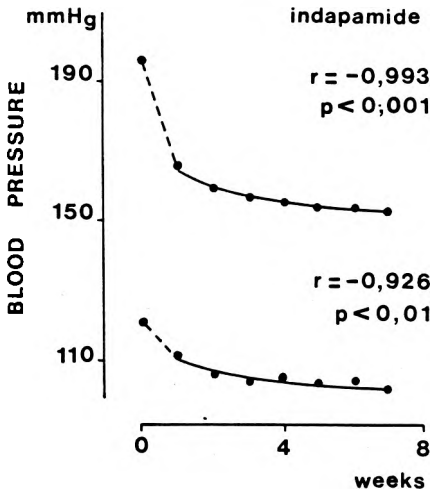

Fig. 2. Evolution of mean supine arterial pressure of 14 patients with essential hypertension. All cases were treated with indapamide 2,5 mg/d as monotherapy. A power function has been fitted to the experimental results. (Adapted from Reyes *et al.*[16])

blockers, reserpine and hydralazine cause a rebound in BP in some patients after sudden withdrawal of therapy. BP values then rise above pretreatment levels. This withdrawal syndrome has not been described for diuretics.

When prolonged treatment with a diuretic is discontinued, a gradual return of BP to pretreatment values occurs over several days.[16–26] This pattern is of value since hypertensives are inclined to discontinue their medication sporadically. In some patients treated with a diuretic for very prolonged periods, a remission of hypertension which may last for several months occurs if treatment is stopped.[1,27]

Antihypertensive action of diuretics in different racial groups

Antihypertensive diuretics are of equal efficacy in the different racial groups,[6] whereas ß-blockers are less effective in Blacks.

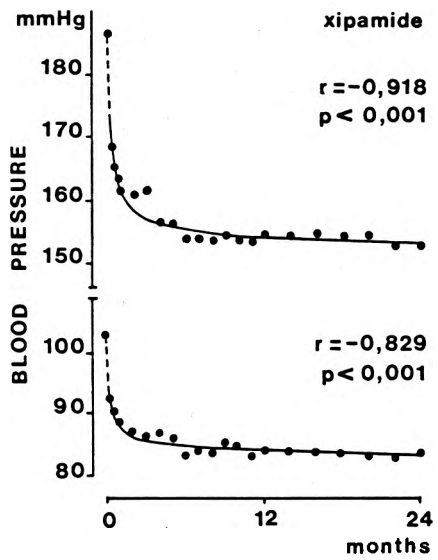

Fig. 3. Evolution of mean supine arterial pressure of 25 patients with essential hypertension treated with xipamide as monotherapy. In data for the 8th, 11th and 12th months 24 subjects are included, 23 subjects in the 18th and 20th months, 22 in the 22nd and 24th months and 20 in the 14th and 16th months. Xipamide 20 mg/d was given during the first 4 weeks and thereafter 10 mg in 2 patients, 20 mg in 16, 30 mg in 4 and 40 mg/d in 3 patients. A power function has been fitted to the experimental data. (Adapted from Castro and Reyes,[18] by courtesy of *Sistole*.)

Antihypertensive dose of diuretics

The dose-response curves for diuretic and antihypertensive actions of diuretics are not parallel. The maximal antihypertensive effect is

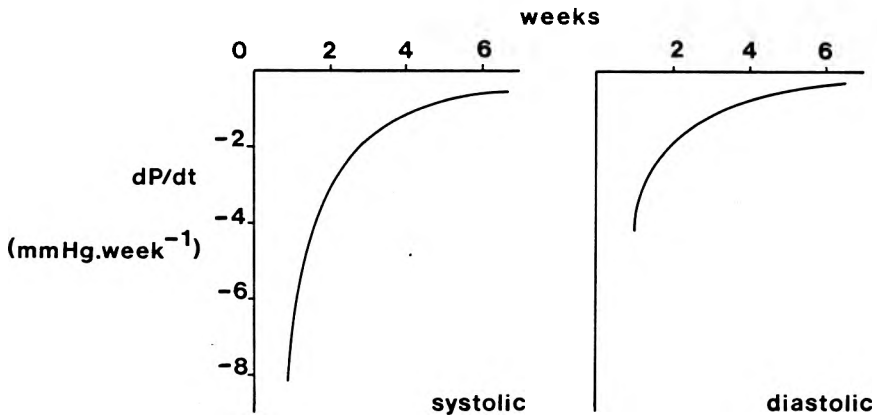

Fig. 4. Evolution of the rate at which arterial blood pressure changes (as a function of time) dP/dt, in response to monotherapy with indapamide. The curve has been derived from the functions represented in Fig. 2.

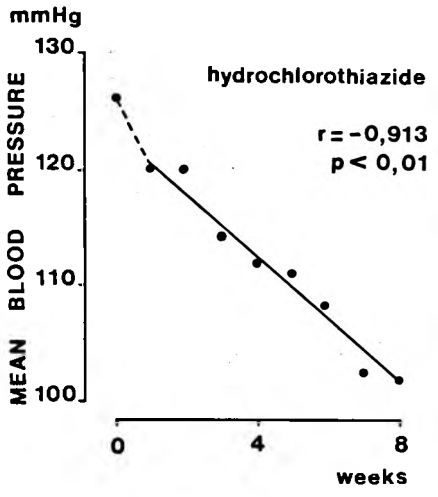

Fig. 5. Evolution of mean supine arterial pressure of 15 patients with essential hypertension. All cases were treated with hydrochlorothiazide alone; in all cases the dosage was 25 mg/d during the first 4 weeks and in 8 patients during the last 4 weeks. A dosage of 50 mg/d was given to 7 patients during the last 4 weeks. The arterial pressure as a function of time was fitted by a linear function. (Adapted from Leary *et al.*,[21] by courtesy of *Current Therapeutic Research.*)

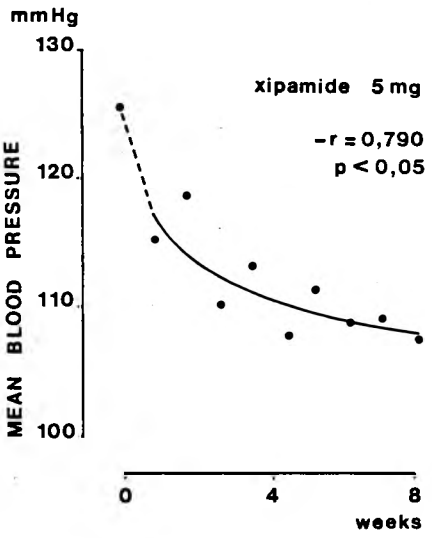

Fig. 6. Evolution of the mean supine arterial pressure of 23 patients with essential hypertension (data from 22 patients at the 3rd, 6th and 7th weeks, and 21 at the 8th and 9th weeks). All cases were treated with xipamide 5 mg/d as a monotherapy. The arterial pressure as a function of time was fitted by a power function. (Adapted from Leary and Reyes.[19])

exerted at a dose which is much lower than the standard diuretic dose (i.e., one which provokes a 24-hour urinary sodium excretion similar to that caused by 50 mg of hydrochlorothiazide). An added antihypertensive effect is not obtained when daily doses of hydrochlorothiazide, chlorthalidone, xipamide or indapamide exceed 12,5 , 25, 10 or 2,5 mg respectively.[19,28-33] Furthermore, xipamide (Fig. 6) and indapamide both maintain their power antihypertensive effect at doses well below their standard diuretic dose. When these diuretics are given at doses below the maximal antihypertensive dose, they exhibit a dose-related antihypertensive effect, an example being chlorthalidone 12,5 mg/d.[31] When power diuretics are administered at doses within their power antihypertensive dosage range (e.g. xipamide 5-40 mg/d), the rate at which BP falls in response to treatment decreases with the dose (A. J. Reyes and W. P. Leary, unpublished observations). For many years hypertensive patients have been overdosed with diuretics because the commercially available formulations generally contain a standard diuretic dose per tablet.

The dose that has a maximal antihypertensive effect should not be exceeded when treating uncomplicated hypertension, because larger doses usually provoke significant urinary excretion of potassium, magnesium and zinc and alterations in lipid, carbohydrate and urate metabolism.

Selection of an antihypertensive diuretic

The difference between BP values during treatment with a power and a linear diuretic depicted in Fig. 1 appears irrelevant when applied to individual cases, especially since the level of BP finally attained is practically the same for both types of drug. It is, however, more rational to prescribe a power diuretic in all cases of hypertension uncomplicated by reduced peripheral blood flows, and to reserve linear diuretics for patients with clinical impairment of blood flow in the cerebrovascular, renal, coronary or peripheral vasculature. In the latter situation, it would be appropriate to decrease BP smoothly so as to allow organ adaptation to the resulting diminution in perfusion pressure. These criteria also apply to geriatric patients, in whom power diuretics may be used safely provided the limitations already discussed do not exist.

Monotherapy with diuretics decreases the incidence of cardiac insufficiency, cerebrovascular disease, obstructive arterial disease of the limbs and renal insufficiency to the rates which occur in patients with normal BP. However, monotherapy with antihypertensive diuretics does not reduce the high incidence of sudden death and myocardial infarction in hypertensive patients. It is possible that this lack of effect has to do with the fact that chronic administration of these drugs in standard doses of diuretics, which is unfortunately common in practice, causes significant increases in urinary potassium[34,35] and magnesium[36,37] excretion, eventually leading to somatic depletion of these cations. Magnesium deficiency *per se* might explain the development of fatal cardiac arrhythmias during diuretic treatment,[36-39] with potassium deficiency also contributing towards this effect. Magnesium deficiency could also explain the higher incidence of myocardial infarction in hypertensive patients treated with diuretics in standard diuretic doses.[40]

Chlorthalidone 100 mg, hydrochlorothiazide 50 mg, xipamide 5, 10 or 20 mg have been shown to be hypermagnesiuretic when administered acutely to normal volunteers[38,39] (A. J. Reyes and W. P. Leary, unpublished observations). Only two antihypertensive diuretic formulations, indapamide 2,5 mg[41] and the combination of hydrochlorothiazide 50 mg and amiloride 5 mg (W. P. Leary *et al.*, unpublished observations) have so far been found not to induce hypermagnesiuria when administered as monodoses to healthy individuals. Indapamide 2,5 mg acts in this manner because the dose is below its standard diuretic dose, whereas the hydrochlorothiazide-amiloride combination is not acutely hypermagnesiuretic because amiloride counteracts the effects of hydrochlorothiazide to some extent.

Indapamide 2,5 mg stimulates hyperkaliuresis when given in monodoses to healthy volunteers. Nevertheless, the fact that this formulation apparently does not provoke hypermagnesiuria could explain why treat-

ment with indapamide does not induce cardiac arrhythmias, as found in a telemetric study of cardiac rhythm.[42] The combination of hydrochlorothiazide and amiloride leaves urinary excretions of potassium and magnesium unchanged when administered acutely, although a recent study indicates that magnesium depletion may follow prolonged therapy with this formulation (W. P. Leary *et al.*, unpublished observations).

Diuretics usually affect the metabolism of lipids, carbohydrates and urates, factors which increase the risk of cardiovascular diseases.[43,44] Indapamide 2,5 mg/d is an exception, presumably because this dose does not exert a standard diuretic effect.

With the development of new antihypertensive diuretics it may be expected that substances will be found which do not influence cardiovascular risk factors unfavourably.

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PAPER F11

Diuretic-Induced Magnesium Losses

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Diuretic-Induced Magnesium Losses

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Summary

Long term administration of common loop or distal tubular diuretics may cause somatic magnesium depletion. The resultant deficiency of Mg^{++} destabilises the myocardium electrically and is a principal cause of cardiac arrhythmias ascribed to diuretics. The adverse effects of diuretics caused by Mg^{++} depletion can be avoided by selecting diuretics that do not cause magnesium deficiency, minimising the diuretic dose, supplementation of Mg^{++} intake, or the concomitant use of a K^+-retaining, Mg^{++}-sparing diuretic.

Modern diuretics are divisible into 3 main groups. *Loop diuretics*, including frusemide (furosemide), piretanide and muzolimine, act mainly at a common site in the thick ascending portion of Henle's loop and are remarkable for the speed with which they change urinary flows of fluid and solutes. *Distal tubular diuretics* such as the thiazides, chlorthalidone, indapamide and xipamide act in the proximal part of the distal convoluted tubule, inducing relatively smooth changes in urinary flow. A third group is comprised of the *K^+-sparing diuretics* spironolactone, triamterene and amiloride which inhibit normal transparietal interchange between Na^+, and K^+ and H^+ in the final portion of the distal convoluted tubule (Reyes and Leary, 1980).

Commonly used diuretics are those which act principally at the loop of Henle or in the early distal convoluted tubule, and their standard oral diuretic dose is that which provokes a natriuresis equivalent to that induced by 40mg frusemide (loop diuretics) or 50mg hydrochlorothiazide (distal tubular diuretics) given to healthy volunteers under controlled experimental conditions.

Prolonged administration of common diuretics at standard doses has been implicated in the genesis of various cardiac arrhythmias, sudden death and increases in the incidences of atherogenic alterations in lipid metabolism, atheroma, coronary and cerebral vascular accidents, and arrhythmias complicating myocardial infarction (Reyes and Leary, 1980). Potassium deficiency caused by diuretics is usually incriminated as the major pathogenic factor in cardiac arrhythmias provoked by these drugs (Morgan et al., 1980) but recently it has been increasingly recognised that Mg^{++} deficiency secondary to hypermagnesiuria is a principal determinant of diuretic-induced cardiac arrhythmias (Dyckner and Wester, 1979, 1981, 1982; Reyes and Leary 1983b; Sheehan and White, 1982). Clinical experience indicates that cardiac arrhythmias secondary to diuretic administration are suppressed by Mg^{++} supplementation irrespective of somatic K^+ status (Dyckner and Wester, 1979, 1981, 1982; Sheehan and White, 1982).

1. Hypermagnesiuria and Magnesium Deficiency Provoked by Diuretics

At normal plasma concentrations, approxi-

mately 6 mmol Mg^{++} is excreted in the urine daily. The main site of Mg^{++} reabsorption in the kidney is the thick ascending limb of the loop of Henle, possibly driven to some extent by active chloride reabsorption. Other factors influencing Mg^{++} reabsorption, including magnesium deficiency, hypercalcaemia, parathyroid hormone, and loop diuretics, also act at the same site (Mountokalakis, 1983).

Common diuretics may cause significant increases in urinary magnesium losses, depending on the type of diuretic, the dose administered and the duration of treatment.

1.1 Single-Dose Studies

The effects of single standard doses of common diuretics on 24-hour urinary Mg^{++} output have been studied under controlled conditions in healthy, biologically equivalent, volunteers. Chlorthalidone, frusemide, hydrochlorothiazide and xipamide induced significant hypermagnesiuria, whereas the loop diuretic muzolimine 30mg, the distal convoluted tubular diuretic indapamide 2.5mg, and the K^+-sparing diuretic amiloride 5mg or 10mg did not affect urinary Mg^{++} output significantly (fig. 1). The combination of hydrochlorothiazide 50mg and amiloride 5mg also had no effect on 24-hour magnesiuria. All the drugs tested increased 24-hour urinary Na^+ output significantly and chlorthalidone, hydrochlorothiazide, indapamide, muzolimine and clorexolone also increased 24-hour urinary K^+ output significantly (Leary and Reyes, 1982; Leary et al., 1983; Reyes and Leary, 1982a,b; Reyes et al., 1983).

1.2 Long Term Administration

No data exist on the effects of long term administration of diuretics on total body stores of Mg^{++}. In a study in which plasma Mg^{++} was measured before and during treatment with the loop diuretic piretanide 12 mg/day in 9 patients, the plasma

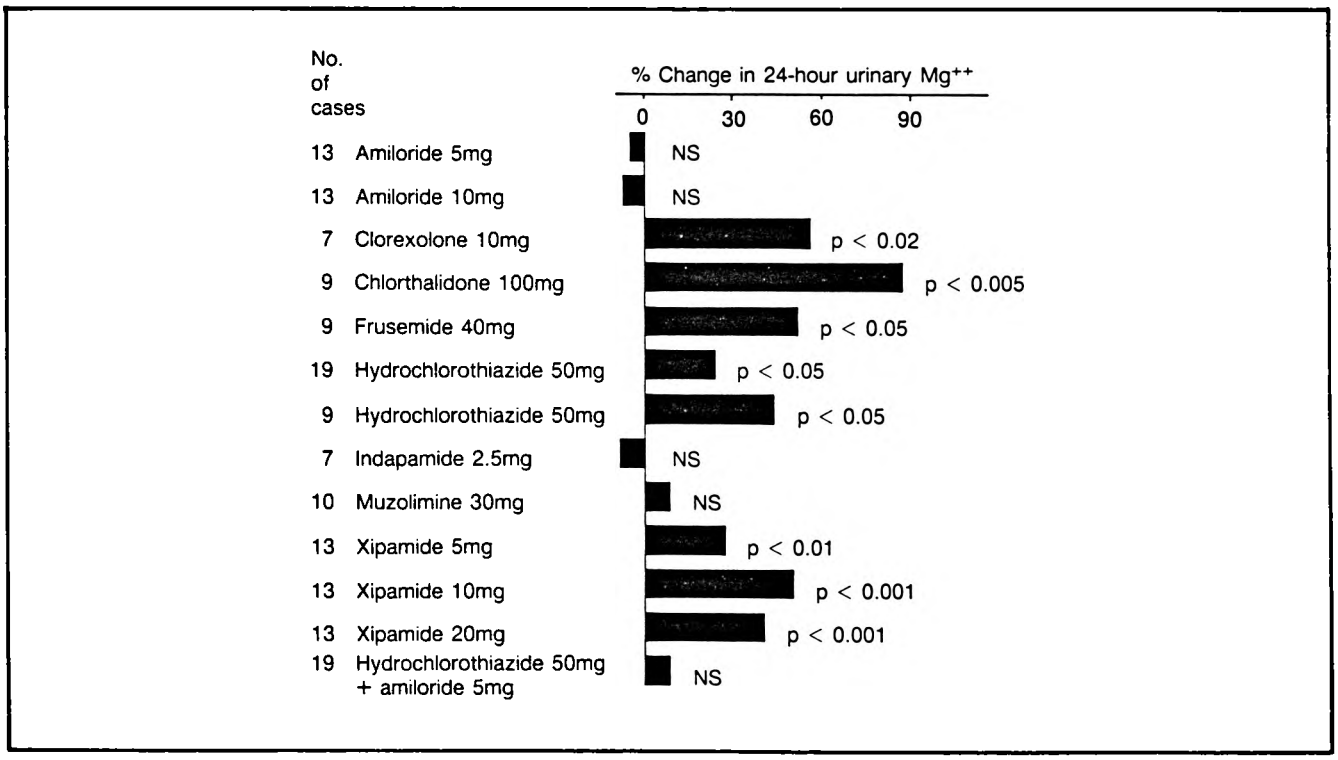

Fig. 1. Percentage changes in 24-hour urinary output of Mg^{++} in response to various diuretics given to healthy volunteers. No significant increases were noted after amiloride 5 or 10mg, indapamide 2.5mg, muzolimine 30mg, or a combination of hydrochlorothiazide 50mg and amiloride 5mg [authors' data].

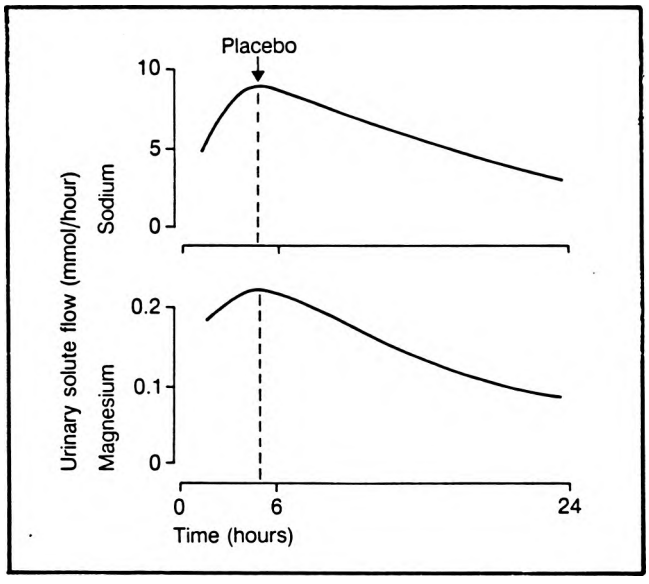

Fig. 2. Mean urinary Na^+ and Mg^{++} flows after the oral administration of placebo to 9 healthy volunteers at the start of the experiment (8 a.m.). The time courses of natriuresis and magnesiuresis are almost parallel; their peaks coincide in time [adapted with permission from Reyes and Leary, 1982a].

concentration was found to be significantly decreased after 12 weeks of therapy (Leary and Reyes, 1981).

2. Mechanism of Magnesium Deficiency Provoked by Diuretics

Under normal circumstances, urinary Mg^{++} output ranges from 4 to 8 mmol/day (100 to 400 mg/day). Most filtered Mg^{++} is reabsorbed in the nephron, 20 to 30% at the proximal convoluted tubule, 50 to 60% at the thick ascending portion of the loop of Henle, and 1 to 5% at the distal convoluted tubule (Quamme, 1981; Quamme and Dirks, 1980). Parathyroid hormone promotes Mg^{++} reabsorption at the loop of Henle, and perhaps at the distal convoluted tubule (Massry, 1981; Quamme and Dirks, 1980).

2.1 Distal Convoluted Tubular Diuretics

Hypermagnesiuria provoked by diuretics acting at the distal convoluted tubule cannot be explained solely by direct blockade of transparietal reabsorption of the cation since only 1 to 5% of filtered Mg^{++} is reabsorbed in the distal tubule. In normal volunteers the mean Cl^-, Na^+, fluid and Mg^{++} urinary flow curves indicate that the time courses of all these urinary excretions are almost parallel after the administration of a placebo (fig. 2). Significant dephasing was found between the time courses of urinary Mg^{++} and urinary Cl^-, Na^+ and fluid after the administration of diuretics such as hydrochlorothiazide (fig. 3) and chlorthalidone (Leary and Reyes, 1982; Reyes and Leary, 1982a).

Long term administration of these diuretics causes hypocalciuria (Bloch et al., 1981), followed by hypercalcaemia, which increases magnesiuria directly and reduces plasma parathyroid hormone secretion (Quamme, 1982). The decrease in parathyroid hormone diminishes the reabsorption of Mg^{++} in the loop of Henle, thus further increasing magnesiuria. The subsequent mechanisms whereby distal convoluted tubular diuretics induce hypermagnesiuria are similar to those associated with loop diuretics.

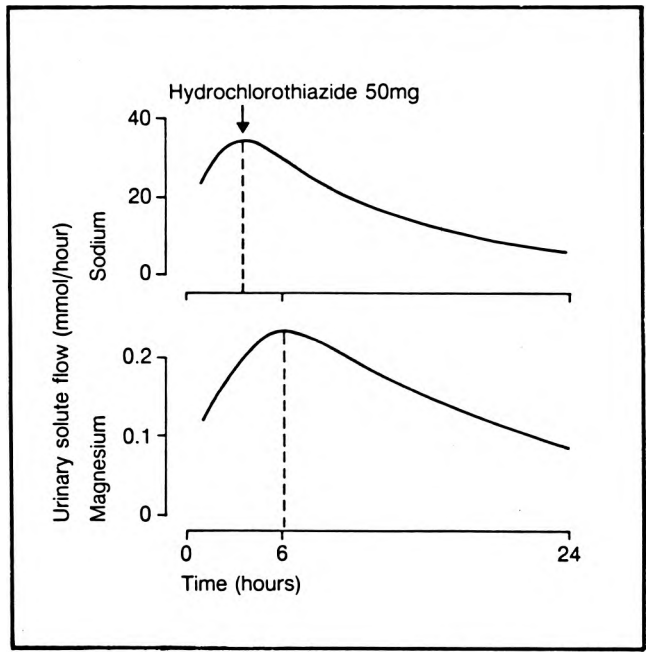

Fig. 3. Mean urinary Na^+ and Mg^{++} flows after the oral administration of 50mg hydrochlorothiazide to 9 healthy volunteers at the start of the experiment (8 a.m.). The time course of magnesiuresis is delayed with respect to that of natriuresis [adapted with permission from Leary and Reyes, 1982b].

2.2 Loop Diuretics

Loop diuretics block transparietal reabsorption of Mg^{++} in the thick ascending portion of the loop of Henle (Quamme, 1981; Quamme and Dirks, 1980). When healthy subjects are given loop diuretics, urinary Mg^{++} flow is delayed with respect to fluid, Cl^- and Na^+ flows which are linked by events occurring coincidentally in the loop of Henle. This suggests that the hypermagnesiuria induced by loop diuretics is partly due to mechanisms other than the direct blockade of Mg^{++} reabsorption at that site (Reyes and Leary, 1983a).

Loop diuretics provoke hypercalciuria (Reyes and Leary, 1980) which decreases calcaemia and thus increases plasma parathyroid hormone (Elmgreen et al., 1980). Parathyroid hormone mobilises Ca^{++} and Mg^{++} from bone, increasing the amount of Mg^{++} filtered at the glomerulus and perpetuating hypermagnesiuria. Magnesium ion deficiency ensues eventually, unless its exogenous supply is increased. This deficiency is manifested by hypomagnesaemia only 8 to 15 weeks after the initiation of diuretic treatment (Leary and Reyes, 1981), presumably because it is initially masked by Mg^{++} mobilisation from bone to plasma.

Magnesium deficiency decreases the release of parathyroid hormone to some extent (Mahaffee et al., 1982) and also causes resistance to the action of parathyroid hormone in bone and in the kidney (Fischer and Fischer, 1981). Both these factors tend to increase hypermagnesiuria. Moreover, frusemide has been found to directly diminish renal sensitivity to parathyroid hormone (Turlapaty and Altura, 1982). The diminution in plasma levels and bone resistance to parathyroid hormone limit Mg^{++} and Ca^{++} mobilisation from the main store, thus reinforcing hypocalcaemia, which diminishes magnesiuria directly, i.e. independently from parathyroid hormone (Quamme 1982; Quamme and Dirks, 1980); this compensating mechanism, however, is of limited clinical importance.

It is of interest that the loop diuretic muzolimine 30mg and the distal tubular diuretic indapamide 2.5mg do not increase 24-hour urinary Mg^{++} output significantly, but both delay urinary Mg^{++} flow with respect to Na^+ in the same manner as frusemide 40mg or hydrochlorothiazide 50mg. This suggests that these diuretics, at their standard doses, stimulate mechanisms that account for diuretic-induced hypermagnesiuria. The possibility that prolonged therapy with these formulations could provoke significant Mg^{++} depletion requires investigation. Higher doses of these diuretics may be expected to induce hypermagnesiuria acutely.

2.3 Potassium-Sparing Diuretics

The K^+-sparing diuretic amiloride reduced urinary Mg^{++} excretion, although this reduction was not statistically significant, and did not alter the time course of its urinary flow in normal individuals (Leary et al., 1983). When a combination of amiloride 5mg and hydrochlorothiazide 50mg was administered to healthy volunteers there was also no significant change in Mg^{++} output (Leary et al., 1984). This suggests that amiloride induces Mg^{++}-sparing when the amount of the cation passing through the distal convoluted tubule is increased by the action of common diuretics at more proximal nephron sites, at least under acute experimental conditions (Leary and Reyes, 1982; Leary et al., 1984).

3. Prophylaxis of Magnesium Deficiency

When the comparative effects of hydrochlorothiazide 50mg, amiloride 5mg or 10mg, and a combination of hydrochlorothiazide 50mg and amiloride 5mg are considered (Leary et al., 1983; Leary et al., 1984), it is clear that excretions of Na^+, Cl^- or fluid are costly in terms of Mg^{++} and K^+ excretion, when hydrochlorothiazide is administered alone. A striking change is effected by the co-administration of amiloride and offers one approach to the prophylaxis of diuretic-induced magnesium deficiency. However, no final conclusions will be reached until the effects of long term administration are assessed. The responses to other K^+-sparing diuretics remain to be evaluated. Other measures which merit consideration include minimising the diuretic dose, avoidance of diuretic

formulations which cause marked Mg^{++} excretion such as chlorthalidone 100mg, and the supplementation of dietary magnesium.

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Résumé

L'utilisation prolongée des diurétiques habituels de l'anse ou du tube distal peut entraîner une hypomagnésémie. Le déficit en Mg^{++} déstabilise électriquement le myocarde et se trouve être ainsi une cause majeure d'arythmies cardiaques dues aux diurétiques. Les effets indésirables liés au déficit magnésien lors d'un traitement diurétique pourraient être évités par l'utilisation préférentielle de médicaments qui épargnent le magnésium, par

le choix d'une dose minimum, par un complément d'apport en magnésium ou par l'utilisation d'un diurétique qui simultanément retient le potassium et épargne le magnésium.

Zusammenfassung

Die Langzeitverabreichung von Schleifendiuretika oder solchen, die am distalen Tubulus angreifen, kann eine Magnesiumverarmung des Organismus verursachen. Der resultierende Mangel an Mg^{++} destabilisiert das Myokard elektrisch und ist eine Hauptursache für die den Diuretika zugeschriebenen Herzarrhythmien. Die durch Mg^{++}-Mangel verursachten Nebenwirkungen der Diuretika können durch Wahl von Diuretika, die keinen Magnesiummangel verursachen, durch Minimierung der Diuretikadosis, zusätzliche Mg^{++}-Aufnahme oder durch die gleichzeitige Verwendung von K^{+}-erhaltenen und Mg^{++}-sparenden Diuretika vermieden werden.

Sommario

La somministrazione a lungo termine di diuretici dell'ansa o del tubulo distale di uso comune può determinare una deplezione corporea di magnesio. La risultante deficienza di Mg^{++} destabilizza elettricamente il miocardio ed è una delle cause principali delle aritmie cardiache imputate ai diuretici. Gli effetti negativi dei diuretici, dovuti ad una deplezione di Mg^{++}, possono essere evitati scegliendo diuretici che non determinano una carenza di magnesio, utilizzando dosi minime del diuretico, aumentando l'assunzione di Mg^{++} o usando contemporaneamente un diuretico risparmiatore di K^{+} e Mg^{++}.

Resumen

La administración prolongada de diuréticos habituales del asa o del túbulo distal puede causar depleción somática de magnesio. El déficit de Mg^{++} resultante desestabiliza eléctricamente el miocardio y es una de las principales causas de arritmias adscritas a los diuréticos. Los efectos nocivos de los diuréticos debidos a la depleción de Mg^{++} pueden evitarse eligiendo diuréticos que no causan déficit de magnesio, minimizando las dosis, suplementando la ingestion de Mg^{++} o mediante el uso simultáneo de un diurético ahorrador de potasio y magnesio.

Resumo

A administração a longo prazo dos diuréticos tubulares pode causar depleção somática de magnésio. A deficiência de Mg^{++} resultante desestabiliza eletricamente o miocárdio, sendo uma das principais causas das arritmias cardíacas atribuídas aos diuréticos. Os efeitos adversos dos diuréticos causados pela depleção de Mg^{++} podem ser evitados em se selecionando diuréticos que não causem deficiência de magnésio, em se minimizando a dose de diurético, com suplementação de magnésio ou com o uso concomitante de um diurético que retenha K^{+} e poupe Mg^{++}.

PAPER F12

Cardiovascular Toxicity of Diuretics Related to Magnesium Depletion

This review was shared by the authors. It is an expanded and more detailed version of the previous publications and repeats many of the statements made in other publications included in this section.

Human Toxicol. (1984), **3**, 351–372

Cardiovascular Toxicity of Diuretics Related to Magnesium Depletion

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The chronic administration of common loop or distal tubular diuretics may lead to somatic depletion of Mg^{2+}. The resultant deficiency of this cation causes an increase in intramyocardial cytosolic Ca^{2+} and aggravates the decrease in intramyocardial K^+ provoked by diuretics through their hyperkaliuretic effect. Thus the myocardium is electrically destabilized and cardiac arrhythmias may develop. Mg^{2+} deficiency positively contributes to the development of atherogenic alterations in lipid metabolism, vasospastic phenomena in the coronary and cerebrovascular territories, myocardial infarction and to the retardation of infarct healing and the occurrence of ventricular arrhythmias during the acute phase of infarction.

Introduction

Modern diuretics may be divided into three categories. Loop diuretics, including the frusemide, bumetanide, muzolimine and piretanide, act within the kidney mainly at a common acceptor in the thick ascending portion of the loop of Henle (Reyes & Leary, 1980; Reyes, 1981). The principal sites of renal action for distal tubular diuretics, including thiazides, chlorthalidone, clorexolone, indapamide and xipamide, are at specific acceptors for each substance, in the first portion of the distal convoluted tubules (Reyes & Leary, 1980; Reyes, 1981). The K^+-retaining diuretics spironolactone, amiloride and triamterene inhibit normal transparietal interchange between Na^+, which is reabsorbed, and K^+ and H^+, which are excreted, in the final portion of the distal convoluted tubule (Reyes & Leary, 1980; Reyes, 1981).

Common diuretics are defined as those which act principally at the loop of Henle or at the distal convoluted tubule. Standard diuretic doses of diuretics are those provoking a natriuresis equivalent to that induced by 40 mg of frusemide (loop diuretics) or to 50 mg of hydrochlorothiazide (distal tubular diuretics), when these drugs are given orally to healthy volunteer subjects under controlled experimental conditions (Reyes, 1981).

The chronic administration of common diuretics at standard diuretic doses may give rise to diverse cardiac arrhythmias, including ventricular fibrillation, or sudden death, and increase the incidences of atherogenic alterations in lipid metabolism, atheroma, vasospastic phenomena in the coronary and

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cerebrovascular territories, myocardial infarction and arrhythmias complicating acute myocardial infarction (Reyes & Leary, 1980; Reyes, 1981).

Classically, K^+ deficiency caused by diuretics has been incriminated as the principal pathogenic factor in arrhythmias provoked by these drugs (Morgan *et al.*, 1980; Nordrehaug, 1981; Olsson, 1981). However, K^+ deficiency is only one of several contributing factors, whereas the principal determinant of diuretic-induced cardiac arrhythmias is Mg^{2+} deficiency, which is secondary to the hypermagnesiuria induced by common diuretics (Dyckner & Wester, 1979,1981a,b,1982a; Rotman, 1981; Whang *et al.*, 1981; Sheehan & White, 1982; Wester & Dyckner, 1981,1982; Reyes, 1983; Reyes & Leary, 1983a,b,c). In all cases of cardiac arrhythmias unequivocally due to common diuretics, in which appropriate laboratory analyses were carried out, Mg^{2+} deficiency was identified as the causative factor and the arrhythmia was suppressed by Mg^{2+} supplementation, irrespective of overall somatic K^+ status (Dyckner & Wester, 1979,1981a,1982b; Sheehan & White, 1982).

The well-known fact that effective control of essential hypertension by monotherapy with distal tubular diuretics does not reduce the incidence of sudden death in these patients could possibly be explained by the deficiency of Mg^{2+} which these drugs cause (Reyes & Leary, 1983b).

Hypermagnesiuria and magnesium deficiency provoked by diuretics

The effects on 24-h urinary Mg^{2+} output of single standard doses of common diuretics were studied under controlled conditions in normal, biologically equivalent, volunteer subjects. Clorexolone, chlorthalidone, frusemide, hydrochlorothiazide and xipamide induced significant hypermagnesiuria, whereas the loop diuretic muzolimine (30 mg), the distal convoluted tubular diuretic indapamide (2.5 mg) and the K^+-retaining diuretic amiloride (5 or 10 mg) did not affect urinary Mg^{2+} output significantly (Figure 1). The combination of hydrochlorothiazide (50 mg) and amiloride (5 mg) also had no effect on 24-h magnesiuria (Figure 1). All the formulations tested increased 24-h urinary Na^+ output significantly and clorexolone, chlorthalidone, hydrochlorothiazide, indapamide, muzolimine and xipamide also increased 24-h urinary K^+ output significantly.

No data exist on the effects chronic administration of diuretics have on total body stores of Mg^{2+}. In a study in which plasma Mg^{2+} was measured before and during treatment with the loop diuretic piretanide (12 mg/day) in nine patients, the variable was found to be significantly decreased after 12 weeks of therapy (Leary & Reyes, 1981b) (Figure 2). Hydrochlorothiazide (50 mg) (9 patients) or a combination of hydrochlorothiazide (50 mg) and amiloride (5 mg) (12 patients) reduced plasma Mg^{2+} significantly in hypertensive patients given these diuretics as monotherapy after an average of 20 weeks (W.P. Leary, A.J. Reyes & K. van der Byl, unpublished work).

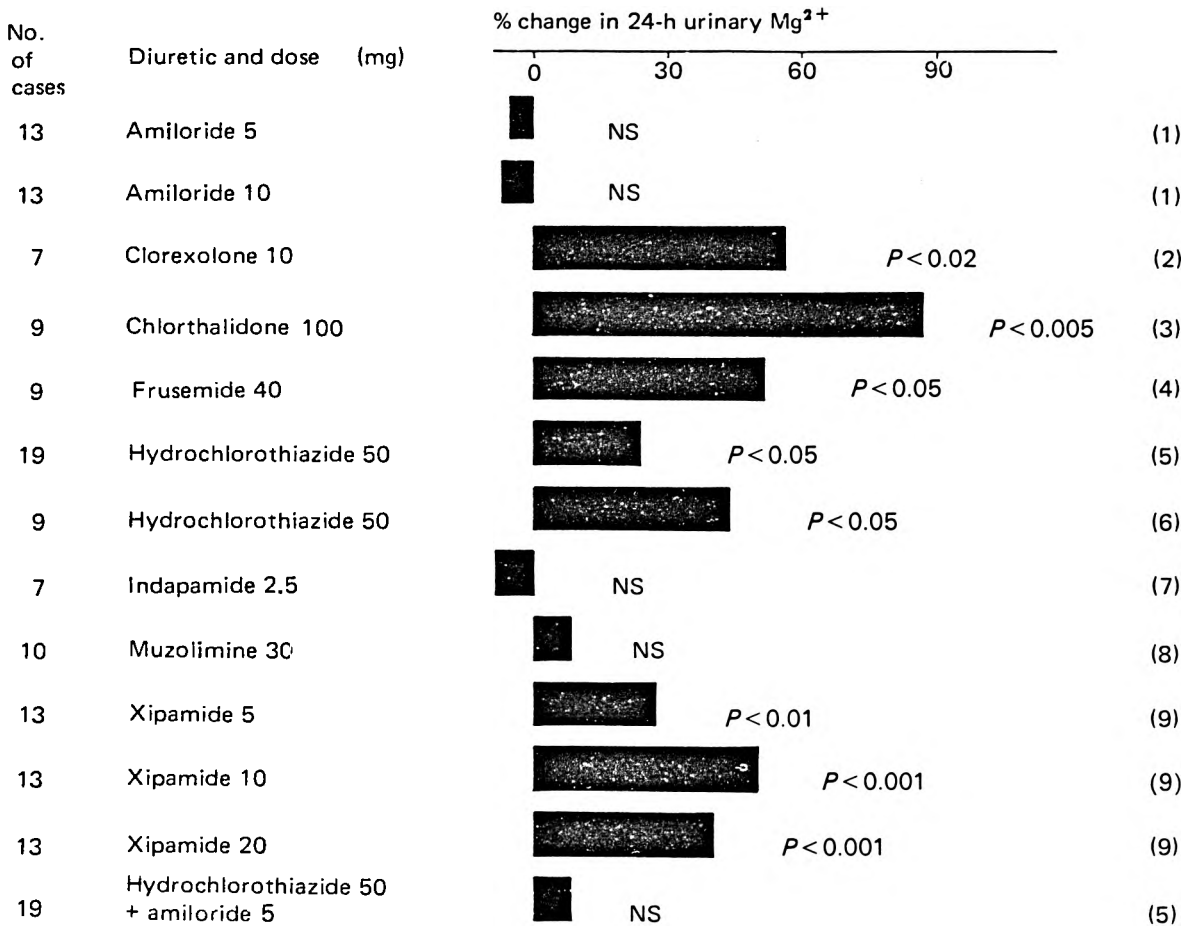

Figure 1 Summary of the results from several studies in which healthy volunteer subjects were given single doses of diuretic formulations. Bars depict percentage changes in 24-h urinary Mg^{2+} output after these diuretics with respect to control 24-h magnesiuria. NS = not significant. (1)Leary *et al.*, 1983a; (2)Leary & Reyes, 1982b; (3)Reyes & Leary, 1982a; (4)Reyes & Leary, 1982b; (5)Leary *et al.*, 1984; (6)Leary & Reyes, 1982c; (7)Reyes *et al.*, 1983a; (8)W.P. Leary, A.J. Reyes & K. van der Byl, unpublished work; (9)A.J. Reyes & W.P. Leary, unpublished work

Mechanism of magnesium deficiency provoked by diuretics

Under normal circumstances, urinary Mg^{2+} output ranges from 4 to 8 mmol/day (100–400 mg/day). Most filtered Mg^{2+} is reabsorbed in the nephron, 20–30% at the proximal convoluted tubule, 50–60% at the thick ascending portion of the loop of Henle and 1–5% at the distal convoluted tubule (Quamme & Dirks, 1980; Quamme, 1981). Parathormone (PTH) promotes Mg^{2+} reabsorption at the loop of Henle and, perhaps, at the distal convoluted tubule (Quamme & Dirks, 1980; Massry, 1981; Olhaberry *et al.*, 1983a). The kidney, which can be regarded as the principal regulatory organ of Mg^{2+} metabolism, handles this cation independently from Cl^-, Na^+ and K^+ (Quamme & Dirks,1980; Massry, 1981; Quamme, 1981,1982; Olhaberry *et al.*, 1983a).

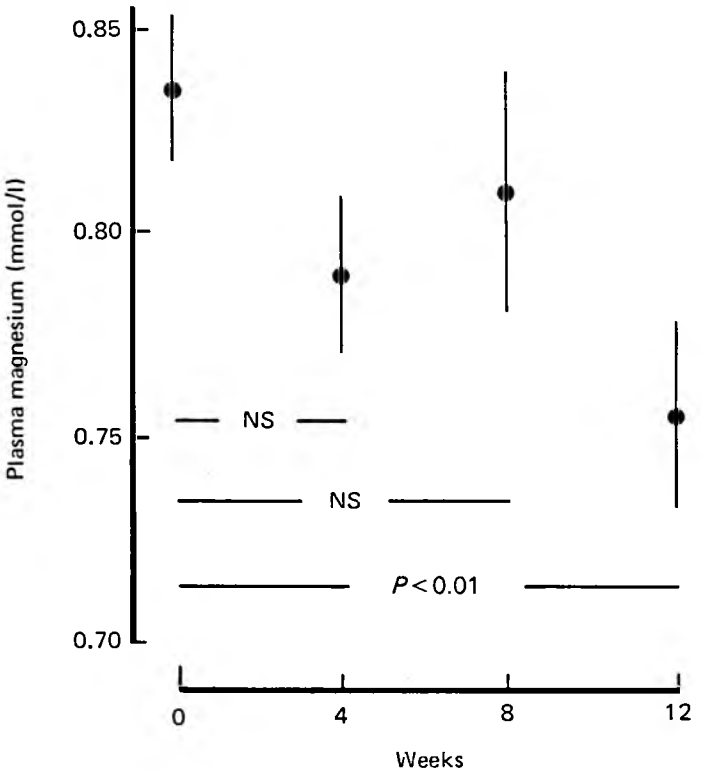

Figure 2 Changes in magnesaemia during the monotherapeutic treatment of nine hypertensive patients with piretanide (12 mg/day). Results as mean ± SEM. NS = not significant. Leary & Reyes (1981b), by courtesy of S. Afr. Med. J.

Loop diuretics

Loop diuretics block transparietal reabsorption of Mg^{2+} in the thick ascending portion of the loop of Henle (Quamme & Dirks, 1980; Quamme, 1981). This is independent from the blockade of Cl^- reabsorption at the same anatomical level, which accounts for the natriuretic effect of loop diuretics (Quamme & Dirks, 1980; Massry, 1981). In normal volunteer subjects the mean Cl^-, Na^+, fluid and Mg^{2+} urinary flow curves, derived from experimental data by a mathematical model (Reyes & Leary, 1981a,b), indicate that the time courses of all these urinary excretions are almost parallel after the administration of placebo (Figure 3). When the same subjects are given loop diuretics, urinary Mg^{2+} flow is delayed with respect to fluid, Cl^- and Na^+ flows (Figure 3). This fact suggests that the hypermagnesiuria induced by loop diuretics is partly due to mechanisms other than the direct blockade of Mg^{2+} reabsorption (Reyes & Leary, 1983a).

Loop diuretics provoke hypercalciuria (Reyes & Leary, 1980; Reyes *et al.*, 1981) which decreases calcaemia (Figure 4) and thus increases serum parathyroid hormone (sPTH) (Elmgreen *et al.*, 1980). PTH mobilizes Ca^{2+} and Mg^{2+} from bone, increasing the amount of Mg^{2+} available for renal excretion in this manner, and perpetuating hypermagnesiuria (Reyes & Leary, 1983a). Mg^{2+} deficiency

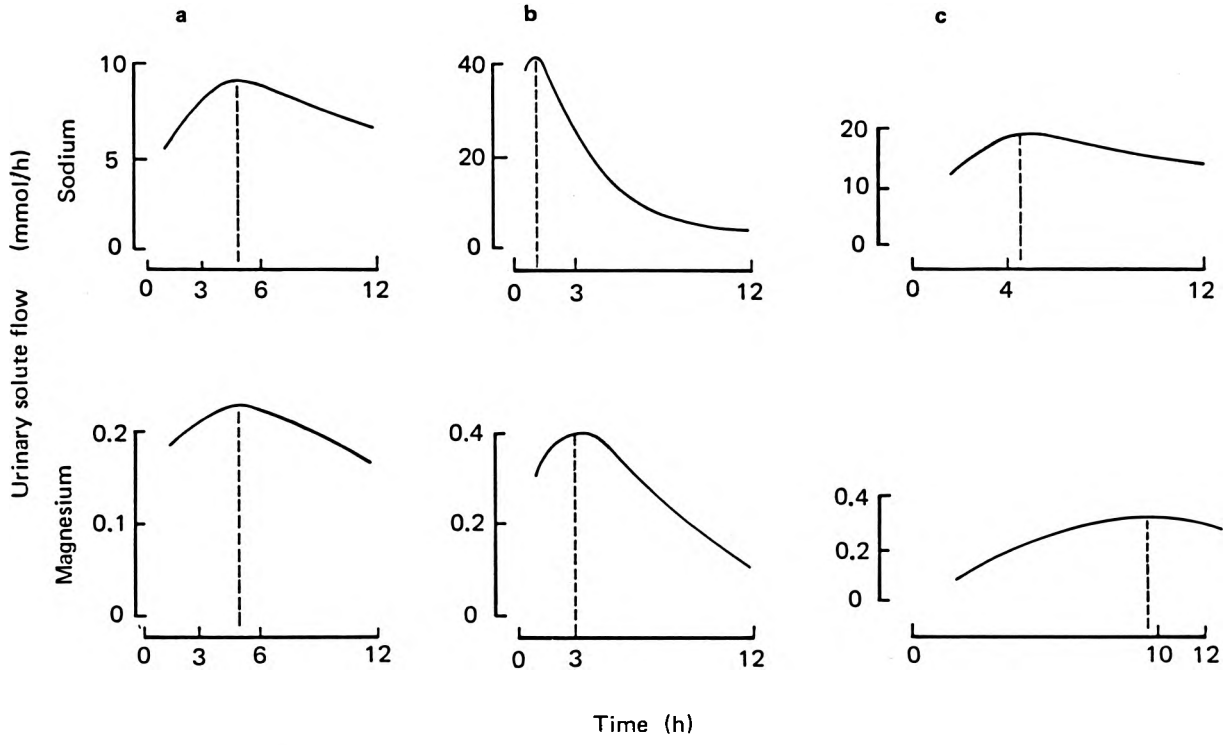

Figure 3 Mean urinary Na^+ and Mg^{2+} flows after the oral administration of placebo (**a**; $n = 10$), frusemide (**b**; 40 mg, $n = 9$) or chlorthalidone (**c**; 100 mg, $n = 9$) to healthy volunteer subjects at hour 0 of the experiments (08.00h). The time courses of natriuresis and magnesiuresis after placebo are almost parallel; their peaks therefore practically coincide in time. After frusemide or chlorthalidone the time course of magnesiuria is delayed with respect to that of natriuria. n = number of volunteer subjects. Adapted from Reyes & Leary (1982a,b), by courtesy of *Curr. Ther. Res.*

ensues eventually, unless its exogenous supply is increased. Mg^{2+} deficiency is manifested by hypomagnesaemia (low plasma Mg^{2+} concentration) only 8–15 weeks after the initiation of diuretic treatment (Leary & Reyes, 1981b) because it is initially masked by Mg^{2+} mobilization from bone to plasma (Reyes & Leary, 1983a).

Mg^{2+} deficiency decreases the release of PTH since the cation normally activates parathyroid adenylate cyclase by competition with Ca^{2+} at the modulating site of the enzyme or by promotion of the synthesis of endogenous guanine nucleotides (Mahaffee *et al.*, 1982). In addition, Mg^{2+} deficiency causes resistance to the action of PTH in bone and in the kidney (Fischer & Fischer, 1981; Fischer & Giroux, 1981; Rude & Singer, 1981), apparently because it obtunds interaction between the hormone's receptor and adenylate cyclase, an enzyme which is positively influenced by PTH (Thode *et al.*, 1981). Thus Mg^{2+} deficiency decreases plasma PTH and causes renal and bone resistance to it; these facts tend overall to increase hypermagnesiuria. Moreover, frusemide has been found to directly diminish renal sensitivity to PTH (Turlapaty & Altura, 1982). The diminution in plasma PTH and bone resistance to it limit Mg^{2+} and Ca^{2+} mobilization from

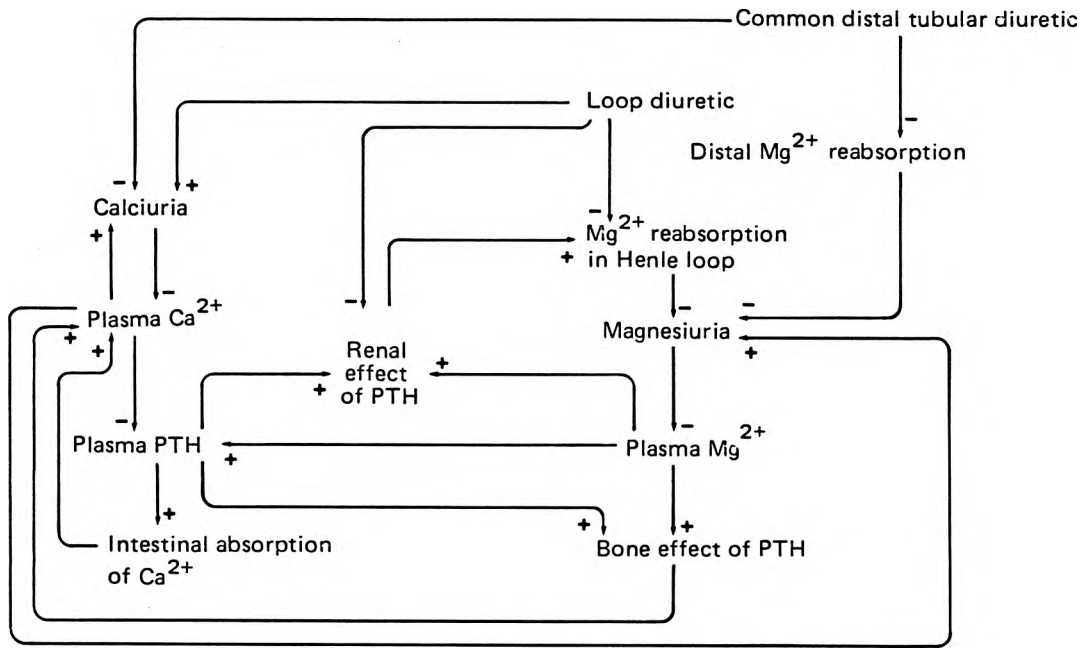

Figure 4 Causal diagram of the determinaton of Mg^{2+} deficiency provoked by loop and distal tubular diuretics. Changes (+ = augmentation; – = diminution) resulting from increases in the variables at which arrows start (system dynamics notation) are depicted. PTH = parathormone. Reyes (1983), by courtesy of *Prensa Med. Argent.*

its main store, thus reinforcing hypocalcaemia, which diminishes magnesiuria directly, i.e. independently from PTH (Quamme & Dirks, 1980; Quamme, 1982); this compensating mechanism is, however, of limited quantitative importance. The decreases in plasma PTH and in its renal effect decrease the activity of renal 1-hydroxylase; the synthesis of 1,25-$(OH_2)D_3$ is therefore decreased and hypocalcaemia aggravated. Hypocalcaemia stimulates the activity of renal 1-hydroxylase and consequently the synthesis of 1,25-$(OH_2)D_3$; hypocalcaemia and hypomagnesaemia are thus partially compensated for; however, this mechanism has no quantitative significance.

Although muzolimine (30 mg) did not increase 24-h urinary Mg^{2+} output significantly (Figure 1), it delayed urinary Mg^{2+} flow with respect to Na^+ in the same manner as frusemide (40 mg) (W.P. Leary, A.J. Reyes & K. van der Byl, unpublished work). This indicates that muzolimine, at its standard diuretic dose, brings into play the mechanisms that account for loop diuretic-induced hypermagnesiuria. The possibility that this formulation could provoke significant depletion of somatic Mg^{2+} on prolonged administration should therefore be evaluated. Higher doses of this substance may be expected to induce hypermagnesiuria acutely.

Distal convoluted tubular diuretics

Hypermagnesiuria provoked by diuretics acting at the distal convoluted tubule cannot be explained solely by direct blockade of transparietal reabsorption of the

cation since only 1–5% of filtered Mg^{2+} is reabsorbed in the distal tubule. In experiments similar to those described for frusemide, significant dephasing was found between the time courses of urinary Mg^{2+} and urinary Cl^-, Na^+ and fluid after the administration of diuretics such as hydrochlorothiazide (Leary & Reyes, 1982c), chlorthalidone (Figure 3), xipamide (A.J. Reyes & W.P. Leary, unpublished work) and clorexolone (Leary & Reyes, 1982b).

The chronic administration of these diuretics causes hypocalciuria (Bloch *et al.*, 1981), followed by hypercalcaemia, which increases magnesiuria (Quamme, 1982) and reduces plasma PTH (Quamme, 1982). The decrease in sPTH diminishes the reabsorption of Mg^{2+} in the loop of Henle thus further increasing magnesiuria. The subsequent mechanisms whereby distal convoluted tubular diuretics induce hypermagnesiuria are similar to those associated with loop diuretics (Figure 4).

Indapamide (2.5 mg) probably did not provoke hypermagnesiuria because the dose given, which exhibits the maximal antihypertensive effect (Berglund & Andersson, 1975; Reyes & Leary, 1981c) of this substance (Reyes *et al.*, 1983b), is well below its standard diuretic dose. However, after indapamide (2.5 mg) urinary Mg^{2+} flow was found to be delayed with respect to that of Na^+ (Reyes *et al.*, 1983a), which suggests that this diuretic provokes hypermagnesiuria at higher doses.

Potassium-retaining diuretics

The K^+-retaining diuretic amiloride reduced urinary Mg^{2+} excretion, although this reduction was statistically not significant, and did not alter the time course of its urinary flow, when monodosed in normal individuals (Leary *et al.*, 1983a). When a combination of amiloride (5 mg) and hydrochlorothiazide (50 mg) was given acutely to healthy volunteer subjects there was also no significant change in Mg^{2+} output (Leary *et al.*, 1984) (Figure 1). This suggests that amiloride induces Mg^{2+} reabsorption (Devane & Ryan, 1983) and therefore acts as a Mg^{2+} sparer when the amount of the cation passing through the distal convoluted tubule is increased by the action of common diuretics at more proximal nephronal sites (Leary & Reyes, 1982a; Leary *et al.*, 1984). However, since amiloride provokes hypocalciuria, on chronic administration its putative Mg^{2+}-retaining direct action would be counterbalanced or overcome by the events that account for the Henle's-loop-mediated hypermagnesiuresis induced by common distal tubular diuretics (W.P. Leary, A.J. Reyes & K. van der Byl, unpublished work).

Pathogenesis of cardiac arrhythmias provoked by common diuretics

The description which follows departs from the electrically 'resting' myocardial cell.

Treatment with common diuretics may significantly diminish the intramyocardial content of Mg^{2+} (Dyckner & Wester, 1979; Whang *et al.*, 1981; Sheehan & White, 1982). This decreases the activity of the Na^+, K^+-ATPase which accounts for the active transport of K^+ into and Na^+ out of the cell (Na^+, K^+ pump), since

Na^+, K^+-ATPase requires Mg^{2+} as a cofactor (Dyckner & Wester, 1979; Olhaberry *et al.*, 1983b). Consequently, the intramyocardial concentration of Na^+ increases and that of K^+ decreases when Mg^{2+} deficiency is present. This reduction in the intracellular concentration of K^+ is added to the somatic K^+ depletion resulting from diuretic-induced hyperkaliuresis (Morgan, 1979; Morgan *et al.*, 1980; Reyes & Leary, 1980, 1982a,b; Leary & Reyes 1981b,1982b,c; Reyes *et al.*, 1983a). Thus the net Mg^{2+} plus K^+ output after a diuretic, as depicted in Figure 5, would be indicative of its arrhythmogenic potential. Diuretics increase the amount of Na^+ reaching the final portion of the distal convoluted tubule and therefore the normal interchange between Na^+ and K^+, H^+ is increased. In addition, hyponatraemia and the relative decrease in heart output, elicited by the natriuretic action of diuretics, increases aldosteronaemia which further elevates the Na^+–K^+,H^+ interchange in the distal convoluted tubule (Haalboom, 1980; Reyes & Leary, 1980; Tucker *et al.*, 1980). In familial hypokalaemic alkalosis with tubulopathy (Güllner *et al.*, 1981) hypermagnesiuria occurs in association with

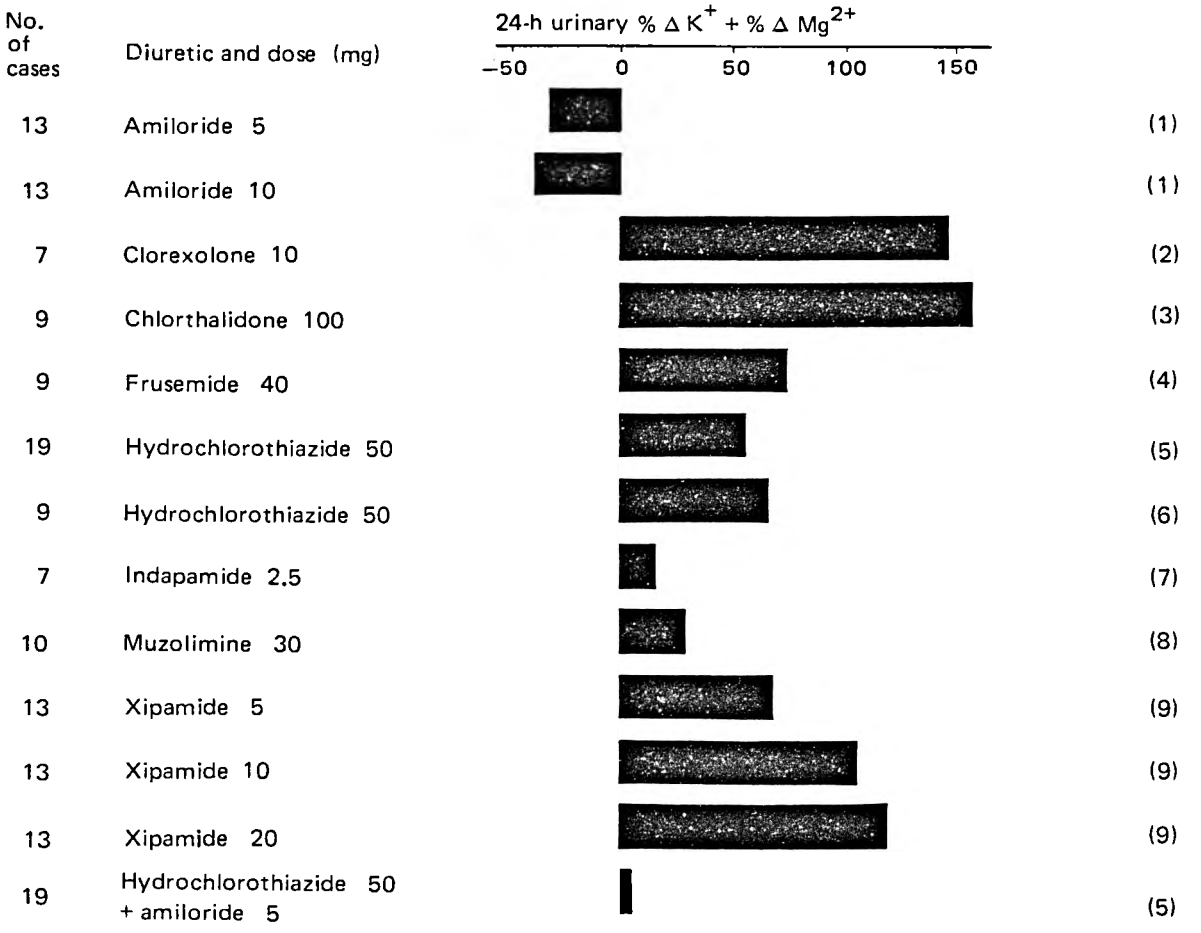

Figure 5 Summary of results from several studies in which healthy volunteer subjects were given single doses of diuretic formulations. Bars depict the sums of percentage increases in 24-h urinary Mg^{2+} and K^+ outputs with respect to control values. For references (1) – (9) see legend to Figure 1

hyperaldosteronaemia and hypomagnesaemia increases aldosteronaemia independently from the renin–angiotensin system, but these mechanisms are of limited importance during the treatment of ordinary patients with common diuretics (Massry, 1981). Increased proton excretion in urine elevates the extracellular pH, thus facilitating the entrance of K^+ into the cell by diffusion; however, the factors that decrease intracellular K^+ predominate (Figure 6). Cytosolic Ca^{2+} concentration within the myocardium increases when Mg^{2+} deficiency is present (Wester & Dyckner, 1981). The transmitochondrial interchange of Na^+ and Ca^{2+} rises because of the increase in cytosolic Na^+ secondary to reduced Na^+, K^+ pump activity, Na^+ passing into the mitochondria and Ca^+ from them into the cytosol. Cytosolic Ca^{2+} also increases as a result of changes

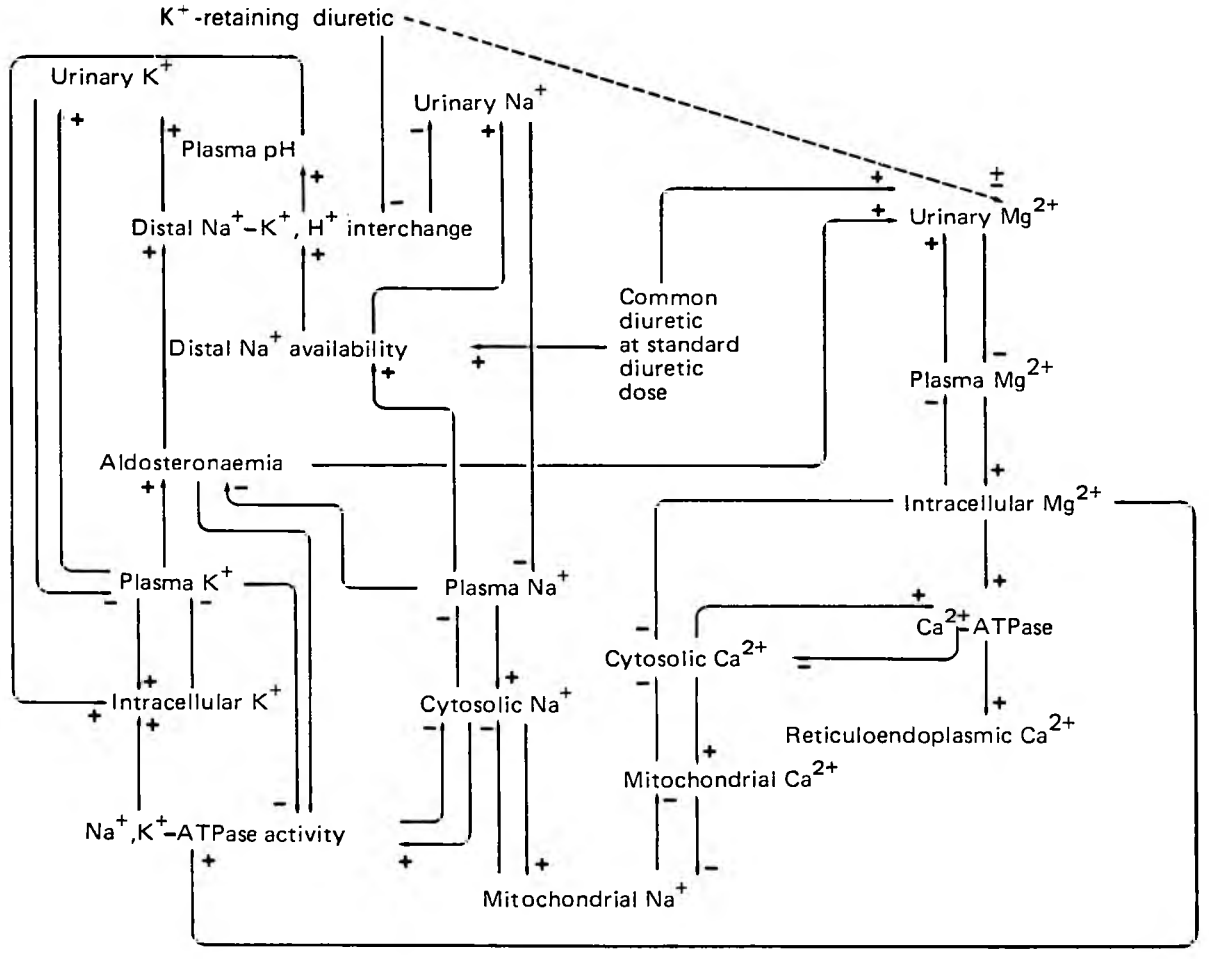

Figure 6 Causal diagram of the principal mechanisms whereby diuretics alter the intramyocardial concentrations of Mg^{2+}, K^+, Ca^{2+} and Na^+. Changes (+ = augmentation; – = diminution) resulting from increases in the variables from where arrows depart (system dynamics notation) are depicted. The broken line indicates an effect which becomes clinically relevant only at doses above those generally prescribed in practice. Reyes (1983), by courtesy of *Prensa Med. Argent.*

in passage of the ion from the extracellular compartment which occur in Mg^{2+} deficiency (Altura, 1982; Altura & Turlapaty, 1982). The fall in intracellular Mg^{2+} concentration decreases the activity of the Mg^{2+}-dependent Ca^{2+}-ATPases that account for the active transport of Ca^{2+} from the cytosol into the endoplasmic reticulum, and from the cytosol to the *milieu intérior*, thus augmenting cytosolic Ca^{2+} (Figure 6). Within the myocardium, the increase in cytosolic Ca^{2+} is the prime factor underlying the occurrence of cardiac arrhythmias during treatment with common diuretics; the decrease in intracellular K^+ and Mg^{2+} and the increase in cytosolic Na^+ contribute to this deleterious effect of diuretics, since all these ionic derangements destabilize the sarcolemma electrically, increasing myocardial excitability (Nayler, 1981).

Other mechanisms exist which contribute to the intramyocardial electrolyte disturbances provoked by diuretics; these processes are less important than those already described (Figure 7). The haemodynamic changes induced by diuretics increase catecholamine secretion by the suprarenal glands. The decrease in intracellular Mg^{2+} and the increase in cytosolic Ca^{2+}, reinforce both this secretion and the release of catecholamines by the sympathetic nerve endings (Altura, B.M. & Altura, B.T. 1982; Altura, B.T. & Altura, B.M., 1982; Altura & Turlapaty,1982). Catecholamines activate adenylate cyclase and promote the synthesis of adenosine 3′,5′-cyclic monophosphate (cAMP) from adenosine 5′-triphosphate (ATP). The increased adenylate cyclase activity is augmented because catecholamines decrease the secretion of insulin, which normally inhibits the activity of the enzyme. A decrease in intracellular Mg^{2+} would cause a fall in the activity of adenylate cyclase, since Mg^{2+} promotes the activity of the enzyme directly and is a cofactor for enzymes involved in the cyclation of guanosine nucleotide which precede the activation of adenylate cyclase (Olhaberry *et al.*, 1983); however, the overall effect of the various factors involved is a net increase in the activity of adenylate cyclase (Erdös & Maguire, 1980). An increase in cAMP availability is promoted by diuretics like hydrochlorothiazide (Furman, 1981) that inhibit phosphodiesterase, the enzyme which catalyses cAMP. The amount of cAMP produced depends on ATP availability. Catecholamines reduce tissue PO_2 and thus decrease the amount of ATP synthesized; however, increased glycolysis and lipolysis secondary to the rise in cAMP (Figure 7) provide ATP through the production of free fatty acids.

An increase in ATP tends to diminish glycolysis by reducing the activity of phosphofructokinase, which catalyses the rate-limiting step of the glycolytic chain. However, glycolysis is stimulated by the cAMP-dependent activation of the glycogenolytic enzyme phosphatase and also because the decrease in intracellular Mg^{2+} activates phosphoenolpyruvate carboxykinase, an enzyme that catalyses glyconeogenesis (McNeill *et al.*, 1982). Free fatty acids tend to diminish glycolysis through inhibition of the activities of various enzymes.

Free fatty acids bind ionic Mg^{2+} in plasma, thus removing it from the pool ionic Mg^{2+} which is transferable to the cell (Flink *et al.*, 1981). This perpetuates and further aggravates the intracellular Mg^{2+} deficiency provoked by diuretics.

Increased cAMP decreases the activity of lipoprotein lipase, thus limiting further free fatty acid liberation. Raised free fatty acid levels decrease the activity of

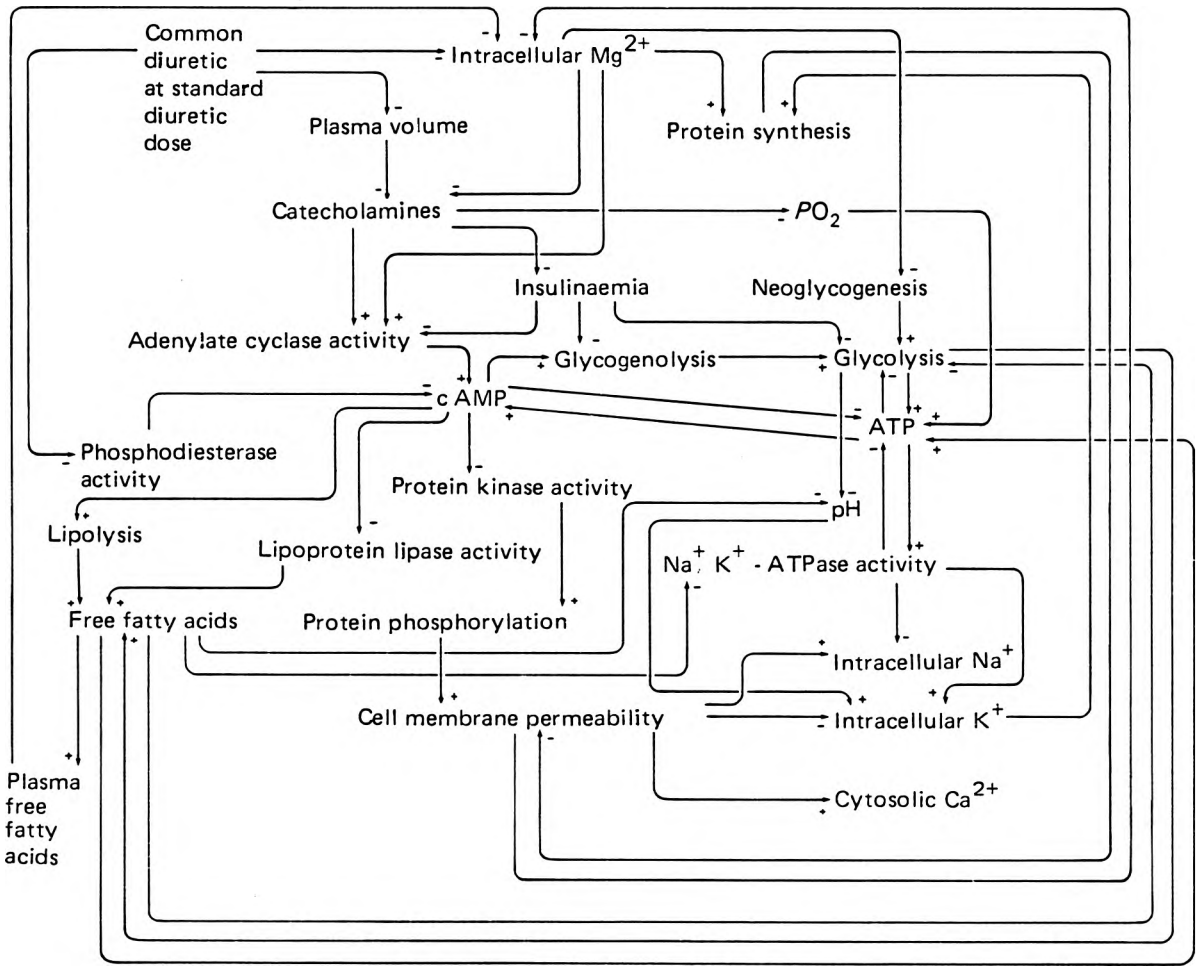

Figure 7 Causal diagram of the complementary mechanisms whereby diuretics alter the intramyocardial concentrations of Mg^{2+}, K^+, Ca^{2+} and Na^+. Changes (+ = augmentation; − = diminution) resulting from increases in the variables at which arrows start (system dynamics notation) are depicted. Reyes (1983), by courtesy of *Prensa Med. Argent.*

Na^+,K^+-ATPase, further altering the intramyocardial electrolyte balance (Reyes & Leary, 1983b). cAMP also activates cellular protein kinase, which promotes the phosphorylation of sarcolemmal proteins; the cell membrane permeability to Ca^{2+}, Na^+ and K^+ and Mg^{2+} is thus increased in favour of their electrochemical gradients. Consequently, the intramyocardial concentrations of Ca^{2+} and Na^+ rise and those of Mg^{2+} and K^+ fall. This is aggravated because protein synthesis is altered in Mg^{2+} deficiency, since the cation acts as a cofactor for enzymes involved in transcription, and because the fall in intracellular K^+ also affects protein synthesis (Günther, 1981; Olhaberry *et al.*, 1983a).

Diuretics, magnesium deficiency and coronary and cerebrovascular diseases

Mg^{2+} deficiency has been identified as a coronary risk factor.

The incidence of coronary heart disease varies widely in different geographical regions and serious epidemiological studies have been carried out to identify variables that could explain this fact. In Finland (Karppanen, 1981; Luoma *et al.*, 1983) and in South Afrcia (Leary *et al.*, 1983b) it was found that the incidence of death ascribed to ischaemic heart disease is inversely correlated with the concentration of Mg^{2+} in drinking water. This ranges from 0.5 to 30 mg in different regions and can be used as an index of the Mg^{2+} content of foods produced in the same geological area. In addition, it is possible that the amount of Mg^{2+} present in drinking water is in itself an important determinant of total bodily Mg^{2+} (Marier, 1982). Post-mortem studies in England have demonstrated significantly reduced intramyocardial and coronary arterial wall Mg^{2+} levels in road accident victims who lived in areas where water supplies have a low Mg^{2+} content (Chipperfield & Chipperfield, 1978). A comprehensive review by Marier (1982) indicates all these findings have been confirmed in the USA and Canada by various authors.

Plasma Mg^{2+} concentration is significantly lower in patients with moderately severe coronary heart disease, diagnosed by arteriography, than in subjects with mild or absent radiographic changes (Manthey *et al.*, 1981).

Experimentally, Mg^{2+} deficiency increases coronary (Turlapaty & Altura, 1980,1982; Siegel *et al.*, 1981; Turlapaty *et al.*, 1981; Altura, 1982; Altura, B.M. & Altura, B.T., 1982; Altura & Turlapaty, 1982) and cerebrovascular tone (Altura, B.T. & Altura, B.M., 1980,1982), whereas Mg^{2+} supplementation has a corrective effect. Mg^{2+} deficiency increases vascular reactivity to catecholamines, angiotensin, K^+, serotonin, vasospastic peptides and neurohumoral stimuli and attenuates vasodilatation induced by prostaglandins or isoproterenol (Altura, 1982). Mg^{2+} and verapamil block the same Ca^{2+}-entrance channel to the vascular smooth muscle cell (Turlapaty *et al.*, 1981). The overall mechanisms accounting for these vascular changes are probably similar to those depicted in Figures 6 and 7. The administration of Mg^{2+} accelerates the rate of myocardial recovery from ischaemia in experimental animals (Bersohn *et al.*, 1982).

Experimental Mg^{2+} deficiency causes characteristic structural changes in the myocardium (Günther *et al.*, 1981) and in skeletal muscle (Robeson *et al.*, 1980). In myocardial fibres, there in enlargement of the interstitial space of the transverse tubular system and a substance, possibly calcium phosphate, precipitates in vesicles of the longitudinal system of the endoplasmic reticulum. Mitochondrial oedema with loss of mitochondrial matrix also occurs, the interstitial space is enlarged, filaments and fibres of isolated collagen are seen in the vicinity of the interstitial capillaries and the fibrocyte endoplasmic reticulum is increased (Günther *et al.*, 1981).

Stress increases catecholamine release and therefore augments Mg^{2+} deficiency, which further increases catecholamine levels (Classen, 1981). Experimentally, stress has been found to provoke hypermagnesiuria (Altura, 1980) and factors, such as noise, deplete somatic Mg^{2+} stores (Classen, 1981; Ising, 1981). Type-A behaviour (Altura, 1980), stress (Classen, 1981), catecholamines (Classen, 1981) and

alterations in plasma lipids (Rayssiguier, 1981) potentiate each other as factors determining Mg^{2+} deficiency and this interplay could explain the high incidence of coronary heart disease in the population with type-A behaviour.

When diuretics are given to patients who are under stress or have coronary heart disease, particular care should be exercised to ensure that Mg^{2+} deficiency does not develop.

Diuretics, magnesium deficiency and hypertension

The possibility that Mg^{2+} deficiency contributes to the development of arterial hypertension has been postulated on the basis of indirect experimental evidence (Altura, B.M. & Altura, B.T., 1982). Unfortunately, no adequate epidemiological studies on the relation between the incidence of hypertension and dietary Mg^{2+} content exist (McCarron, 1983).

In a recent study of hypertensive patients given prolonged diuretic therapy resulting in stable blood pressure values, subjects developed hypomagnesaemia. Oral Mg^{2+} supplementation in these patients resulted in an increase in magnesaemia and a significant reduction in blood pressure (Dyckner & Wester, 1983), suggesting that the antihypertensive effect of diuretics may be hampered by the decrease in somatic Mg^{2+} which these substances induce. The mechanism whereby this occurs is unknown but may involve processes in vascular muscle resembling those depicted in Figures 6 and 7 (Altura, 1982). Plasma Mg^{2+} concentration should always be measured in hypertensive patients treated with common diuretics, and oral Mg^{2+} supplements should be prescribed in cases of hypomagnesaemia before considering the effect of a diuretic maximal and adding another antihypertensive to the therapeutic regimen.

Diuretics, magnesium deficiency and acute myocardial infarction

The myocardial content of Mg^{2+} has been found to be low in necrotic and perinecrotic zones (Condorelli, 1973), both in human necropsy specimens and after coronary artery ligation in several species. Both Mg^{2+} and K^{+} concentrations are low in the infarcted myocardium (Sarker & Heggtreit, 1982) and the administration of both cations was found to accelerate healing more than medication with either of them given alone (Lehr, 1981). Ventricular arrhythmias occurring during acute myocardial infarction respond better to Mg^{2+} than to other antiarrhythmic agents (Condorelli, 1973; Morton *et al.*, 1981; Rotman,1981). The mechanisms that underlie these phenomena are unknown; it might be speculated that hypoxia favours the exit of Mg^{2+} from the cell through an increase in permeability to the ion. Mg^{2+} status should be monitored in patients who are under treatment with common diuretics, when they suffer myocardial infarction or who are given diuretics during the acute phase of myocardial infarction.

Diuretic treatment, alterations in lipid and carbohydrate metabolism and magnesium deficiency

Common loop and distal tubular diuretics generally cause clinically significant alterations in plasma lipids, when they are given at standard diuretic doses to

normal volunteer subjects or hypertensive patients for more than 4 weeks. The changes include increases in total cholesterol (Ames & Hill, 1978; Falch & Schreiner, 1980; Glück *et al.*, 1980; Joos *et al.*, 1980; Güllner *et al.*, 1981; Meier *et al.*, 1982) and/or triglycerides (Ames & Hill, 1978; Falch & Schreiner, 1980; Joos *et al.*, 1980; Güllner *et al.*, 1981; Leary & Reyes, 1981a; Reyes *et al.*, 1981) and increases of the β/α-lipoprotein (LDL/HDL) ratio (Glück *et al.*, 1979, 1980; Grimm *et al.*, 1981). These alterations in plasma lipids are consistent with an increase in the risk of cardiovascular disease. It is possible that Mg^{2+} depletion is one of the factors leading to diuretic-induced dyslipaemias (Reyes & Leary, 1983c), since similar derangements of lipid metabolism occur in experimental Mg^{2+} deficiency when animals are fed a high-lipid or high-carbohydrate diet (Rayssiguier, 1981). Most investigations of the effects which diuretics have on lipid metabolism (Ames & Hill, 1978; Glück *et al.*, 1979,1980; Falch & Schreiner, 1980; Joos *et al.*, 1980; Boehringer *et al.*, 1981; Grimm *et al.*, Güllner *et al.*, 1981; Robinson *et al.*, 1981; Weidmann *et al.*, 1981; Meier *et al.*, 1982) have been biased by improper control of diet or failure to take other factors, such as familial history of diabetes or dyslipaemia, into account. Some of the effects of diuretics on lipid metabolism may be explained by the increase in catecholamines elicited by these drugs (Figure 7); other effects could be related to the alteration in protein synthesis provoked by diuretics through increased K^+ and Mg^{2+} excretions (Reyes *et al.*, 1983a) (Figure 7).

Both diuretics (Furman, 1981) and Mg^{2+} deficiency reduce glucose tolerance thus causing a pathophysiological picture that resembles diabetes mellitus, a condition that is usually accompanied by depletion of bodily Mg^{2+} stores and that may be aggravated by diuretics or by Mg^{2+} deficiency of any origin (Johansson *et al.*, 1981). Patients with dyslipaemias induced by diuretics have been shown to respond favourably to supplementation of the diet with Mg^{2+} chloride tablets (W.H. Davis, A.J. Reyes & W.P. Leary, unpublished work; A.J. Reyes, T.N. Acosta-Barrios & W.P. Leary, unpublished work).

Magnesium deficiency provoked by diuretics

The diagnosis of Mg^{2+} deficiency in its early stages is difficult (Cronin & Knochel, 1983; Dyckner & Wester, 1982b; Erdös & Maguire, 1980; Fischer & Fischer, 1981; Flink, 1981; Halidmann, 1982; Roux & Courtois, 1981; Ruiz-Palomo *et al.*, 1983). Its existence should be suspected whenever diuretic treatment in standard doses have been prolonged, especially if any other factor exists that may precipitate or aggravate diuretic-induced Mg^{2+} deficiency or cause Mg^{2+} deficiency *per se* (Table 1). Many of these factors may also provoke K^+ deficiency, which frequently coexists with Mg^{2+} deficiency in patients treated with diuretics. Moreover, when diminished serum K^+ and Mg^{2+} values are found in patients under treatment with diuretics, they correlate with each other (Kohvakka *et al.*, 1982).

Clinical manifestations of Mg^{2+} deficiency include anorexia, nausea, apathy, muscular weakness, fatigue, excitation and, in some cases, delirium or coma. Other clinical signs are tetany, peripheral tremor involving muscles of the tongue, face and limbs, ataxia, vertigo, lateral and vertical nystagmus, tetany and convulsions.

Occasionally positive Chvostek and Trousseau signs, myoclonia or spontaneous carpopedal spasms occur. Advanced Mg^{2+} deficiency may be confused with hypocalcaemia. Atrial fibrillation is the most frequent cardiac arrhythmia, followed by ventricular and supraventricular extrasystoles (Sheehan & White, 1982) and ventricular fibrillation. The electrocardiographic signs of Mg^{2+} deficiency are non-specific prolongation of the PQ, QTc and QUc intervals and flattening of the T waves (Davis & Ziady, 1978). Muscular derangements of the inferior portion of the oesophagous may lead to dysphagia.

Microcytic anaemia with decreased erythrocytic half-life, reticulocytosis and spherocytosis may be present in blood.

Hypocalcaemia and hypokalaemia resistant to supplementation therapy have been reported in association with Mg^{2+} deficiency and metabolic alkalosis also occurs. Total plasma lipids may be elevated (Erdös & Maguire, 1980; Fischer & Fischer, 1981; Flink, 1981; Rude & Singer, 1981; Dyckner & Wester, 1982b; Halidmann, 1982; Cronin & Knochel, 1983; Ruiz-Palomo *et al.*, 1983).

No routine procedure exists for the evaluation of total Mg^{2+} stores. Measurement of the plasma Mg^{2+} concentration is the procedure of choice for diagnosing Mg^{2+} deficiency, although experimental error is such that atomic absorption spectrometry is the only acceptable method routinely available at the moment. This technique measures total plasma Mg^{2+}; diffusible Mg^{2+} may be evaluated, in plasma and tissues, by means of a selective electrode (Hess & Weingart, 1981), which is not yet used in the clinical laboratory.

Mg^{2+} deficiency is conventionally diagnosed when the total plasma Mg^{2+} concentration, referred to as magnesaemia, is < 0.75 mmol/l (Rude & Singer, 1981; Dyckner & Wester, 1982a). The upper limit of normality is 1.05 mmol/l. It must be stressed that a normal plasma Mg^{2+} level does not necessarily exclude somatic Mg^{2+} deficiency, since in the early stages of the condition Mg^{2+} is mobilized from bone, retarding any fall in Mg^{2+} concentration.

The concentration of Mg^{2+} in striated muscle has been used to evaluate the ion content in the soft tissue (Dyckner & Wester, 1979), but it bears no linear relation to myocardial Mg^{2+}, since the cation is more readily retained in the myocardium than in skeletal muscle. Lymphocytes provide an adequate means for estimating somatic Mg^{2+} status, because they are metabolically active, can be studied repeatedly (Ryan *et al.*, 1981) and intralymphocytic and intramyocardial Mg^{2+} contents correlate linearly.

Table 1 Conditions that may provoke magnesium deficiency *per se* or may precipitate or aggravate magnesium deficiency during diuretic treatment. Adapted from Reyes (1983), by courtesy of *Prensa Med. Argent.*

1 Conditions in which Mg^{2+} supply to the *milieu intérieur* is decreased
- 1.1 Inadequate intake
  - 1.1.1 Low Mg^{2+} content in all foods
  - 1.1.2 Diet poor in Mg^{2+}
  - 1.1.3 Decreased food intake
  - 1.1.4 Parenteral nutrition with low Mg^{2+} content
- 1.2 Altered absorption
  - 1.2.1 Malabsorption syndromes
    - 1.2.1.1 Gluten enteropathy
    - 1.2.1.2 Pancreatic insufficiency with steatorrhoea
    - 1.2.1.3 Tropical sprue
    - 1.2.1.4 Other
  - 1.2.2 Extensive enteral resection
  - 1.2.3 Biliary and enteric fistuli
  - 1.2.4 Excessive intake of oxalate and phytate

2 Conditions in which Mg^{2+} losses are increased
- 2.1 Enteric
  - 2.1.1 Vomiting
  - 2.1.2 Diarrhoea
  - 2.1.3 Repetitive gastric aspiration
- 2.2 Excessive sweating
- 2.3 Renal
  - 2.3.1 Renal insufficiency with hypermagnesiuria
  - 2.3.2 Osmotic diuresis (glucose, mannitol, urea)
  - 2.3.3 Alcohol
  - 2.3.4 Drug-induced hypermagnesiuria
    - 2.3.4.1 Antineoplastics: cisplatin
    - 2.3.4.2 Antibiotics: amphotericin B, carbenicillin, gentamicin, ticarcillin
    - 2.3.4.3 Cardiac glycosides
  - 2.3.5 Chronic parenteral nutrition with liquids
  - 2.3.6 Renal tubular acidosis
  - 2.3.7 Diuretic phase of acute tubular necrosis
  - 2.3.8 Postobstructive polyuria
  - 2.3.9 Essential familial hypermagnesiuria
  - 2.3.10 Essential sporadic hypermagnesiuria

3 Conditions in which various factors coexist
- 3.1 Hungry bone syndrome
- 3.2 Hyperparathyroidism
- 3.3 Hyperthyroidism
- 3.4 Excessive lactation
- 3.5 Protein malnutrition
- 3.6 Diabetes mellitus
- 3.7 Phosphate deficiency
- 3.8 Metabolic acidosis
- 3.9 Primary hyperaldosteronism
- 3.10 Hypercalcaemia of any origin
- 3.11 Acute pancreatitis
- 3.12 Third-term pregnancy

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SECTION G : REVIEWS (SPANISH)

Two publications are presented. These papers are based on original lectures delivered in Argentina. Adverse reactions to antihypertensive drugs and the pharmacology of vasoactive substances are reviewed. No other author is involved but the script was translated into Spanish by editorial staff at the journal.

PAPER G1

Tratamiento De La Hipertension: La Farmacologia De Los Agentes Vasoactivos

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TRATAMIENTO DE LA HIPERTENSION: LA FARMACOLOGIA DE LOS AGENTES VASOACTIVOS

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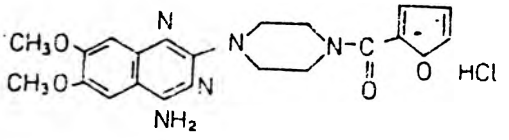
La presión arterial sistémica es controlada por mecanismos homeostáticos complejos, y puede ser afectada por agentes vasoactivos endógenos o medicamentos utilizados para regular la presión arterial. Las sustancias endógenas son de particular interés en vista de su posible papel en la patogénesis de la hipertensión. Este trabajo está limitado a la farmacología de los agentes vasoactivos usados en el tratamiento de la presión arterial elevada.

Los compuestos vasoactivos empleados en esta terapéutica incluyen todas aquellas sustancias que actúan directamente sobre el músculo liso de la pared de los vasos sanguíneos. No se discutirán los bloqueantes de los receptores adrenérgicos y la vasodilatación producida por mecanismos neurogénicos distales o proximales. Las drogas: prazosin, hidralazina, minoxidil y diazóxidos, tienen particular importancia, y se analizará detalladamente su farmacología, incluyendo comentarios sobre sus mecanismos de acción y sus efectos colaterales. Además, se hará una breve referencia a varios otros compuestos incluyendo los antagonistas de la angiotensina y las prostaglandinas con efectos hipotensores.

El prazosin (Fig. 1), es un derivado de la quinazolina que reduce la presión arterial por la combinación de un bloqueo funcional de los receptores y una relajación directa del músculo liso vascular, mediada

PRAZOSIN-ClH



2-[4-2-furoil piperazin]-4 amino-6,7-dimetoxi quinazolina

Fig. 1

por la inhibición de la fosfodiesterasa.[1] El prazosin fue desarrollado como un inhibidor de esta enzima, a partir de numerosas sustancias sintéticas cuyas estructuras recuerdan al 3'5'-adenosín monofosfato cíclico (AMP cíclico), al guanosina monofosfato cíclico (GMP cíclico), a la teofilina y a la papaverina.

El AMP cíclico regula numerosos cambios biológicos en células vivas incluyendo la relajación muscular lisa. La síntesis de AMP cíclico a partir del ATP aumenta por la estimulación de los receptores β-adrenérgicos o por el bloqueo de los α-adrenoreceptores; sus niveles también pueden ser aumentados por inhibición de la fosfodiesterasa, la enzima responsable de la degradación del AMP cíclico. Un aumento

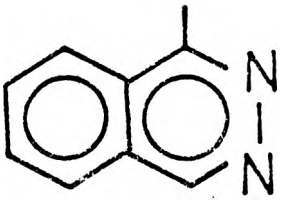
en los niveles de AMP cíclico por cualesquiera de estos mecanismos debería, teóricamente, relajar el músculo liso y producir una caída en la presión arterial sistémica. Un segundo nucleótido cíclico, el GMP cíclico, está también presente en muchos tejidos y es controlado por enzimas similares. La elevación de los niveles de GMP cíclico en el corazón, en respuesta a la acetilcolina o a la inhibición de la fosfodiesterasa, produce una disminución de la frecuencia cardíaca y de la fuerza de contracción.[11, 17]

Estudios preliminares *in vitro* indicaron que el prazosin inhibe la degradación de ambos nucleótidos cíclicos por la fosfodiesterasa, y podría además disminuir la presión arterial sin inducir taquicardia refleja. *In vivo*, los efectos periféricos de este compuesto son más marcados en las arteriolas, sugiriendo una distribución selectiva del prazosin o una susceptibilidad aumentada a la inhibición de la fosfodiesterasa en este tejido, donde la enzima está presente en concentraciones relativamente bajas. El prazosin-2 ^{14}C es fijado bastante fácilmente por el corazón, permitiendo la inhibición de la fosfodiesterasa GMP cíclica, como se ha descripto anteriormente.[4]

El prazosin es en su mayor parte excretado después de ser metabolizado por o-demetilación, hidrólisis de la unión amida, hidroxilación y apertura del ciclo por oxidación seguida por N-dealquilación. Este compuesto tiene una vida media plasmática corta (1-2 horas); como en el caso del minoxidil y de la α-metil-dopa, sus efectos anti-hipertensivos persisten después de la caída de sus niveles plasmáticos, indicando una actividad mantenida a nivel de los receptores.[11]

En el hombre, el prazosin reduce la resistencia periférica total con pocos efectos sobre la frecuencia cardíaca, el índice sistólico y el índice cardíaco. La droga es generalmente administrada tres veces por día y pueden necesitarse hasta cuatro semanas para que comience a manifestarse el efecto máximo de una cierta dosis. El efecto antihipertensivo es mayor en la posición erecta que en la supina, pero la hipotensión ortostática severa y los mareos son relativamente poco frecuentes y no se observan modificaciones del flujo sanguíneo renal. La eficacia del prazosin puede ser aumentada por la combinación con un diurético o con un agente bloqueante β-adrenérgico.[3, 18]

HIDRALAZINA



1-hidrazinoftalazina

Fig. 2

Se ha descripto una variedad de efectos colaterales incluyendo debilidad, palpitaciones, náuseas, boca seca, visión borrosa, edema, depresión, constipación y diarrea. Una pequeña proporción de pacientes pierde la conciencia y sufre colapso después de la administración inicial de prazosin; este efecto está relacionado con la dosis y puede ser evitado estudiando en todos los individuos el efecto de una pequeña dosis de prueba.[22]

La hidralazina (Fig. 2) ha sido usada en el tratamiento de la hipertensión desde 1950 y es probablemente el más común de los vasodilatadores.

Los mecanismos a través de los cuales la hidralazina causa vasodilatación son inciertos, aunque se sabe que suprime las respuestas vasculares a varios agentes vasoconstrictores sugiriendo una inhibición de los mecanismos contráctiles celulares, posiblemente mediados a través del calcio. Estudios *in vitro* indican que la hidralazina inhibe la hidrólisis del AMP cíclico por la fosfodiesterasa, pero carece aparentemente de la capacidad del prazosin para inhibir la degradación del GMP cíclico. En el hombre la hidralazina relaja la musculatura lisa arteriolar, especialmente en las circulaciones esplácnica, cerebral, coronaria y renal, sin aumentar la capacitancia venosa. La respuesta simpática refleja secundaria a la vasodilatación produce aumento de la frecuencia cardíaca, de la ve-

locidad de eyección del ventrículo izquierdo y del volumen minuto cardíaco. Estos cambios pueden aumentar la presión en la arteria pulmonar en pacientes con enfermedad mitral o causar dolor anginoso en pacientes con insuficiencia coronaria, a menos que se administre un bloqueante beta con el vasodilatador. Posiblemente por estas respuestas cardíacas reflejas se desarrolla tolerancia al efecto hipotensivo de la hidralazina.[1, 11]

La hidralazina se absorbe bien después de la administración oral, tiene una gran afinidad por las paredes de las arterias musculares, alcanza sus niveles máximos plasmáticos a las 3 ó 4 horas y puede persistir en el plasma hasta 24 horas. Los mayores cambios metabólicos después de la absorción son la hidroxilación del anillo cíclico, la siguiente conjugación con el ácido glucurónico y la N-acetilación. Si la N-acetilación se halla disminuida, puede producirse la acumulación de la hidralazina con los efectos adversos consecuentes.[22] Se forman pequeñas cantidades de hidrazona que podrían combinarse posiblemente con piridoxina produciendo una neuropatía periférica. El efecto de la hidralazina se desarrolla después de 15-20 minutos, alcanza la máxima actividad en una hora y dura alrededor de 6 horas. La dilatación preferencial de las arteriolas minimiza los cambios posturales, pero pueden ocurrir otras respuestas adversas. Entre ellas se han descripto dolor de cabeza, mareos, náuseas, palpitaciones, sudor, congestión nasal, parestesias, calambres y temblores. La administración crónica de hidralazina, en altas dosis, puede producir manifestaciones de artritis reumatoidea o de lupus eritematoso sistémico.

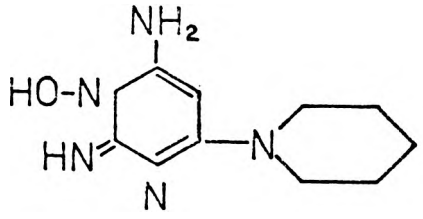
La hidralazina, como otros vasodilatadores, puede causar retención de agua y sal con la consiguiente ganancia de peso y edema. Estos cambios son posiblemente mediados a través del sistema renina-angiotensina-aldosterona, ya que el flujo sanguíneo renal y la filtración glomerular no disminuyen por la hidralazina. La administración concomitante de un diurético y un bloqueador β debería contrarrestar estos efectos.[15]

El minoxidil (Fig. 3) es un derivado de la piperidino - pirimidina capaz de disminuir la presión arterial por vasodilatación y, posiblemente, por atenuación de la función nerviosa simpática.

Después de la administración oral se absorbe rápidamente y tiene una vida media plasmática corta de alrededor de cuatro horas, siendo excretado por la orina como un glucurónido conjugado, durante las primeras doce horas después de la ingestión, y como otros dos metabolitos después de las doce horas. El efecto máximo ocurre cuatro horas después de la administración del minoxidil y dura 24 horas o más, dependiendo de la dosis dada. La aparente falta de correlación entre los niveles circulantes en plasma y la duración de la actividad sugiere su permanencia en los receptores vasculares.[9] Afecta en forma semejante las presiones supina y erecta, de manera que la hipotensión postural no es un problema habitual.[14]

El bloqueo colinérgico, histaminérgico y beta-adrenérgico no interfiere con los efectos del minoxidil sobre el músculo vascular, y fuera del sistema cardiovascular este compuesto tiene efectos farmacológicos mínimos.[7] La libido y la eyaculación no son afectadas, pero otros efectos adversos han sido registrados: la taquicardia refleja puede ser algunas veces bastante severa como para precipitar una angina de pecho, pero ésta es controlada rápidamente por el bloqueo beta. La retención de agua y sal puede alcanzar dimensiones considera-

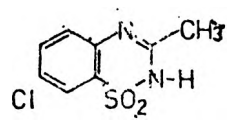
MINOXIDIL



6-amino-1.2-dihidro-1-hidroxi-2-imino-4-piperidinopirimidina

Fig. 3

DIAZOXIDO



7-Cloro-3-metil-1,2,4-benzotiodiazina-1,1-dióxido

Fig. 4

bles, pero es controlada generalmente con la administración de diuréticos. También se ha observado hipertricosis en la cara, frente y sienes y rigidez de los rasgos faciales en pacientes tratados con minoxidil.[14]

La actividad renínica plasmática está aumentada por la administración de minoxidil y este efecto, que es parcialmente controlado por el propranolol, ha sido atribuido a un aumento de la actividad adrenérgica y a una disminución en la presión de perfusión renal. Además es importante hacer notar que la eliminación de la droga es principalmente una función de su metabolismo; la depuración del minoxidil corporal, como tal, no depende de la filtración glomerular, sugiriendo que no es probable la excesiva acumulación en pacientes urémicos.[9] Comunicaciones preliminares confirman este hecho habiéndose descripto respuestas excelentes en pacientes refractarios a otros tratamientos.[20]

El diazóxido (Fig. 4) es un potente vasodilatador estructuralmente muy semejante a los diuréticos benzotiodiazínicos.

El diazóxido intravenoso reduce la presión arterial sistémica y pulmonar dentro de los 3 a 5 minutos y aumenta el volumen minuto cardíaco, la frecuencia cardíaca, el volumen sistólico y la velocidad de eyección del ventrículo izquierdo. La filtración glomerular cae y hay una reducción en la excreción urinaria de sodio, potasio, bicarbonato, ácido úrico y agua. Estos cambios duran entre 4 y 12 horas y pueden precipitar insuficiencia cardíaca en hipertensos urémicos, que puede generalmente superarse con la administración de un diurético tiazídico o con furosemida. La relajación del músculo liso uterino y la hiperglucemia que se observan son debidas a la disminución en la secreción de insulina y al aumento en la liberación de catecolaminas. La hiperglucemia responde a la administración de insulina o de una sulfonilurea. El diazóxido aumenta la secreción de renina.

El mecanismo exacto por el cual el diazóxido produce vasodilatación es desconocido, aunque existen evidencias de que, de alguna manera, inhibe la fosfodiesterasa e interfiere con los receptores vasculares al calcio. De este modo, aunque el diazóxido inhibe, no competitivamente, la constricción inducida por la noradrenalina en las preparaciones arteriales "deplecionadas" de calcio, la respuesta vascular es restaurada por el agregado de calcio. Esta acción puede ser mediada por el bloqueo de los receptores al calcio, la "depleción" intracelular de calcio o el bloqueo de su liberación intracelular.

El diazóxido tiene la característica, poco común, de que su vida media plasmática es considerablemente mayor que su efecto hipotensor. Aunque el mecanismo no es claro, puede estar relacionado con un alto grado de unión reversible con las proteínas plasmáticas. La droga es excretada principalmente como tal.

Los efectos colaterales pueden variar desde condiciones severas, tales como la insuficiencia cardíaca, debida a la retención de agua y electrolitos, y la hipotensión severa, marcada, que responde a la noradrenalina, hasta angina, arritmias, vómitos, dolor abdominal y una sensación de quemazón a lo largo de la vena usada para la administración de la droga. El edema debido a la retención de agua y sodio responde a los diuréticos tiazídicos; éstos, sin embargo, pueden agravar los efectos hipotensores e hiperglucemiantes del diazóxido. La angina, la taquicardia y las arritmias cardíacas pueden controlarse generalmente con la administración de agentes bloqueantes beta.[14, 19]

Las indicaciones terapéuticas del diazóxido en la hipertensión son indefinidas hasta el momento, pero puede emplearse para tratar la encefalopatía hipertensiva y, combinado con furosemida, en la hipertensión severa asociada con nefritis aguda o insuficiencia renal. El diazóxido puede combinarse exitosamente con un agente bloqueador beta adrenérgico en el tratamiento

de la disección aguda de la aorta, pero debe usarse con gran precaución en la hipertensión con insuficiencia coronaria. La caída de la presión arterial que ocurre en respuesta al diazóxido no es siempre dosis dependiente, y respuestas hipotensoras peligrosas pueden ocurrir en pacientes de edad y en pacientes con enfermedad cerebrovascular. El diazóxido oral es útil, pero de eficacia incierta.[22]

El nitroprusiato de sodio (Fig. 5) es un antagonista funcional de la noradrenalina, la histamina y la acetilcolina, con una acción muy fugaz debido a su rápida conversión en tiocianato, que es excretado por la orina. A diferencia de otros vasodilatadores usados en el tratamiento de la hipertensión, el nitroprusiato de sodio relaja el músculo liso de las venas y arteriolas aproximadamente en igual grado. Se observa acumulación de sangre en las venas y ésta es responsable de la caída en el volumen minuto, particularmente en la posición erecta, y de un aumento reflejo de la frecuencia cardíaca. El flujo sanguíneo renal y la filtración glomerular difícilmente cambian, pero la actividad renínica del plasma aumenta.[14, 21]

Las soluciones de nitroprusiato de sodio deben ser preparadas en el momento y guardadas, sólo por pocas horas, en la oscuridad.

Después de la administración prolongada de nitroprusiato, especialmente en pacientes con insuficiencia renal, puede acumularse tiocianato, que algunas veces produce debilidad, náuseas, campanilleo, psicosis e hipotiroidismo. La excesiva vasodilatación e hipotensión puede producir síntomas tales como sudoración, vómitos,

NITROPRUSIATO DE SODIO DIHIDRATO

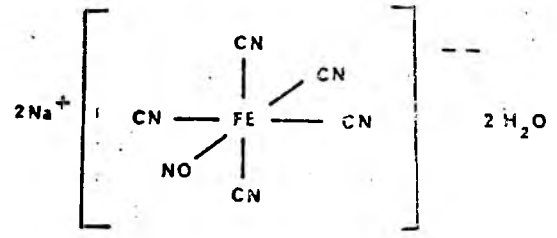
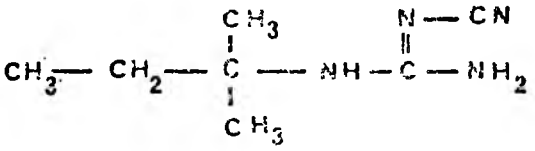


Fig. 5

GUANCIDINA



1-CIANO-3-T-AMILGUANIDINA

Fig. 6

insomnio, dolor de cabeza, palpitaciones y dolor torácico, que pueden evitarse con el cuidadoso registro de la presión arterial.

El nitroprusiato es usado en diversas crisis hipertensivas y disminuye rápidamente la presión arterial a los niveles deseados. La respuesta es regulada fácilmente alterando la velocidad de infusión, pero los pacientes deben ser cuidadosamente controlados. La falta de efecto sobre el volumen minuto y el volumen sistólico representa una desventaja cuando se necesita un aumento en la perfusión tisular o cuando existe coartación de la aorta, pero es una ventaja importante en pacientes con disección de la aorta o severa enfermedad coronaria.[14, 21]

Otros vasodilatadores han sido sintetizados y usados en el tratamiento de la hipertensión durante los últimos diez años. La guancidina (Fig. 6) neutraliza el efecto vasoconstrictor de la renina, de la angiotensina, y en cierto grado, el de la adrenalina y la noradrenalina.[23] Produce dilatación tanto de los vasos de resistencia como de capacitancia, y los reflejos simpáticos no son afectados. En consecuencia, puede aparecer taquicardia, pero la hipotensión ortostática no representa un problema. Como otros vasodilatadores, la guancidina produce retención de líquido.[10]

La bupicomida tiene una acción similar a la de la hidralazina. Produce relajación del músculo liso arteriolar, con la consiguiente caída de la resistencia periférica vascular y aumento reflejo de la frecuencia cardíaca, del volumen minuto y de la velocidad de eyección del ventrículo izquierdo. La filtración glomerular no es afectada por la

bupiconida, indicando que determina una significativa dilatación de los vasos aferentes y eferentes.[5]

Los vasodilatadores discutidos hasta aquí son familiares a los clínicos que se dedican a la hipertensión; algunos han sido usados durante años. Investigaciones en curso indican que una variedad de nuevos compuestos relacionados con las prostaglandinas y con la angiotensina II pueden ser desarrollados a corto plazo para el uso clínico.

Las prostaglandinas E_2, F_1 y A reducen la presión arterial en los individuos hipertensos [10] aunque los efectos cardiovasculares de las prostaglandinas varían según las especies. La caída de la presión arterial es probablemente debida a vasodilatación; las prostaglandinas dilatan las arteriolas del músculo esquelético y de la piel, y la prostaglandina E_1 antagoniza los efectos vasculares de las catecolaminas, de la vasopresina y de la angiotensina.[12, 13] La inyección intravenosa de prostaglandina E_1 produce taquicardia en el hombre, pero reduce el volumen minuto cardíaco.[2] Las prostaglandinas naturales como agentes hipotensores tienen una aplicación más bien limitada por su acción breve y sus efectos sistémicos desagradables; sin embargo, existe la posibilidad de obtener la síntesis de análogos adecuados de las prostaglandinas.

El papel de la angiotensina en la regulación de la presión arterial no está claro, a pesar de los años invertidos en estudios cuidadosos tanto en el laboratorio como en la clínica. Sin embargo, presenta gran interés el desarrollo de antagonistas de la angiotensina capaces de reducir la presión arterial en ciertas condiciones. El 1-sarcosina-8-alanina-angiotensina II es un antagonista de la angiotensina que actúa en el receptor del músculo liso vascular y disminuye la presión arterial en el hombre o en los animales de laboratorio si la actividad renínica del plasma está elevada por encima de los niveles controles por la "depleción" salina o por patología vásculorenal.[6, 8]

Existen varios otros análogos, pero su uso estará limitado por algún tiempo al campo de la investigación.

Resumen

Se discute la farmacología de los vasodilatadores usados en el tratamiento de la hipertensión. Los agentes usados más comúnmente son: el prazosin, la hidralazina, el minoxidil, el diazóxido y el nitroprusiato, presentando distintas características en lo que se refiere a la vía de administración, la duración de su actividad y los efectos colaterales que determinan. La mayoría de los vasodilatadores produce taquicardia refleja y edema debido a la retención de agua y sodio. Nuevos compuestos derivados de las prostaglandinas y de la angiotensina pueden desarrollarse en el futuro para su uso farmacológico en el hombre.

Summary

TREATMENT OF HYPERTENSION: THE PHARMACOLOGY OF VASOACTIVE AGENTS.

Vasodilators used in the treatment of hypertension are discussed in terms of their pharmacology. Prazosin, hydralazine, minoxidil, diazoxide and nitroprusside are the agents most commonly used and vary in their route of administration, duration of action and side effects. Most vasodilators cause reflex tachycardia and edema due to sodium and water retention. New compounds derived from prostaglandins or angiotensin may be developed in the future.

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Discusión

Dr. Conway: El diazóxido es estructuralmente similar a los diuréticos tiazídicos; en realidad, si se lo administra intraarterialmente es un diurético. También es un potente vasodilatador. La acción diurética se puede demostrar por experimentos de "stop-flow", y es similar al efecto de la clorotiazida. Cuando se lo administra por vía oral o endovenosa predomina la acción vasodilatadora y, en consecuencia, se producen una serie de efectos reflejos que anulan su capacidad diurética y llevan a la retención de sodio. Para determinar su acción diurética se lo infunde en la arteria renal de una preparación inervada; produce vasodilatación renal y el efecto diurético aparece a nivel de los túbulos, aparentemente en el mismo sitio de acción de la clorotiazida.

Dr. Robertson: En dos pacientes tratadas con prazosin he observado la aparición de un deseo urgente y doloroso de orinar, que desapareció al suspender la droga. No estaba relacionado con infección urinaria. Sé de otro caso similar también en una mujer. Por otra parte, en una paciente tratada con diazóxido observé la aparición de hirsutismo. Dr. Leary, ¿tiene usted alguna otra información al respecto?

Dr. Leary: Con respecto al primer problema, no lo he observado en mis pacientes y no sé cuál puede ser el mecanismo. Con respecto al hirsutismo, se ha pensado que puede ser consecuencia del mayor flujo sanguíneo a nivel de la piel. Sin embargo, es difícil explicar la limitada distribución del pelo. Pienso que estas drogas pueden tener un efecto común, posiblemente a nivel endocrino.

Dr. Langer: Dr. Leary, ¿tiene usted alguna explicación para el período de latencia, de alrededor de cuatro semanas, entre el comienzo del tratamiento con prazosin y la verificación de efectos clínicos de naturaleza antihipertensiva, y si durante ese período de latencia se puede demostrar el desarrollo del efecto vasodilatador y del bloqueo alfa?

Dr. Leary: No, no tengo explicación para el período de latencia, sobre todo teniendo en cuenta la rápida eliminación de este compuesto. Existe un pequeño porcentaje de pacientes que responden agudamente y, en estos casos, se pueden producir accidentes graves. Uno de nuestros pacientes de edad avanzada falleció aparentemente por este motivo.

Dr. Langer: Quisiera saber si al suspender la droga se observa persistencia de los efectos antihipertensivos por cierto tiempo. Eso sucede muchas veces con drogas que

tienen un período de latencia prolongado antes de establecerse sus efectos terapéuticos.

Dr. Leary: En mi experiencia la respuesta es no. No parece haber una prolongación de los efectos después de suspender la droga comparable con el período de latencia. Puede existir un período de prolongación del efecto de unos pocos días a una semana, pero no más.

Dra. Basso: En el tratamiento ambulatorio de la hipertensión arterial con las drogas vasoactivas disponibles, ¿cuál es, en su opinión, la más efectiva y con menor incidencia de efectos colaterales?

Dr. Leary: Creo que es muy difícil contestar a esa pregunta. No administraría como rutina vasodilatadores, a menos que el paciente no responda a la terapéutica más simple, como puede ser un diurético con o sin el agregado de un beta bloqueante. En este momento mi esquema es agregar, en caso de que sea necesario, prazosin a la combinación mencionada. No utilizamos hidralazina porque en nuestra área vemos muchos casos de lupus eritematoso diseminado, y no empleamos el minoxidil porque no está disponible en nuestro mercado. Las desventajas de los vasodilatadores orales son comunes a todos. Tampoco usamos diazóxido por razones regionales, ya que el 25-35 % de nuestros pacientes de origen hindú tiene diabetes mellitus y esta droga posee un efecto antiinsulínico.

PAPER G2

Effectos Colaterales Adversos En El Tratamiento De La Hipertension Humana

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EFECTOS COLATERALES ADVERSOS EN EL TRATAMIENTO DE LA HIPERTENSION HUMANA

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La incidencia de la hipertensión arterial en el mundo es de tal magnitud que los médicos suelen frecuentemente enfrentarse a ella haciendo necesario un acabado conocimiento de las reacciones adversas que pueden ocurrir durante su tratamiento. Estas reacciones pueden aparecer como cambios en los signos clínicos, en los síntomas o en los datos del laboratorio relacionados con la enfermedad hipertensiva o ser producidas por los medicamentos utilizados en el manejo de la enfermedad. Tales reacciones pueden ser predecibles dadas las características farmacológicas de la medicación utilizada o impredecibles y no relacionadas con las acciones farmacológicas de los medicamentos.

En líneas generales, las reacciones adversas a los agentes antihipertensivos pueden estar: *a)* relacionadas con los agentes antihipertensivos *per se; b)* producidas por interacciones entre ellos o, *c)* causadas por interacciones entre los fármacos antihipertensivos y las drogas utilizadas para el tratamiento de enfermedades independientes.

El tratamiento de elección para la hipertensión varía ligeramente de un lugar a otro, pero la aspiración común de todos los médicos es controlar la enfermedad y remediar los síntomas evitando los efectos colaterales. Esto es particularmente importante, dado que la hipertensión es una enfermedad potencialmente letal que generalmente sólo presenta sintomatología después de largo tiempo de evolución y por ello, los pacientes, sin una hipertensión complicada, se resisten a continuar la terapia si ésta les causa síntomas desagradables. A continuación, se revisarán algunas de las reacciones a los medicamentos, que deben ser reconocidas por los médicos para el bienestar de sus pacientes.

Cierto número de efectos colaterales son comunes a varios agentes antihipertensivos, por lo tanto no serán mencionados repetidas veces. La hipotensión, el letargo, la depresión, la congestión nasal o rinitis, síntomas gastrointestinales, el dolor de cabeza, vértigos y manifestaciones alérgicas pueden ocurrir con variada frecuencia y han sido asociados con los bloqueantes adrenérgicos y con los vasodilatadores [1].

Efectos adversos asociados con las distintas clases de drogas

Los diuréticos son ampliamente utilizados en el inicio del tratamiento de la hipertensión; la mayoría de los médicos prefieren los derivados de las tiazidas o aquellos que tienen menor efecto perdedor de potasio. Las reacciones adversas al tratamiento con estas drogas son bien conocidas y están resumidas en la Tabla 1.

Además de estos efectos indeseados de los diuréticos han sido descriptas varias interacciones con otras drogas [2]. La ototoxicidad producida por los aminoglucósi-

TABLA 1. — *Reacciones adversas a los diuréticos*

Derivados benzotiadizínicos y furosemida:	
Diabetes	
Hipoglucemia neonatal	
Hiperuricemia	
Hipocalemia	
Hiperlipidemia	
Síndrome hiperosmolar	
Diuréticos de pobre efecto perdedor de potasio:	
Hipercalemia	
Ginecomastia	
Carcinoma de mama	Espironolactona
Anemia megaloblástica	Triamtirene

dos puede ser aumentada por la furosemida o el ácido etacrínico, aunque este último rara vez es usado para el tratamiento de la hipertensión. También los diuréticos refuerzan la tendencia a la hiperglucemia producida por el diaxózido y potencian marcadamente el efecto hipotensor de los bloqueantes adrenérgicos, lo que puede causar problemas en pacientes no suficientemente vigilados.

En la literatura médica se han descripto más de 50 reacciones adversas al tratamiento de la hipertensión con los *bloqueantes beta* [1-3]. Sin embargo, las complicaciones más comúnmente registradas en los pacientes que utilizan estos medicamentos son el espasmo bronquial, el desencadenamiento de insuficiencia cardíaca y alteraciones circulatorias periféricas que especialmente aparecen si se utiliza un compuesto no selectivo para receptores beta y carente de actividad simpaticomimética intrínseca. Los bloqueantes beta se asemejan estructuralmente entre sí y tienen efectos colaterales similares, aunque han sido descriptas ligeras diferencias farmacológicas entre ellos. Estas diferencias pueden ser muy marcadas en el laboratorio, pero esto no garantiza su real comportamiento en el hombre. De este modo, drogas con actividad intrínseca demostrable, tales como el oxprenolol, pueden desencadenar insuficiencia circulatoria en aquellos pacientes cuya función ventricular está alterada [3]. En los pacientes asmáticos, los bloqueantes beta relativamente selectivos tales como el acebutolol y el metoprolol ocasionalmente producen espasmo bronquial. La incidencia de frío en las extremidades y la ausencia de pulsos periféricos asociada con los bloqueantes beta varía ligeramente y hay cierta evidencia que tanto la posesión de actividad intrínseca como la selectividad beta, disminuyen el riesgo de esta complicación. Parece ser que la liposolubilidad de los bloqueantes beta o de sus metabolitos, facilitan la penetración en el sistema nervioso central y podría explicar la relativa frecuencia de sueños y alucinaciones que aparecen cuando se usa el propranolol y el pindolol. Las evidencias acumuladas sugieren que las complicaciones oculares y la peritonitis esclerosante rara vez se relacionan con el uso de otros bloqueantes beta que no sea el practolol.

Los bloqueantes beta rara vez se ven envueltos en serias complicaciones por interacción con otras drogas, aunque se pueden observar severas respuestas hipertensivas, debido a la liberación de noradrenalina, por la administración de fenilpropanolamina comúnmente presente en los medicamentos para el resfrío.

También existe la posibilidad teórica de que los hipertensos asmáticos, tratados con bloqueantes beta, puedan responder adversamente a los efectos alfa adrenérgicos de la adrenalina administrada para el espasmo bronquial. A dichos hipertensos asmáticos solamente se les debería administrar bloqueantes selectivos beta y nunca antes de haber probado otra terapia. Se han presentado evidencias sugiriendo que los hipertensos diabéticos deberían ser tratados con bloqueantes selectivos beta tales como el metoprolol y el acebutolol más bien que con drogas no selectivas como el propranolol.

El bloqueo de los receptores $beta_2$ puede enmascarar signos y síntomas de hipoglucemia aguda y demorar los mecanismos reguladores de la homeostasis. La administración de anestésicos generales a pacientes cuya presión arterial está controlada por los bloqueantes beta adrenérgicos está en controversia, pero de cualquier modo deberían monitorearse las respuestas cardiovasculares durante la anestesia.

Los *vasodilatadores* han reingresado a

la terapéutica favorecidos por la aparición del prazosin y el minoxidil. Los vasodilatadores puros generalmente producen retención de sodio y agua con ganancia de peso y edema, taquicardia refleja y las respuestas no específicas a los agentes antihipertensivos previamente nombradas, incluidos los trastornos gastrointestinales y la depresión [4-5]. La hidralazina también ha sido asociada a un síndrome similar al lupus eritematoso, pero esto generalmente ocurre con pacientes que han sido tratados con más de 200 mg por día. Esta situación se ha asociado con la baja acetilación de la hidralazina, siendo más común en las mujeres blancas que en cualquier otra clase de pacientes y es revertida por la interrupción de la terapia. El minoxidil produce hipertricosis en más del 50 % de los pacientes y en las mujeres puede tomar la forma de marcado hirsutismo. Hipotensión postural severa y colapso pueden ocurrir dentro de la primera hora de la ingesta de prazosin. Por esta razón, la mayoría de los clínicos piensan que una pequeña dosis de prueba, no mayor de 0.25 mg, debe ser dada cuando el prazosin es lo primero que se prescribe y que los pacientes deben permanecer bajo vigilancia médica por lo menos durante tres horas después de administrada la primera dosis [5].

Los diuréticos disminuyen el edema y la retención de líquidos y también potencian el efecto hipotensivo de los vasodilatadores. El uso concomitante de los agentes bloqueadores beta anula la taquicardia refleja y también puede añadir sus efectos aditivos beneficiosos sobre la hipertensión. La administración simultánea de fenilbutazona, corticoesteroides, carbenoxolona y anticonceptivos orales puede interferir con los efectos de estos y de otros medicamentos antihipertensivos por la producción de retención líquida. El alcohol, los sedantes e hipnóticos, los tranquilizantes, la L-dopa y los anestésicos generales potencian el efecto de todos los fármacos antihipertensivos, inclusive los vasodilatadores.

En la mayoría de los pacientes, la elevada presión arterial puede ser satisfactoriamente controlada mediante la administración criteriosa de diuréticos, bloqueantes beta o una combinación de estos fármacos con o sin el agregado de vasodilatadores [6].

Sin embargo, varios de los otros agentes antihipertensivos son aún usados frecuentemente y merecen mencionarse en una revisión de esta naturaleza.

Los *bloqueantes adrenérgicos*, tales como la guanetidina, producen frecuentemente hipotensión asociada con los cambios posturales y el ejercicio. Se han descripto anomalías en la función hepática, trombocitopenias, neutropenias, reacciones dermatológicas tales como prurito, eritemas multiformes, rush penfigoide y dermatitis exfoliativa.

Durante el tratamiento con debrisoquina puede presentarse edema y dolor parotídeo y una reacción hipertensiva a la tiramina. Las perturbaciones de la función sexual en el hombre pueden ser particularmente importantes.

Gran número de fármacos suelen interactuar con los medicamentos similares a la guanetidina. Una vasodilatación adicional, debida al alcohol o a otros vasodilatadores, puede ser particularmente inconveniente. La combinación con diuréticos o agentes bloqueantes beta adrenérgicos potencia el efecto de los bloqueadores neuronales adrenérgicos sobre la presión arterial, y puede agravar la hipotensión postural. Los antidepresivos tricíclicos interfieren con la captación de las guanidinas por la neurona adrenérgica, por lo tanto interfieren con la acción antihipertensiva de estas drogas. Este efecto es también compartido, en cierto grado, por los derivados fenotiazínicos [5].

Las anfetaminas, la efedrina, la fenilefrina y el anorexígeno dietilpropión desplazan a la guanetidina de sus receptores adrenérgicos, interfiriendo de este modo con sus efectos hipotensores y aumentando el riesgo de arritmias cardíacas. La guanetidina también potencia el efecto de la cocaína, del curare y de algunos anestésicos gaseosos.

La *alfa metildopa* produce efectos colaterales en un 70-75 % de los pacientes [1] pero estas reacciones generalmente no son específicas ni severas. Pueden aparecer dificultades en la capacidad de concentración y en la realización de operaciones

simples, pero las interferencias con la regulación simpática generalmente son menos severas que las observadas con los bloqueantes de las neuronas adrenérgicas. El test de Coombs se encuentra positivo en hasta un 30 % de los pacientes tratados con alfa metildopa durante más de seis meses, especialmente si las dosis usadas son altas. La anemia hemolítica es rara, y cuando ocurre no es severa y desaparece espontáneamente después de suspender la terapia. En un tratamiento ininterrumpido por más de un año pueden elevarse las transaminasas del plasma y aparecer signos de colestasis. Generalmente se retorna a la normalidad cuando se suspende el tratamiento, pero han sido descriptas hepatitis crónicas que pueden llevar a la necrosis hepática si la droga es nuevamente administrada. Esta forma de hepatitis probablemente tenga un componente inmune o de hipersensibilidad y en algunos pacientes el fenómeno L. E. es positivo [5].

Las interacciones con los diuréticos y los bloqueantes beta adrenérgicos generalmente son favorables con la consecuente disminución de la presión arterial y la posibilidad de reducir la dosis de alfa metildopa. Sin embargo, la acción hipotensora de la alfa metildopa puede disminuir por la acción central de la reserpina y los antidepresivos tricíclicos. La alfa metildopa anula la respuesta terapéutica de la L-dopa, presumiblemente por una inhibición competitiva, y la presión arterial puede aumentar inesperadamente si drogas de acción simpaticomimética indirecta son administradas a los pacientes tratados con alfa metildopa.

La *reserpina* es aún ampliamente usada en los países en desarrollo dada su relativa eficacia y el bajo costo en comparación con otros preparados. Es bien sabido que esta droga puede producir depresión y otros cambios de la personalidad y también se la ha asociado con el desarrollo del parkinsonismo. Aparte de los trabajos del grupo de Boston [5], hasta el momento no hay evidencia cierta de que produzca desarrollo de cáncer de mama en las mujeres postmenopáusicas. La reserpina potencia los efectos de las drogas sedantes y antihipertensivas, pero ha sido descripto antagonismo con el curare, los anestésicos locales y los antidepresivos tricíclicos. La combinación de la reserpina con los digitálicos puede desencadenar arritmias cardíacas.

La clonidina y sus compuestos relacionados pueden controlar la presión arterial sin producir hipotensión ortostática, pero cuando causan una marcada sedación suelen interferir con las actividades diarias. La suspensión brusca de la terapéutica, en pacientes sin complicaciones o en aquellos con signos de toxicidad tales como vómitos o severas diarreas, puede producir un peligroso aumento de la presión arterial, alteraciones nerviosas, temblores, sudoración y dolores de cabeza.

La clonidina potencia los efectos de los depresores incluyendo el alcohol y los sedantes, y puede antagonizar los efectos de los bloqueantes beta adrenérgicos y de los antidepresivos tricíclicos.

Los fármacos descriptos hasta aquí, son los usados generalmente hoy para el manejo de la hipertensión. *El diazóxido y el nitroprusiato de sodio* son utilizados comúnmente en aquellas situaciones en que una peligrosa elevación de la presión arterial requiere su inmediata reducción. La rápida disminución de la presión arterial mediante el diazóxido, puede producir dificultades en la perfusión tisular incluyendo el cerebro y las coronarias y marcada hiperglucemia que precede a la cetoacidosis. Este preparado también provoca reacciones hematológicas y atraviesa la barrera placentaria con la consiguiente toxicidad para el feto. El uso prolongado de este medicamento produce hiperglucemia, retención de sodio y agua, hipertricosis e hiperuricemia. También han sido descriptos accidentes de pancreatitis aguda.

Los efectos antihipertensivos, hiperuricémicos e hiperglucemiantes están potenciados por el uso concomitante de las tiazidas. Los bloqueantes beta adrenérgicos aumentan los efectos hipotensivos de esta droga pero bloquean el aumento de la frecuencia cardíaca y del volumen minuto. In vitro, el diazóxido desplaza a la warfarina de sus sitios de unión con las

proteínas plasmáticas, por lo cual haría necesario modificar las dosis de anticoagulantes en el hombre. La clorpromazina potencia los efectos hiperglucemiantes del diazóxido.

La infusión de nitroprusiato tiene un efecto hipotensor casi inmediato producido por vasodilatación y acompañado por taquicardia refleja y un incremento en el volumen minuto [7]. Si la infusión es demasiado rápida, pueden aparecer sudoraciones, náuseas, vómitos, ansiedad y espasmos musculares. El nitroprusiato se convierte en cianógenos, cianidos y tiocianatos. Si los niveles de cianidos llegan a valores relativamente altos, puede ocurrir la muerte por envenenamiento. La velocidad de infusión no debe exceder los 10 μg/min.

En esta revisión se ha enfatizado sobre los efectos colaterales y las interacciones de las drogas más corrientemente utilizadas en la terapia de la hipertensión. No se ha hecho mención de algunos nuevos compuestos, porque no es posible suministrar detallada información de todas las drogas accesibles y porque las reacciones adversas a algunos de estos agentes sólo se harán aparentes cuando hayan sido usados durante un largo período. En este momento es razonable sugerir que los médicos que tratan la hipertensión tengan información de tres o cuatro buenas drogas y se aseguren un acabado conocimiento de sus efectos colaterales más frecuentes.

De todas maneras, deben estar atentos para modificar los regímenes terapéuticos cuando nuevas y efectivas drogas, con menos efectos colaterales, estén a su alcance. Yo sugeriría, sin embargo, que tales cambios no sean hechos a la ligera sino cuando la experiencia general en numerosos centros haya indicado las características de las nuevas preparaciones desarrolladas.

Resumen

La incidencia de la hipertensión es de tal magnitud que los médicos la tratan frecuentemente y a menudo aparece en pacientes que padecen otras enfermedades. Por lo tanto, es necesario que las reacciones adversas a los agentes antihipertensivos sean del conocimiento del médico práctico. En términos generales tales reacciones pueden estar: a) *relacionadas con los agentes antihipertensivos* per se; b) *producidas por interacciones entre ellos, o* c) *causados por interacciones entre los fármacos antihipertensivos y las drogas utilizadas para el tratamiento de enfermedades independientes. Las reacciones adversas de la clase* a) *consisten en aquellas vinculadas con la dosis dada y las que dependen de las propiedades farmacológicas del medicamento. Por ejemplo, los bloqueantes beta adrenérgicos efectivamente disminuyen la presión arterial, pero sus efectos sobre los receptores beta pueden causar cierto grado de espasmo bronquial. Este efecto se hace más manifiesto con el aumento de la dosis. Los agentes antihipertensivos también pueden causar una gran variedad de efectos colaterales debidos a la pérdida de su especificidad, lo cual se traduce en acciones sobre un gran número de receptores no relacionados con el control de la presión arterial. Estas respuestas incluyen síntomas bien conocidos, tales como sequedad de la boca, fatiga, dificultades sexuales y trastornos gastrointestinales. Las interacciones entre los agentes antihipertensivos ocurren menos frecuentemente de lo que podría preverse en una enfermedad generalmente tratada con la polifarmacia. Cuando se agregan nuevos fármacos a un tratamiento en curso, el problema más frecuente es la sobredosis, con la consiguiente severa hipotensión. La hipertensión puede aparecer en pacientes con tratamiento medicamentoso para diversas patologías, tales como la depresión, artritis, epilepsia, parkinsonismo, obesidad y aun el resfrío común. Ocurren importantes interacciones entre los fármacos antihipertensivos y los agentes antidepresivos tanto los tricíclicos como aquellos que actúan indirectamente a través de una acción simpatomimética. El médico clínico debería tratar de evitar la posibilidad de que ocurran estos efectos indeseables en sus pacientes hipertensos.*

Summary

DRUG INTERACTIONS AND ADVERSE REACTIONS IN THE TREATMENT OF HYPERTENSION.

The incidence of hypertension is such that medical practitioners treat the condition frequently, often in patients suffering from other diseases in addition. Thus, it is imperative that adverse responses to antihypertensive agents be common knowledge. In general terms such responses may be, a) *related to antihypertensive agents* per se; b) *due to interactions between different antihypertensives or,* c) *caused by interactions between antihypertensives and drugs given as treatment for unrelated complaints. Adverse reactions in class* a) *above, may be further subdivided depending upon their relationship to dose given and to the intrinsic pharmacological properties of the medicine. Thus beta-adrenergic blockade may effectively lower blood pressure, but effects upon beta-2-receptors can be expected to cause some degree of bronchospasm. This effect becomes more apparent with increasing dose. Hypotensive agents may also cause a variety of side effects due to their lack of specificity which results in actions upon a wide range of receptors unrelated to blood pressure control. These responses include well-known symptoms such as dryness of the mouth, fatigue, sexual difficulties and gastrointestinal upsets. Interactions between antihypertensive agents occur less commoply than might be anticipated in a condition frequently treated by polypharmacy. The commonest problem is over-dosage with consequent severe hypotension occurring when new agents are added to an existing treatment regimen. Hypertension may occur in patients undergoing drug treatment for a variety of conditions including depression, arthritis, epilepsy, Parkinsonism, obesity and even the common cold. Important interactions occur between antihypertensive agents indirectly acting sympathomimetically and tricyclic antidepressants, which diminish antihypertensive drug effects. The practicing physician should attempt to avoid the possibility of all these undesirable responses occurring in his hypertensive patients.*

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