Thomas Hamilton Bothwell

Tributes and Biographical Notes

Thomas Hamilton Bothwell was born in Johannesburg on the 27th of February 1926. He won a scholarship to St John’s College and this set the pattern for his highly successful academic career. He graduated from the Witwatersrand Medical School in 1947 having won the Bronze Medal of the Southern Transvaal Branch of the Medical Association of South Africa for the most distinguished medical graduate of the year as well as the Medical Graduates Association Prize for the best final year student in the subject of Medicine. Twenty years later he was appointed Professor and Head of the Department of Medicine, a post which he filled with great distinction until his retirement at the end of 1991. However, both the Faculty of Medicine and the University prevailed upon him to accept the onerous post of Dean so that his numerous talents would be available to lead the Faculty into the new South Africa.

Tom Bothwell has all the requirements of a professor in abundant measure. He is an internationally renowned researcher, a lucid inspiring teacher of under- and post-graduates, a highly skilled compassionate physician and an excellent administrator. It is indeed rare to have all these talents combined in a single individual.

His research career was greatly boosted by a Nuffield Travelling Fellowship to Oxford and a Lederle Travelling Fellowship to the University of Washington (1954-56), where his research skills were honed to the cutting edge of knowledge in his chosen field - iron metabolism. Over the last 40 years the research programme he developed covered virtually every facet of iron metabolism and he exploited fully the unique opportunities provided for this in South Africa: major disturbances of iron balance in different population groups, such as iron overload in Black males and iron deficiency in Indian females. These are problems affecting large segments of the population; in fact iron deficiency is the commonest nutritional disease in the world today. Every aspect of iron metabolism was explored including the biochemical and molecular processes involved in cellular iron transport. He has made highly significant contributions to medical science with the help of young men and women whom he has trained. For his research, Professor Bothwell has received many awards, including an honourary fellowship of the American College of Physicians, an honourary doctorate from the University of Cape Town and the Gold Medal of the South African Medical Research Council.

He is an outstanding teacher and his lectures are a model of clarity, fluency and logic. He received the PV Tobias Award for Excellence in Clinical Teaching and has supervised about 20 doctoral theses. During his tenure of the chair, the standard of teaching in his department has risen steadily, as well as the quantity and quality of research output. The clinical services rendered by the Department of Medicine with its generalists and specialists in the various teaching hospitals is of the very highest standard and the product of our Medical School is one of the best in the world. Professor Bothwell has made a major contribution to these achievements.

In addition, he has played seminal roles in administration and organization within his hospital, medical school and university as well as internationally, reaching peak positions in every sphere. Through this position on three important South African bodies, the Medical and Dental Council, the Medical Research Council and the Scientific Advisory Council of the Prime Minister he helped shape research, professional policy and ethical behaviour. He has been an expert adviser to the World Health Organization, the International Atomic Energy Agency and the International Nutritional Anaemia Consultative Groups amongst many others.

At the present time he is a member of the University Council, the Council of the College of Medicine and the National Health Advisory group of AIDS and numerous other committees.

What sort of man can accomplish so much? Tom Bothwell is a warm, caring, compassionate person with a good sense of humour who has an open door policy making his advice, counsel and knowledge freely available to all who seek it. We are indeed fortunate to have him as Dean of the Faculty of Medicine.

Saul Zwi
Professor of Pulmonology
Department of Medicine
University of the Witwatersrand
An Element of Life - Iron in the Souls - a tribute to Thomas Hamilton Bothwell

In haem and globin smoothly wrapped
Red to its ferrous core
Oxygen is tightly trapped
Niched in metallic jaw.
Iron carries breath to brain
Nourishment for thought
Traversing artery and vein
Haemic ships to port.
Electrons in a redox trance
Spin in transition state
Orbiting they leap and dance
Up to frenetic rate.
Life's from an element of Mars
Struck from thee cosmos and the stars.

Thomas H Bothwell - lamplighter

Most of us who enter the medical profession encounter a mentor who ignites in us a spark that kindles a lifelong flame to illuminate our way through the dark and fascinating mysteries of medicine. Tom Bothwell lit such a flame in me during my research fellowship in the Department of Medicine. It created a glow that stimulates me to shed light on the shadowy perimeter of our circle of growing knowledge and understanding. I am a small part of the beacon that is biomedical research. Though my light sometimes flickers in the gusts of day-to-day existence, it is not extinguished. Tom Bothwell is a master lamplighter.

Ralph Green
Chairman, Department of Laboratory Hematology,
The Cleveland Clinic Foundation
Cleveland
Ohio, USA
Former Clinical Research Fellow (1964-67)
Iron and Red Cell Metabolism Unit
Department of Medicine
University of the Witwatersrand

Although many medical students dream of becoming famous researchers the path to that goal is difficult and requires much encouragement and guidance, particularly at the early stages. My first introduction to research in medicine came in 1972 when I joined the Department of Medicine as a physician and became involved in the care of patients with haematologic illness and with the investigation of the problems of iron nutrition in Southern Africa.

Professor Bothwell was already renowned for his work in these areas, particularly that leading to the understanding that all non-haeme dietary iron forms a common pool in the gut and that absorption of any iron added to the diet is from this common pool. In addition much work had already been done by the Iron and Red Cell Metabolism Unit, under his leadership on the influence of exogenous ligands on the absorption of iron. The project I started was to look at the effects of gastro-intestinal secretions on the availability of dietary iron. I remember having something like 600 test tubes in racks in front of me. All were to have a variety of reagents added to them, the solubilised radiolabelled iron then to be extracted and analysed. When Tom Bothwell appeared at my side at the laboratory bench and fell to with pipettes and tubes I realised that the willingness to do hands on, and even tedious work, is one of the qualities needed in research. Of course his involvement wasn't just that of messing about with test tubes. The next phases, of analyzing the data and turning it into a comprehensive and elegant scientific report owed even more to his efforts. It was during that process that I saw that it is not just the initial idea but the stamina to see a project through to completion as well as the ability to modify the initial hypothesis in the light of the experimental findings that are all requirements in research. I count myself fortunate in having been associated with the Iron and Red Cell Metabolism Unit and having Professor Bothwell as my mentor.

Professor WR Bezwoda
Associate Professor
Department of Medicine
Clinical Head Division of Haematology/Oncology
Johannesburg Hospital and University of the Witwatersrand
Professor Bothwell’s name was well known to me long before we first met. As a clinical fellow in hematology, I have used his classical textbook on Iron Metabolism as a standard reference in my daily work. Later, as a research fellow at the department of Clem Finch in Seattle I have been able to work with some of the scientists and technicians who were closely associated with him during his long stay in the States. His work on Bantu siderosis, the series of outstanding publications on the role of ascorbic acid in iron overload, and his beautiful chapter defending the hereditary nature of idiopathic hemochromatosis are in my view some of the most important milestones in the evolution of clinical research in our generation. In addition to his outstanding ability as a scientist Tom has a unique ability to communicate with people, to get to the essence of things within the shortest time possible, and an exceptional ability to express himself in writing. When we first met in 1974 Tom had asked me whether I have regretted returning from the States to a small country with limited resources. He has certainly proved that truly great research has to do with the clinical problems unique to one's environment, and Tom has been instrumental in bringing this to the attention of the international community and elucidating the pathogenesis of a chapter in medicine which could be studied nowhere else but in South Africa.

Chaim Hershko
Professor of Medicine
Hebrew University Hadassah Medical School
Chairman, Department of Medicine
Shaare Zedek Medical Centre
Jerusalem, Israel

The privilege of working for a man like Professor Tom Bothwell is inestimable. I was granted that privilege and during the years 1980 and 1981, with the late Professor John Torrance, helped to transform the laboratory into a mixture of "Juicy Lucy" and a curry emporium. Out of this unlikely pot pourri, scientific papers emerged - but it was always under Professor Bothwell’s incisive direction that perspective about one's research endeavours was maintained, and its value extracted. His open office door meant that one could always approach him. This may have been to get advice about difficulties arising from the day’s work or may have been in response to a summons - usually a note on one's desk, sometimes with a skull and cross-bones drawn at the top! In either case one was met by the unfailing patience, guidance and encouragement of Prof Bothwell, and by advice frequently laced with his acerbic wit. I have known him not only as a mentor and brilliant teacher but also as a doctor whose insight, compassion and wisdom are inspirational. His retirement from the department of medicine marks the end of an era and I wish him a very happy and fulfilling retirement.

MA Gillooly
Ex Research Fellow
MRC Iron and Red Cell Metabolism Unit
Department of Medicine
University of the Witwatersrand.

I had the extraordinary privilege of knowing Professor Bothwell personally and working for him as an intern, registrar, research fellow and junior member of the faculty at the University of the Witwatersrand. I remember with nostalgia the excitement of those formative years of my career. I was stimulated by his intellect, marvelled at his incisive approach to complex clinical and laboratory problems, and enjoyed his friendship, dry sense of humour and the warmth of his personality. Although I regard myself as particularly fortunate to have had this association with Professor Bothwell, I realize that I am only one of thousands of Wits graduates whose career successes are the result of his inspiration and unselfish support. Over the years I have come to appreciate fully what a remarkable role model he has been for me and for a generation of South African doctors.

Professor Bothwell’s scientific contributions over the past forty years have earned him an international reputation as a pre-eminent leader in research related to iron metabolism and iron nutrition. His pioneering studies, leadership in promoting international collaboration and numerous authoritative publications including "Iron Metabolism in Man" which remains the standard reference work in the field, have been key elements in the extraordinary advances made over the last several decades and their application to alleviating human suffering.

Professor Bothwell, I join in congratulating you on your remarkable achievements in both medical research and in the teaching of clinical medicine. I thank you personally for your friendship, unwavering support and for inspiring me to share a small part of your "Adventures in the Iron Trade". I wish you and Alix every happiness and fulfilment in the new challenges you choose after your retirement.

Sean R Lynch
Professor of Medicine
Eastern Virginia Medical School
Norfolk, Virginia, USA.
Tom Bothwell: physician, researcher, friend

To have unusual abilities and put them to use for other; to recognise abilities in others and develop them to full potential; these are the hallmarks of men who achieve greatness. Tom Bothwell is such a man: As undergraduate student he excelled in his studies. I was able to observe this being one year ahead of him at Medical School. He emerged as an innovative researcher in 1948, when he encountered and was able to conclusively diagnose haemochromatosis in a patient. This set him on his course of research in Iron Metabolism in which he has become a world leader. He recognised talent, often latent at the time, and encouraged its development to produce outstanding researchers and academicians.

Tom Bothwell has always remained a clinician, humble because of his depth of knowledge. He has trained many men and women who are now devoted doctors. Tom Bothwell has been loyal to medicine, to research, to his University and to his many colleagues, students, friends and to his country. A man at his best!

Professor AJ Brink
Emeritus Professor of Cardiology and
Former Dean,
University of Stellenbosch Medical School
Former Director MRC

Whilst still a second year medical student, the late Professor Guy Elliott suggest that, in order to continue laboratory work, I approach Dr Thomas H Bothwell who had recently returned from the United States. This turned out to be a fortunate event that significantly influenced my future. It was an opportunity to acquire the discipline of sound clinical practice from a talented physician, and to benefit from his caring approach to patients. Concurrently, the fundamentals of good quality research were systematically but patiently instilled into me by a man of uncompromising principle and whose humility set a standard that remains hard to match. Foremost amongst the many pragmatic lessons learnt, often the hard way, was not to try and be something for which I was unqualified. This same philosophy if reflected in his monumental studies of iron metabolism where carefully selected laboratory-based techniques were applied to solving clinical problems. He never felt the need to be a biochemist, a statistician or a molecular biologist but nevertheless was always able to select from these options that method best suited to resolving a particular problem. The relevance of both his work and personal stature are internationally acknowledged. I consider it an honour and a privilege to have spent more than fifteen years as a beneficiary of his kind and wise counsel.

Peter Jacobs
Professor and Head
Department of Haematology
University of Cape Town

I first came across the thorough and effective work of Tom Bothwell in 1969 when I began some studies on the subcellular distribution of iron in the small intestinal mucosa. Since then we have met on many occasions usually at scientific conferences or workshops. His work has always been carefully planned and executed as well as being beautifully presented. His lectures are a model of clarity and he has always managed to pack a lot of information into a lecture without seeming to hurry. He was a founder member of the International Committee for Standardization in Haematology (Expert Panel on Iron) and has been a major contributor in terms of developmental work and at meetings of the Panel until his retirement from the Committee this year. His contribution will be greatly missed.

Mark Worwood
Reader in Haematology
University of Wales College of Medicine
Cardiff, Wales.

The majority of people can retired disappointed by the knowledge that their influence wanes with their departure. No such anonymity awaits Professor Bothwell. Illustrious academics live on, not only in their publications, but in the careers of other people who were fortunate enough to come under their influence.

Merlin H Sayers
Associate Professor of Medicine
Division of Hematology
University of Washington,
Seattle, USA.
I write to congratulate Professor Bothwell on his retirement as head of the Department of Medicine. I had both the good fortune and unique privilege to complete my medical internship and clinical research graduate student training under his guidance. I was further privileged to do post-graduate research work with him. He is, without doubt, the foremost South African medical academic of our time. His contributions include numerous pioneering yet definitive research publications in the area of iron metabolism, the effective teaching of under- and graduate students, a national and international leadership role in medicine, education and scientific research but most important, the non-compromising maintenance of academic excellence. These were made despite considerable internal and external forces mitigating against success. Professor Bothwell leaves a legacy of great intellectual achievement and inspiration but most of all, he leaves to both the University and the Department the challenge of maintaining and advancing academic standards.

RD Baynes  
Professor of Medicine  
University of Kansas  
Kansas City, Kansas, USA.

I have had the privilege to know Tom Bothwell for more than 30 years. Over many years he has made huge contributions in several important fields related to iron metabolism, clinical medicine and iron nutrition. His name is associated with many key papers on iron overload and his research comprises both clinical and experimental works. He and his group have done fundamental work in the field of iron absorption and he has translated his knowledge into difficult practical studies on iron fortification which have been elegantly carried through.

He belongs to that group that is never slandered. The reason for this is that he is a scientist, a teacher and doctor, who is a gentleman in its best meaning, devoted to all his tasks, absolutely straight forward, always honest and not the least having a wonderful sense of humour.

I really wish him well for the future.

Leif Hallberg  
Professor of Medicine  
University of Goteberg  
Sweden

Wits, more than most institutions, owes much of its excellence and most of its momentum to a small band of "giants". These giants are at one and the same time leaders, role models, known internationally for their research and known at home for their teaching. To their patients they are beloved physicians; to their colleagues, wise counsellors, good friends. They are givers; to their departments, their hospitals, their universities, the College of Medicine, the Medical Council. Tom Bothwell has deserved the "giant" rating ever since he first joined the Department of Medicine; we know because we were there. That he has been able to keep it up ever since while maintaining his "cool" and his sense of humour is an extraordinary achievement, and for this, credit must be shared with the other half of the team, Alix. We appreciate this opportunity to paying tribute to Tom and of thanking him for what he has given to institutions which we all care for.

Maurice McGregor  
Ex Dean, Prof of Cardiology  
University of McGill Medical School  
Margaret R Becklake  
Professor of Epidemiology and Pulmonology  
University of McGill Medical School

I remember a young physician who came to Seattle and made major contributions to the iron research program there, who subsequently became a leader in the field. While I may have taught you little about iron, I do remember with pleasure that I was able to show you Rome as you sat perched on the back of our rented vespa.

It is enough to publish seminal papers on iron absorption and iron overload, but to do this while successfully running a department of medicine is an accomplishment indeed. Your light has illuminated the shadowy world of iron metabolism, but more, you have been a pathfinder and inspiration for so many. Those of us who have been your peers join the many outstanding fellows you have parented in congratulating you and wishing you well in the future challenges that await you.

Clem Finch  
Emeritus Professor of Haematology  
University of Washington  
Seattle, Washington, USA.
It is a privilege and pleasure for me to extend my best wishes and warmest regards to Professor TH Bothwell who is about to retire from a truly distinguished carer. As my first mentor, he taught me academic rigor, the importance of a careful, honest and meticulous approach to research design, data, collection and interpretation, and the kind of commitment required to pursue an academic career in internal medicine. He has remained an inspiration to me and an individual in whom I hold in the highest respect. Any successes that I have can be attributed in large part to the excellent training I received while working with Professor Bothwell and his colleague, Professor RW Charlton. Professor Bothwell, I wish you health, happiness and hope that in your retirement you continue to remain in touch with your many students and protege's. You will always be remembered as one of the true leaders in the field of internal medicine and for your pioneering work on iron metabolism.

David A Lipschitz
Professor of Medicine
Director, Geriatric Research, Education and Clinical Center
John L McClellan Veterans Hospital
Head, Division on Aging University of Arkansas for Medical Sciences
Arkansas, USA

One of the great unadvertised perks of a career in academic medicine is the opportunity to meet scientific colleagues periodically, often in attractive and interesting parts of the world. As important as these meetings are in stimulating an interchange of research ideas, they are even more satisfying in allowing for the formation of lasting friendships. For me, the opportunity of getting together with Tom Bothwell once a year is among the best aspects of belonging to the "Iron Club". I have long admired the many lasting research contributions that Tom and his group have made to the field of iron nutrition and iron metabolism. Especially satisfying is his ability to apply information he has gained about iron absorption to developing practical means for the prevention of iron deficiency. I also have a great deal of respect for Professor Bothwell's admirable qualities as a communicator and writer. He is able to synthesize a complex body of information and to convey it with extraordinary clarity in lectures and articles. Perhaps even more than all these fine attributes, I appreciate his warmth and his talent as the best story teller of the Iron Club. I always look forward to the dry humour, insight of his anecdotes and to his updates on the rapidly changes and challenging circumstances in South Africa. He is one of my most inspiring and talented colleagues, and I am sure that you will miss his idealism particularly when he retires as head of the Department of Medicine.

Peter R Dallman
Professor of Pediatrics
University of California-San Francisco
San Francisco, California, USA

We recall with great appreciation Professor Bothwell's great contributions to the practice of medicine in South Africa, and to the development of the Medical School and the Department of Medicine. We particularly remember the rewarding years of Professor Bothwell's association with the SA Institute for Medical Research, whose pathologists and biochemists called attention to the prevalence of haemosiderosis in this region. Professor Bothwell's studies reveal the full picture of this condition and many other aspects of iron metabolism, research whose value has been internationally acclaimed.

I recall with gratitude Professor Bothwell's caring attendance on my brother Jock, the first full time lecturer in the Department of Medicine, during a long illness following his military service in East Africa in World War 2. Professor Bothwell's great skill as a physician was also evident when, as chairman of an ad hoc committee, he supervised the treatment of the patients affected by Marburg virus disease in the Johannesburg Fever Hospital in 1975. We have valued his friendship very highly and wish him many years of happiness in the future.

JHS Gear
Honorary Research Professorial Fellow
Department of Virology
University of the Witwatersrand
Previous Director SAIMR.

I was very fortunate that my first internship was with Professor Guy Elliott, whose registrar was Tom Bothwell. Not only did I learn a lot from him during that six months, but I am sure it was his influence that led me subsequently to specialise in medicine, and as a registrar when he was a junior consultant, become involved in laboratory research and follow an academic career - none of which I have ever regretted. In due course I was privileged to join him as a member of his Iron and Red Cell Metabolism Unit, and spent many happy and productive years working with him.
Looking back on his early years in research, it is salutary to note how much he was able to achieve on a level of funding which even after allowing for inflation, would be regarded as derisory today. He earned international acclaim before that funding became substantial, and in defiance of Parkinson’s law, increased his productivity as the support grew. However, he continued to run a frugal, efficient research operation which surely ranked as one of the most cost-effective among the laboratory disciplines.

It is fitting indeed that his illustrious career should be crowned by the Deanship.

RW Charlton  
Vice-Chancellor and Principal  
University of the Witwatersrand

I have had the privilege of being associated with Professor Bothwell as colleague and friend for most of my professional life, sharing a common interest in haematology, and more particularly in the nutritional anaemias. His contribution to our knowledge of iron metabolism is immense; the "Bothwell Book" is the standard reference text on the subject, and is likely to remain unchallenged for years to come. Perhaps less well known is his significant input over the years into the formulation of policy at national level on important medical and scientific matters. A man of quite understanding intellect, he has inspired a whole generation of physicians in the practice of clinical investigation. As scientist, teacher, caring doctor and humanist, he leaves a legacy as one of the few all-time greats to have graced our medical school. I count myself very fortunate indeed to have enjoyed the generous support and friendship of this wonderful warm man, who has always been an inspiration to me.

J Metz  
Emeritus Professor  
Formerly Professor of Haematological Pathology and Chairman, School of Pathology  
University of the Witwatersrand  
Former Director of the SAIMR.

Tom Bothwell and I worked together in Guy Elliott’s department in the early nineteen-fifties; his sterling qualities as clinician and medical scientist were already apparent. He has the rare gift of going to the heart of a problem, whether it be at the bedside (when compassionate understanding is so important) or in planning and executing a project in clinical research (where a clear mind and ability to isolate the scientific issues are mandatory). These qualities later combined to make him a great teacher of medicine, guiding light of a very fine department, pillar of Wits university, and a man of international renown.

In clinical research he has few equals. His early work on iron overload was followed by immaculate studies in a wide area of iron metabolism and, with Clem Finch of Seattle, the publication of an impressive monograph which is now the standard reference. His later work has been international with a team of distinguished collaborators abroad, tackling the prevention of iron deficiency anaemia by ascertaining how best to alter standard diets so that they are both acceptable and yield the optimum amount of iron for absorption.

He has surely been helped in his outstanding medical career by Alix, his intelligent, charming and supportive wife. Their home is a delight, as is Tom’s dry sense of humour. It is an honour to have been asked to contribute to this commemorative issue of The Leech.

EB Adams  
Emeritus Professor of Medicine  
University of Natal

For almost forty years Tom Bothwell has been a dominant figure in the field of clinical and experimental iron metabolism. His research has encompassed almost all aspects of the subject, iron deficiency to excess, iron absorption and excretion, iron transport in the plasma, cells and placenta. None of his contemporaries could have failed to be impressed and influenced by the quality of his publications and their pertinence to current research and clinical problems; and also by the long line of outstanding scientists and clinicians who have emerged from his department.

Personally, Tom’s first effect on me was to induce fear - the fear that I might have to speak after him at a meeting, when I could not hope to match the quality of his material or presentation. That aside, I soon enjoyed the encouragement
and help he gave to me as a younger scientist, and later the benefit of many valuable discussions on scientific and other matters, and the great hospitality provided by Alix and Tom in their home in Johannesburg. I hope that many more opportunities for meetings and discussions will occur in the future.

EH Morgan
Professor and Head
Department of Physiology
University of Western Australia
Perth, Australia.

It has been a singular privilege and honour to have been associated with Professor Tom Bothwell for as long as 30 years or more. Observing his clinical approach has been a rare medical education and working with him in iron metabolism was an intellectual treat. I am humbly aware of and grateful for the beneficial influence Tom has had on my own medical career. He has combined his outstanding achievements in medical research with equal excellence in teaching and patient care. His sense of humour and wit can only be described as Bothwellian. I am fortunate indeed to know him as a colleague and friend.

Congratulations Tom on your outstanding contributions to international medical research, to our Medical School, to countless students and doctors and to humanity through not only your own medical wisdom but also the inspiration you have been to your colleagues. I wish you well in your retirement and future endeavours.

Edward Gale
Part-time Physician
Department of Medicine
University of the Witwatersrand

When I joined Professor Tom Bothwell's laboratory in 1977 as a Research Fellow, he was putting together a book called "Iron Metabolism in Man". As each contribution arrived he would set about it with a pair or scissors in his left hand and a pen in the other, rewriting the text, cutting a piece out here, joining it on somewhere else, turning the manuscript into a long length of paper joined together with bits of sticky-tape. then, cut into unequal lengths, it went to the typist only to get the same treatment when it came back. The final result, a distillation of a research career that has produced significant contributions to almost every aspect of iron metabolism, is a masterpiece that, more than a decade later, remains a major reference in the field. Nowhere will you find a clearer explanation of the intricacies of iron metabolism. All our offerings were subjected to the same treatment, spiced with wit and not a little sarcasm. An unaltered paragraph was a major achievement. This collection of his writings in The Leech exemplifies the art of clear, unambiguous scientific penmanship for which Tom Bothwell is renowned.

Patrick MacPhail
Associate Professor
Department of Medicine
University of the Witwatersrand
This Week's Citation Classic

TH Bothwell, Pirzio-Biroli G & CA Finch

(The Citation Classic appeared in *Current Contents*, May 26, 1986)

The regulation of the absorption of iron was studied in animals and in man. Both the size of iron stores and the rate of erythropoiesis were shown to influence absorption. The larger the iron stores, the less iron was absorbed, whereas the greater the red cell production, the greater the amount of iron absorbed. (The Scientific Citation Index indicates that this paper has been cited in over 280 publications).

The Fifth Congress of the International Society of Hematology was held in Paris in 1954, and it was at breakfast in a sidewalk café at that Congress that I met Clem Finch for the first time. Out of this meeting came the offer of a job in his hematology laboratory at the University of Washington. At the time, I was working as a Nuffield Travelling Fellow at the University of Oxford and was pursuing an interest in iron metabolism that had started while I was training as a medical resident in South Africa. The reason for the interest was not difficult to explain, since a large proportion of the adult black population in the country suffered from various degrees of iron overload as a result of drinking fermented beers prepared in iron containers. My own initial studies had, therefore, been directed toward comparing external and internal iron exchange in the local variety of iron overload with the other forms that were recognized elsewhere. It was a field in which Finch's pathophysiological studies had already made a major impact.1

When I arrived in Seattle in 1955, a major objective of the iron program was to define those factors responsible for controlling iron transport, and during the 18 months I spent there, the particular areas of interest included not only iron absorption but also iron transport to the erythroid marrow2 and to the fetus.3

Insofar as internal iron exchange was concerned, the field had been revolutionized by the availability of radioiron of high specific activity and by the pioneering ferrokinetic studies of Rex Huff and his colleagues.4 These techniques were refined and extended in Seattle, and the dominant role of erythropoietic activity in dictating plasma iron turnover was clearly defined.2

The same approach was applied to iron absorption. By combining animal and human studies, it was possible to show that the absorptive process was responsive to two major influences - it was inversely related to the size of the iron stores and directly related to the rate of erythropoiesis. In addition, the intraluminal effects of iron dosage and of valency on absorption were also defined in quantitative terms. In a companion investigation, the absorption of food iron was measured in a large number of subjects with various hematologic disorders.5 In this latter study, the size of the iron stores seemed to be more important in modifying iron absorption than did the rate of erythropoiesis.

The article has probably been frequently cited because it provided a framework for future studies. Any explanation of the control of iron absorption at a molecular level would have to compatible with the observed effects of storage iron status and erythropoietic activity. Thus far, no really satisfactory explanation has been forthcoming.

From a personal standpoint, it has been an ongoing privilege to continue to work with Finch over the years in collaborative ventures, the most recent of which was the production of a book that covers many aspects of iron metabolism, including absorption.6 (TH Bothwell, April 22, 1986).

REFERENCES

Iron Absorption

I. Factors influencing absorption

TH Bothwell, MD, G Pirzio-Biroli, MD and CA Finch, MD
with the technical assistance of B Loden and A Melly.


When the iron content of the body is altered by some cause, such as blood loss, balance is re-established mainly by changes in the amount of iron absorbed from the gut. Because of its capacity to respond to the needs of the body, the gastrointestinal mucosa has been regarded as the chief regulator of iron balance. The mechanisms by which this control is exerted are, however, still in doubt. One problem has been the technical difficulty in accurately measuring iron absorption. Recently, precise methods employing radioiron have been developed, but only limited observations have been made concerning the influence of anemia, anoxia, plasma iron level, iron stores, and altered erythropoiesis on iron absorption.

The present paper reports the results of studies involving several aspects of iron absorption. Experiments in animals were carried out in a variety of situations and the hypotheses which emerged were tested in man. The investigation was broadened to include observations on absorption of iron salts by normal subjects, by individuals after acute blood loss, and by patients with iron deficiency anemia. Information was obtained on the relative importance of certain internal factors in the regulation of iron absorption and, in addition, a rational approach to the problem of iron therapy was developed.

MATERIALS AND METHODS

Sprague Dawley rats weighing 250 to 350 grams, New Zealand white rabbits weighing 2.5 to 3.5 kilograms, and a strain of adult field mice with hereditary spherocytosis were employed in various studies. In each experiment control animals of the same sex, age, weight, and dietary background were employed. The purpose was to indicate whether a specific factor altered absorption in these animals as compared to control animals. Variations, particularly in the amount of iron administered, made it impractical to inter-relate individual studies.

Radioiron was administered directly by gastric intubation. Rats were rendered anemic by removing 5 ml of blood at 5 and 3 days prior to study, or by the parenteral administration of an ethanolic solution of phenylhydrazine (30 mg/Kg). Hemolytic anemia was also induced by the prior injection of methyl cellulose intraperitoneally twice weekly as described elsewhere. Excess body iron stores were produced in rats, rabbits, and mice by the intramuscular injection of iron-dextran (Imferon*). Exchange transfusion was performed in rats by the insertion of plastic cannulae into the femoral artery and vein with the simultaneous removal of circulating blood and the injection of blood previously obtained from donor animals.

Normal human subjects studied had plasma iron concentrations between 80 and 160 µg/100 ml plasma and hematocrits between 42 and 48 per cent. In patients the criteria of iron deficiency were plasma iron levels below 50 µg, the absence of marrow hemosiderin, and a response to iron therapy.

Absorption studies were performed on normal volunteer subjects, fasting or after meals, as specified under results in individual experiments. Phlebotomy was also employed in normal subjects to enhance iron absorption. Unless otherwise specified, such subjects were bled 400 ml on the ninth and seventh days before the absorption

* Obtained through the courtesy of the Benger Company, England and Lakeside Laboratories, Milwaukee, Wis.
study was performed.

Absolute iron absorption was measured by the double isotope method described by Saylor and Finch. In this technique a weighed amount of Fe\(^{59}\) either as iron citrate or as iron bound to fresh plasma** was injected intravenously and at the same time a solution of iron (Fe\(^{59}\)) was given orally. Standards were then made from both solutions given. Fourteen days after administration of the two isotopes, a blood sample was collected and wet-ashed with sulfuric and perchloric acids. Samples were precipitated with the addition of carrier iron to a total of 10 mg and electroplated as described previously. Counting for Fe\(^{55}\) was done with an argon-filled geiger tube having a beryllium filter, whereas Fe\(^{59}\) was determined with a helium-filled tube. The cross-counting for which appropriate correction was made was less than 4 per cent in either direction. Calculation of results:

Percentage absorbed = \(\frac{y_1 (x) \times 100}{x_1}\)

When

\[
\begin{align*}
y_1 &= \text{activity of Fe}^{55} \text{ in blood sample (cpm)}\\
y &= \text{activity of Fe}^{59} \text{ given by mouth (cpm)}\\
x_1 &= \text{activity of Fe}^{59} \text{ in blood sample (cpm)}\\
x &= \text{activity of Fe}^{59} \text{ injected intravenously (cpm)}
\end{align*}
\]

In several studies iron absorption was computed by measuring stool radioiron content. To determine stool activity, a 10-day stool collection was transferred from waxed cartons to a large weighed beaker. The bulked specimen was then covered with concentrated sulfuric acid for 10 to 14 days with occasional stirring and addition of acid to a total of about 5,000 ml. Gentle heating was applied when necessary to produce a homogenous mixture. Ten gram aliquots were electroplated in the usual manner prior to counting. Since the validity of methods such as these must ultimately rest on direct comparisons, results of absorption by the stool and double isotope methods in 4 normal subjects are summarized in Table 1.

In certain studies on human volunteers the major interest was centered not so much on the absolute amount of iron absorbed as on the relative amounts absorbed under two different sets of conditions. One isotope of iron in one form was given by mouth on the first morning, while the second was given by the same route on the following morning. Analysis of activity in the blood drawn two to three weeks later permitted a comparison of absorption between the two test doses in the same subject. In order to estimate the absolute absorption, the per cent of red cell utilization of absorbed iron must be predictable. This was assumed to be 90 percent in normal and iron deficient subjects and the blood volume was estimated at 68 ml/Kg. Estimated per cent absorption then equalled:

\[
\frac{\text{activity per ml blood x blood volume (ml) x 100}}{0.9 \times \text{total radioactivity administered}}
\]

Table 1: Comparison of stool recovery and double isotope methods for measuring iron absorption in 4 human subjects.

<table>
<thead>
<tr>
<th></th>
<th>Stool method (per cent absorption)</th>
<th>Double isotope method (per cent absorption)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.3</td>
<td>4.8</td>
</tr>
<tr>
<td>2</td>
<td>15.2</td>
<td>12.5</td>
</tr>
<tr>
<td>3</td>
<td>5.5</td>
<td>2.6</td>
</tr>
<tr>
<td>4</td>
<td>20.9</td>
<td>16.6</td>
</tr>
</tbody>
</table>

** It is essential that there be either adequate capacity of the recipient’s plasma to bind the injected iron citrate, or that iron be bound to a plasma with adequate binding capacity before its injection.
Table 2: Effects of increased erythropoiesis on iron absorption

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number of animals</th>
<th>Dosage of iron (μg)</th>
<th>Absorption (± SD) (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylhydrazine in rats</td>
<td>5</td>
<td>50</td>
<td>59 ± 19</td>
</tr>
<tr>
<td>Controls</td>
<td>6</td>
<td>50</td>
<td>21 ± 8</td>
</tr>
<tr>
<td>Phenylhydrazine in rabbits</td>
<td>8</td>
<td>1000</td>
<td>30 ± 19</td>
</tr>
<tr>
<td>Controls</td>
<td>8</td>
<td>1000</td>
<td>12 ± 11</td>
</tr>
<tr>
<td>&quot;Methyl Cellulose&quot; in rats</td>
<td>6</td>
<td>3.5</td>
<td>16.2 ± 6.2</td>
</tr>
<tr>
<td>Controls</td>
<td>4</td>
<td>3.5</td>
<td>10.3 ± 1.9</td>
</tr>
<tr>
<td>Congenital spherocytosis in mice</td>
<td>7</td>
<td>3.5</td>
<td>7.2 ± 2.2</td>
</tr>
<tr>
<td>Controls</td>
<td>7</td>
<td>3.5</td>
<td>4.8 ± 1.9</td>
</tr>
</tbody>
</table>

The actual amount of radioactivity given varied in different studies depending upon the anticipated absorption and on the total amount of administered elemental iron. In man, the amount of Fe59 varied between 50 and 100 μc when carrier iron of between 100 and 500 mg was added. Iron59, in doses of approximately 1 μc, was given intravenously as ferrous citrate or was bound in vitro to the normal plasma when the patient's plasma iron binding capacity was inadequate. The specific activity of the two isotopes used varied between 1 and 3 μc/μg. With the double isotope method of measuring absorption, the dosage of each isotope was designed to provide nearly equal counts in the circulating blood. Plasma iron was estimated by the method of Bothwell and Mallet, and of Kitzes, Elvehjem, and Schuette. Bone marrow iron was estimated from sternal aspiration as described by Rath and Finch. Hemoglobin was determined as oxyhemoglobin in an Evelyn colorimeter and hematocrits were spun at 1800 g for 30 minutes. Reticulocytes were stained with cresyl blue and the number in 1000 erythrocytes counted.

RESULTS

A. Internal factors regulating iron absorption

The object of these studies was to delineate the effect of alterations within the body on iron absorption. In order to minimize the possible interference which local gut factors might have on such absorption, initial studies were done with animals and experimental subjects in a fasting state. Iron was given as a freshly prepared solution of ferrous sulfate. The usual dosage of iron was standardized at 0.25 mg/Kg of elemental iron in experimental animals. Data obtained may be summarized under four headings.

1. Effect of the rate of erythropoiesis on iron absorption

Increased erythropoiesis was produced in several different ways. One injection of phenylhydrazine was given to rats and absorption was measured one week later when the reticulocyte count was high and the hematocrit was...
Table 3: The absorption of a dose of 25 mg elemental iron before and after phlebotomy of 8 ml per kilogram in 12 human volunteers.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Plasma Iron</th>
<th>Basal Absorption (per cent)</th>
<th>Days after Phlebotomy</th>
<th>Plasma Iron</th>
<th>Second Absorption (per cent)</th>
<th>S.A. x 100*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>113</td>
<td>12.4</td>
<td>2</td>
<td>140</td>
<td>8.2</td>
<td>66</td>
</tr>
<tr>
<td>2</td>
<td>135</td>
<td>12.5</td>
<td>2</td>
<td>128</td>
<td>11.4</td>
<td>91</td>
</tr>
<tr>
<td>3</td>
<td>81</td>
<td>9.9</td>
<td>3</td>
<td>72</td>
<td>14.6</td>
<td>147</td>
</tr>
<tr>
<td>4</td>
<td>111</td>
<td>13.6</td>
<td>3</td>
<td>92</td>
<td>21.7</td>
<td>160</td>
</tr>
<tr>
<td>5</td>
<td>142</td>
<td>7.8</td>
<td>4</td>
<td>64</td>
<td>5.6</td>
<td>72</td>
</tr>
<tr>
<td>6</td>
<td>123</td>
<td>8.8</td>
<td>4</td>
<td>96</td>
<td>7.1</td>
<td>81</td>
</tr>
<tr>
<td>7</td>
<td>133</td>
<td>13.4</td>
<td>5</td>
<td>50</td>
<td>18.3</td>
<td>137</td>
</tr>
<tr>
<td>8</td>
<td>215</td>
<td>9.5</td>
<td>5</td>
<td>94</td>
<td>15.5</td>
<td>163</td>
</tr>
<tr>
<td>9</td>
<td>118</td>
<td>17.4</td>
<td>7</td>
<td>71</td>
<td>27.0</td>
<td>155</td>
</tr>
<tr>
<td>10</td>
<td>113</td>
<td>12.6</td>
<td>7</td>
<td>83</td>
<td>15.0</td>
<td>119</td>
</tr>
<tr>
<td>11</td>
<td>193</td>
<td>5.0</td>
<td>11</td>
<td>103</td>
<td>11.5</td>
<td>230</td>
</tr>
<tr>
<td>12</td>
<td>112</td>
<td>9.0</td>
<td>11</td>
<td>76</td>
<td>12.0</td>
<td>133</td>
</tr>
</tbody>
</table>

* Second absorption

Rising rapidly. In rabbits, multiple injections were given and absorption was measured at variable times thereafter. Methyl cellulose injected rats which had increased red cell production and destruction, but little anemia, and mice with increased erythropoiesis due to hereditary spherocytosis were also studied. These results are summarized in Table 2 and consistently indicated an increase in iron absorption in animals with increased erythropoiesis as compared to normal animals of the same species given the same dose of iron. Additional studies in rabbits given multiple injections of phenylhydrazine, while again indicating increased absorption with increased erythropoiesis, showed no correlation between a rising or falling hematocrit or with the level of plasma iron.

In an attempt to clarify further the time relationship between increased absorption and erythropoiesis, studies were carried out in experimental subjects before and after phlebotomy. As shown by stool measurements as well as by blood activities in two subjects, increased absorption occurred within 5 days of phlebotomy (Figure 1). Absorption was also measured in normal fasting subjects given 25 mg of iron (Fe59 sulfate) and phlebotomized 8 ml/Kg on the following day. Repeat absorptions were then done with Fe59 under identical conditions through the following week and the pre- and postphlebotomy ratio was determined (Table 3). In the first four days values were variable, but thereafter absorption showed a moderate but consistent increase.

The effect of decreased erythropoiesis was also studied. To retard red cell production, rats were made

Table 4: Effect of decreased erythropoiesis on iron absorption in rats

<table>
<thead>
<tr>
<th>Animals</th>
<th>Days following Transfusion</th>
<th>Dosage (μg)</th>
<th>Hemoglobin (gm)</th>
<th>Reticulocyte count (per cent)</th>
<th>Absorption (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2</td>
<td>20</td>
<td>14.8</td>
<td>2.8</td>
<td>19.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>15.5</td>
<td>2.1</td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>20.2</td>
<td>1.1</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>20.6</td>
<td>1.4</td>
<td>2.4</td>
</tr>
<tr>
<td>Polycythemic</td>
<td>2</td>
<td>20</td>
<td>19.3</td>
<td>1.4</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>16.3</td>
<td>1.5</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>17.8</td>
<td>1.5</td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>16.7</td>
<td>0.8</td>
<td>15.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>16.9</td>
<td>1.8</td>
<td>14.3</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>100</td>
<td>21.0</td>
<td>0.3</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>19.4</td>
<td>0.1</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>19.6</td>
<td>0.3</td>
<td>4.6</td>
</tr>
<tr>
<td>Polycythemic</td>
<td>5</td>
<td>100</td>
<td>19.4</td>
<td>0.1</td>
<td>2.0</td>
</tr>
</tbody>
</table>
polycythemic by exchange transfusion with packed erythrocytes. The hemoglobin level after exchange in these animals was above 20 Gm per cent. As a control, another group of rats was submitted to the same procedure except that they were exchanged-transfused with normal whole blood. Reticulocyte counts were depressed in the polycythemic animals, although the time lag in marrow response made this less conspicuous at two days. Absorption studies (Table 4) showed a reduction in iron absorption by polycythemic animals as compared to controls.

2. **Effect of change in body iron stores on absorption of iron**

Iron deficiency in rats was produced by feeding a low-iron diet from the time of weaning, and excessive iron stores were created by the injection of 100 mg of iron 3 months before the study. Absorption in these animals was compared to that in normal animals (Figure 2). In the group of 6 animals with excessive iron, absorption was decreased to 3.9 ± 0.9 per cent as compared to the normal group of animals with 20 ± 7 per cent absorption. A comparable increase in absorption of iron deficient patients as compared to that in normal subjects is shown later.

3. **Combined effect of iron stores and erythropoiesis on absorption**

The studies previously described indicated that increased erythropoiesis was associated with increased absorption of iron and decreased erythropoiesis with decreased absorption. The interplay of two opposing factors was illustrated by the following experiment. The erythroid marrow was activated by bleeding two groups of animals, one having previously had normal iron stores and the other having been loaded with 100 mg of iron by parenteral injection one month previously. As compared to the normal absorption of 20 percent, 5 phlebotomized animals with hematocrits of 40 ± 2 per cent absorbed 40 ± 17 per cent. The absorption by the 6 animals who were both iron loaded and phlebotomized was within the normal range (15 ± 9 per cent) (Figure 3). Thus, with

**Figure 2:** Effects of changes in body stores on iron absorption in rats

**Figure 3:** The combined effects of increased erythropoiesis and increased stores on iron absorption in rats. As compared with the group of normal animals, increased erythropoiesis increased iron absorption. However, the composite effect of a similar increase of erythropoiesis in animals with excess body iron was a normal absorption.
one factor tending to increase iron absorption and another to decrease it, the results fell within the normal range.

4. Effect of the plasma iron level on absorption from the gut

In the studies on normae and phlebotomized subjects there was no evident correlation between plasma iron and the amount of iron absorbed. To obtain more definite information, absorption studies were performed before and after the plasma iron had been raised to saturation of the iron-binding protein in 3 rabbits and in 2 normal subjects who had been previously phlebotomized. This elevation of plasma iron was accomplished by the infusion of erythrocytes rendered non-viable through in vitro storage in sodium citrate for 3 weeks. The marked increase in plasma iron which occurred between 2 and 8 hours after injection of the cells, due to the catabolism of their hemoglobin, did not significantly affect absorption (Table 5).

B. Effects of some local factors on iron absorption

Many questions which fall outside the scope of this paper arise concerning local factors within the gastrointestinal lumen. However, certain observations relating to the form and amount of administered iron and the effects of food and ascorbic acid are presented because of their practical importance in iron therapy.

1. Form of ingested iron

The absorption of a solution of ferrous sulfate was compared with that of ferrous sulfate in the form of quickly disintegrating and slowly disintegrating spansules.* The spansule and ferrous sulfate preparations were fed to fasting phlebotomized subjects on alternate mornings. Results (Table 6) indicated little difference between the iron solution and the rapid release spansule, but did show a marked reduction in the amount of iron absorbed from the

---

* These were obtained through the courtesy of Smith, Kline & French Laboratories, Philadelphia, Pa.
** This material, ferrous calcium citrate with tricalcium citrate, distributed as Rarical, was obtained through the courtesy of the Ortho Pharmaceutical Corporation, Raritan NJ.
### Table 6: Effect of form of iron on absorption in phlebotomized normal subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>Dose of iron (mg)</th>
<th>Absorption (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ferrous sulfate solution</td>
<td>&quot;Quick&quot; release spansules</td>
</tr>
<tr>
<td>1</td>
<td>16</td>
<td>18.8</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>36.8</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>15.0</td>
</tr>
<tr>
<td></td>
<td>Ferrous sulfate solution</td>
<td>&quot;Slow&quot; release spansules</td>
</tr>
<tr>
<td>4</td>
<td>48</td>
<td>24.0</td>
</tr>
<tr>
<td>5</td>
<td>48</td>
<td>17.4</td>
</tr>
<tr>
<td>6</td>
<td>48</td>
<td>38.0</td>
</tr>
<tr>
<td></td>
<td>Ferrous sulfate solution</td>
<td>Calcium iron citrate</td>
</tr>
<tr>
<td>7</td>
<td>50</td>
<td>9.9</td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td>10.7</td>
</tr>
<tr>
<td>9</td>
<td>50</td>
<td>20.8</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
<td>4.7</td>
</tr>
<tr>
<td>11</td>
<td>100</td>
<td>4.5</td>
</tr>
</tbody>
</table>

preparation with a slower release through the lower intestinal tract. Studies comparing ferrous sulfate with iron in the form of a calcium citrate chelate** showed little difference between these two compounds.

2. **Relation between dosage of iron and amount absorbed**

   Data on 6 normal male volunteers, 13 normal volunteers who had previously been phlebotomised, and on 8 iron other deficient patients are summarized in Figure 4. All tests were done with subjects in a fasting state and all were given freshly prepared solutions of ferrous sulfate varying in dosage from 5 to 500 mg of iron. Normal subjects showed such individual variations as to obscure the effect of dosage on absorption. However, in phlebotomized and iron deficient subjects, the higher absorption with increases in iron ingestion was better defined. The 3 groups studied appeared to differ largely in the capacity of the mucosa to transport iron. The absorption ratio between the normal, phlebotomized, and iron deficient subjects was approximately 1:3:9.

3. **The effect of ascorbic acid and cobalt on iron absorption**

   In animals given a constant amount of ascorbic acid and increments of oral iron (Figure 5), it was possible to

![Figure 5: The effect of 10 mg of ascorbic acid given with varying doses of iron was compared with absorption of iron alone.](image)
Table 7: Effects of ascorbic acid on iron absorption in phlebotomized normal subjects

<table>
<thead>
<tr>
<th>Method of Fe administration</th>
<th>Dose of ascorbic acid (mg)</th>
<th>Absorption (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Fasting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dose of 1 mg/kg</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>1 000</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>100</td>
<td>6.9</td>
</tr>
<tr>
<td>Dose of 2 mg/kg</td>
<td>200</td>
<td>7.3</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>6.1</td>
</tr>
<tr>
<td></td>
<td>1 000</td>
<td>8.2</td>
</tr>
<tr>
<td>After meals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dose of 1 mg/kg</td>
<td>100</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>13.0</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>18.0</td>
</tr>
<tr>
<td></td>
<td>1 000</td>
<td>7.0</td>
</tr>
</tbody>
</table>

* Demonstrate an enhancement in iron absorption. In man, smaller dosages of ascorbic acid, in keeping with amounts feasible for clinical administration, were given. The dosage of ascorbic acid varied from 100 to 1 000 mg, and iron was given in amounts of either 1 or 2 mg/kg. The subjects were phlebotomized prior to absorption tests and studied either fasting or post-prandial. The effect of ascorbic acid was not pronounced, as indicated by an average absorption of 12 per cent in all studies as compared with control values of 9 per cent (Table 7).

The effect on iron absorption of 80 to 120 mg per day of cobalt given as cobalt chloride was also determined. In two subjects absorption studies were carried out after 7 days and in two others after 14 days of cobalt administration. Absorptions before and after cobalt were 3.7 versus 9.8 per cent, 2.3 versus 5.5 per cent, 9.4 versus 9.2 per cent, and 9.8 versus 8.1 per cent.

Discussion

Ingested iron is absorbed in the ferrous form largely by the duodenum. The absorbed iron passes through the mucosal cells into the bloodstream where it is bound by a beta-1 globulin of the plasma. Normally about 90 per cent of this iron is incorporated into the hemoglobin and is found in the circulating red cell mass within 2 or 3 weeks; in iron deficiency anemia absorbed iron may be almost quantitatively recovered from circulating erythrocytes.

The most accurate early information in the literature of iron absorption related to iron deficiency anemia, where the amounts of iron absorbed were great, and absorption could be measured by the increase in circulating hemoglobin. Initial measurements employing radioiron followed the same principle of measuring the radioactivity in the circulating red cell mass. This measurement was found to be unreliable in certain diseases due to a significant and variable distribution of radioactive iron into tissues other than the erythrocyte. This led to the development of a stool recovery method where absorption was determined from the difference between iron administered and that recovered in the feces.

While there is no reason to question the validity of this method on theoretical grounds, since excretion of absorbed iron into the gut is negligible, there are practical difficulties. Not only is it a problem to ensure complete collection over a period of 8 to 12 days, but the per cent absorption is often so small that technical errors are magnified.* At these low levels it is possible to determine absorption more accurately by a double isotope method, whereby the second isotope measures distribution between tissues and red cells. The ratio of the two isotopes then indicates the total amount absorbed without the necessity of a blood volume determination. The reliability of this method at a dose

* In certain conditions, e.g. infections, lymphomas and hemolytic anemias, absorption as judged by stool recoveries has been found sometimes to be a good deal greater than would have been apparent from blood levels alone. Such results would presume that only a small portion of the radioiron entering the plasma is utilized for hemoglobin production in these conditions. In recent years, however, it has been possible, using iron of high specific activity, to tag the plasma iron-binding globulin without exceeding its binding capacity and so find out what proportion of the plasma iron is concerned with red cell production. Under these circumstances with the exception of certain hemolytic anemias, it has been shown that red cell utilization is only rarely below 50 per cent and that it is usually a good deal higher. These findings suggest the need for re-evaluating some of the earlier results for absorption obtained by stool recovery techniques.
range of 0.5 mg/Kg iron in measuring isotope distribution between red cells and other tissues has been previously reported. These studies have been extended over a wide dose range up to 10 mg/Kg with similar results. Combined stool and double isotope measurements on 4 subjects gave an average of 12 per cent and 9 per cent, respectively (Table 1). In another subject given radioiron every 5 days over a 42-day period, average daily absorption was 5.4 per cent by the stool method and 3.9 per cent by the double isotope technique (Figure 1). A further indication of the general reproducibility of the double isotope technique was found in examination of the consistency in repeat measurements in the same individual where the two sets of conditions produced similar absorption (Tables 5, 6 and 7).

It is apparent that the method employed to measure iron absorption must be modified depending on the problem at hand. When the per cent incorporation of radioiron is 80 to 100 as in the normal or iron deficient subject, a determination of total red cell activity at two weeks will suffice. Comparison can then be made of two absorption tests employing different isotopes. If the per cent utilization of radioiron for red cell production is not predictable, either the double isotope method previously described or the stool recovery method must be employed. While the double isotope method has been preferred for most of the studies reported here because of its simplicity and greater accuracy, this method is not feasible when there is a complete failure of erythropoiesis. Accordingly, in these studies a variety of methods have been employed according to the experimental situation.

A further adjunct to the evaluation of absorption in man has been the use of phlebotomy. This has proved particularly useful in testing iron preparations of unknown value, since the amount of iron absorbed in normal subjects is often too small for definitive results. The absorption by phlebotomized subjects over a range of iron dosage was 2 to 3 times normal (Figure 4) and appeared somewhat more uniform from subject to subject.

Normal man was taken as a reference point in studies of iron absorption. It seemed important to define normality as far as possible as it is related to erythropoiesis and body iron content including normal red cell, plasma, and marrow iron values. In such subjects, mean absorption of ferrous salts ranged between 2 and 8 mg as the dose of iron was increased from 20 to 400 mg (Figure 3). One point of interest was the variation in absorption between individual normal subjects. While the explanation for this may lie in changes too subtle to measure, it does emphasize the difficulty of evaluating absorption in the normal subject unless comparative measurements between two compounds are made.

Both the status of iron stores and the level of erythropoiesis have been shown to influence iron absorption. The increased absorption of both iron salts and dietary iron in iron deficiency has been well established. Studies here in animals also indicated depressed iron absorption when iron stores were increased above normal. To what extent this may apply within the narrower limits of expansion and contraction of the normal iron stores is not clear. Available evidence suggests that once the anemia of iron deficiency has been corrected, iron absorption returns to a normal base line despite depleted stores. More data are required on this point.

The second important factor influencing absorption is the activity of erythroid marrow. In the present study, absorption has been shown to be either increased or decreased in direct relationship to the level of erythropoiesis in experimental animals given iron salts. These findings support previous reports of increased absorption related to increased erythropoiesis in experimental animals. In the phlebotomized but otherwise normal subject, absorption has been shown to be consistently elevated (Figure 1). The lack of any relationship between iron absorption and anemia per se is indicated by clinical studies in a following report.

In summary, a variety of experiments has provided abundant evidence to indicate that interplay between body iron content and erythropoietic activity affect iron absorption.

The effectiveness of oral iron in treatment of iron deficiency anemia is indicated by comparing the amount of iron which may be absorbed with the needs of the marrow for erythropoiesis. These marrow requirements, estimated at 25 to 50 mg per day, are met by a single dose of 150 to 200 mg of iron. Comparative studies of different forms of iron indicate that iron must be in a form available to the upper intestinal tract, and that ascorbic acid* in doses of 100 to 1000 mg and cobalt in daily dosage of 80 to 120 mg over one to two weeks do not offer any significant advantage in iron therapy. A citrate-calcium-chelate of iron was shown to be nearly as well absorbed as ferrous sulfate. While these absorption data have no bearing on the tolerance of various preparations, they are consistent with many observations in the literature that ferrous salts in a form available to the upper intestine are the most suitable forms of medicinal iron; and a daily dosage of 0.2 to 0.3 mg iron per kilogram, equivalent to 0.2 Gm exsiccated ferrous sulfate 3 times per day, is usually adequate.

Certain speculations concerning the mechanism of iron absorption seem justified. The role of iron stores and erythropoiesis in modifying absorption has been shown. The time relationship of erythroid marrow and increased iron absorption is of particular interest. Following phlebotomy marrow iron uptake increases within 24 hours, while absorption shows a lag of several days. The connection between marrow and intestinal mucosa is not through the plasma iron or hemoglobin level. Another mechanism whereby the needs of the bone marrow are felt by the mucosa must be operative, although this has not been directly demonstrated.

* The effect of ascorbic acid would appear different when employed with therapeutic doses of iron salt as compared to its administration with food iron, when it has been shown to have a clear-cut effect in enhancing absorption.
SUMMARY

Iron absorption has been quantitated by a double isotope method, by stool recovery and by comparative measurements of iron absorption from two compounds as they appeared within the red cell mass. Employing these isotope measuring techniques, the regulation of absorption of small and large doses of iron has been studied.

Both the size of iron stores and the rate of erythropoiesis have been shown to influence the absorption of iron. The larger the iron stores the less iron absorbed whereas the greater the red cell production, the greater the amount of iron absorbed.

Absorption in normal and phlebotomized subjects and in patients with iron deficiency was measured. The effects of absorption of the amount of iron, the form in which it is administered, and of supplements were also evaluated.

REFERENCES

Effects of iron overload on ascorbic acid metabolism

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Summary

Studies of the ascorbic acid status in two subjects with idiopathic haemochromatosis and in 12 with transfusional siderosis showed that all had decreased levels of white cell ascorbic acid. The urinary excretion of ascorbic acid was also diminished in those subjects in whom such measurements were made. The administration of ascorbic acid was followed by only a small rise in the urinary ascorbic acid output, while the oxalic acid levels (measured in two subjects) showed a significant rise. These findings resemble those described in siderotic Bantu, and support the thesis that increased iron stores lead to irreversible oxidation of some of the available ascorbic acid.

Introduction

Iron overload resulting from prolonged exposure to a high dietary iron intake is extremely common among the Bantu people in Southern Africa (Walker and Arvidsson, 1953; Bothwell and Finch, 1962). The major source of the excessive dietary iron is the home-brewed alcoholic beverages which are consumed in quantity by many adult males (Walker and Arvidsson, 1953; Bothwell, et al., 1964a). It is consequently this section of the population which shows the highest incidence of severe iron overload (Bothwell and Isaacson, 1962). Scurvy is also common among the Bantu. As in other parts of the world it is seasonal, the highest incidence being at the end of winter and in early spring (Seftel, et al., 1966). It is most commonly seen in adult males, and is very rare in children, though other deficiency diseases, such as kwashiorkor, are often encountered (Trowell, 1960).

There is now a considerable amount of evidence indicating that severe iron overload contributes to the development of scurvy in the adult Bantu population. For example, it has been shown that patients presenting with scurvy are invariably heavily siderotic (Bothwell, et al., 1964b; Seftel, et al., 1966). Moreover, the concentrations of ascorbic acid in the leucocytes of non-scorbutic individuals with no clinical evidence of scurvy are much lower than in non-siderotic controls (Lynch, et al., 1967a). The probable reason for the association has also been defined. The large storage depots of iron in the body, which are in the ferric form, represent a massive oxidative potential, and it seems probable that a proportion of the available ascorbic acid is irreversibly oxidized by these deposits (Schulz and Swanepoel, 1962). Evidence supporting this thesis has been obtained by administering large amounts of ascorbic acid to siderotic individuals. In such circumstances the quantities of the vitamin voided in the urine remain low, but large amounts of oxalic acid, which is an oxidation product of ascorbic acid, are passed (Lynch, et al., 1967b). In addition, the clearance rate of ascorbic acid from the plasma is more rapid in siderotic individuals than in control subjects (Lynch, et al., 1967b).

The findings in Bantu with severe siderosis raise the question of whether similar disturbances of ascorbic acid metabolism may not occur in other forms of iron storage disease. Massive iron overload is a feature of the genetic disorder idiopathic haemochromatosis, and has also been noted in certain forms of anaemia. In idiopathic haemochromatosis there is an inappropriate absorption of iron from a normal diet (Charlton and Bothwell, 1967), while multiple factors are involved in the development of the disorder in subjects with anaemia. These include
the administration of multiple transfusions to subjects with refractory anaemias, increased absorption from the gut in conditions where there is increased but ineffective erythroid marrow activity, and the injudicious use of iron therapy in subjects with disordered haemoglobin metabolism (Bothwell and Finch, 1962). In the present study certain aspects of ascorbic acid metabolism were investigated in patients with idiopathic haemochromatosis and in subjects with various refractory anaemias.

Subjects studied

Twelve patients suffering from anaemias requiring multiple transfusions were investigated. Seven had thalassaemia major, one had sickle-cell thalassaemia, three had aplastic anaemia, and one had myelofibrosis. All had received at least 50 pints (23 litres) of blood before the time of study. Two subjects with idiopathic haemochromatosis who had not previously been venesected were also investigated. The diagnosis was established on the basis of the clinical findings, together with the results of desferrioxamine-induced iron excretion (Lundvall and Weinfeld, 1967) and liver biopsy. Neither of the patients gave a history of exposure to excessive dietary or medicinal iron. Since many of the subjects with transfusional haemosiderosis were children, 10 normal children were included in the 32 subjects who were studied as controls. A full dietary history was obtained from both the siderotic and the control subjects.

Chemical methods

White blood cell ascorbic acid concentrations were determined in each patient, and platelet ascorbic acid concentrations were also measured in the majority. (The platelet levels were assessed because it was felt that the apparent white cell values in the thalassaemic patients might not be entirely valid, since some of the subjects had large numbers of erythroid precursors in their peripheral blood.) In addition, urinary ascorbic and oxalic acid determinations were carried out on two subjects with thalassaemia and on two control subjects before and during oral ascorbic acid loading. The basal excretory pattern was established by collecting three 24-hour urine specimens; 250 mg of ascorbic acid was then administered eight-hourly for nine days. During this period the 24-hour urinary excretion of ascorbic acid and oxalic acid was determined each day. The pattern of excretion of ascorbic acid was also studied in the two subjects with idiopathic haemochromatosis before and during ascorbic acid loading.

Urinary ascorbic acid concentrations were measured by the method of Roe (1954). The 24-hour specimens of urine were collected in iron-free vessels containing 100 ml of glacial acetic acid plus edetic acid (0.5 g/l). The oxalic acid concentrations were determined by the method of Powers and Levatin (1944) except that 100 ml aliquots of urine were analysed. Leucocyte ascorbic acid was measured as described by Denson and Bowers (1961); duplicate estimations showed close agreement (within 10%). Because of the low leucocyte counts in the two subjects with aplastic anaemia, several samples of blood were pooled before the estimations were performed. The platelet ascorbic acid concentration was determined on 10 ml of blood mixed with 0.5 ml of 10% edetic acid solution. Erythrocytes and leucocytes were removed by centrifugation at 150 g for 10 minutes, and the platelets in an aliquot of the supernate were counted. A further aliquot (1-2 ml) was centrifuged at 1600 g for 30 minutes; the supernatant was then discarded and the deposit washed with 10 ml of 0.9% NaCl solution. A volume of 1.3 ml of 5% trichloracetic acid was added to the deposit and the ascorbic acid content was determined in the same way as for leucocytes.

The platelet ascorbic acid technique was validated by comparing the ascorbic acid concentrations in leucocytes and platelets in 35 subjects. In order to obtain a range of concentrations for comparison, 13 scorbutic individuals were included. The correlation was good (r = +0.847, P < 0.001) (Figure 1). The mean platelet ascorbic acid concentration in the non-scorbutic individuals was 81 µg/10^6 platelets (range 51-143 µg).

![Figure 1: Correlation between leucocyte and platelet ascorbic acid concentrations (r = +0.847, P < 0.001).](image-url)
Table 1: White cell and platelet ascorbic acid concentrations

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Age in years</th>
<th>White cell ascorbic acid (μg/10⁸ Leucocytes)</th>
<th>Platelet ascorbic acid (μg/10¹⁰ Platelets)</th>
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</thead>
<tbody>
<tr>
<td>Thalassaemia</td>
<td>14</td>
<td>5,9</td>
<td>20,0</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>11,6</td>
<td>37,0</td>
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<td>13</td>
<td>4,2</td>
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<td>10,1</td>
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<tr>
<td></td>
<td>8</td>
<td>14,4</td>
<td>50,0</td>
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<tr>
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<td>40,0</td>
</tr>
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<td></td>
<td>7</td>
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</tr>
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<td></td>
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<tr>
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<td></td>
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<tr>
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<td>40</td>
<td>7,4</td>
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<tr>
<td></td>
<td>12</td>
<td>7,8</td>
<td></td>
</tr>
<tr>
<td>Myelofibrosis</td>
<td>50</td>
<td>9,0</td>
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<tr>
<td>Mean</td>
<td>Mean 31</td>
<td>Mean 30,2</td>
<td>Mean 81,0</td>
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<td>Normal adults (22 subjects)</td>
<td>(range 24-55)</td>
<td>(range 20,3-39,6)</td>
<td>(range 51-143)</td>
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<td>Normal children (10 subjects)</td>
<td>Mean 10</td>
<td>Mean 29,9</td>
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<tr>
<td></td>
<td>(range 7-12)</td>
<td>(range 22,5-41,0)</td>
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</tr>
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</table>

Results

The dietary histories of both the siderotic and the control subjects were unremarkable. They consumed normal amounts of milk, fresh fruit, and vegetables, and the alcoholic intake of the adult subjects was low. None of the siderotic individuals showed any clinical evidence of scurvy. Both the leucocyte and platelet ascorbic acid concentrations in the siderotic subjects were lower than in any of the normal control individuals (Table 1). The mean leucocyte ascorbic acid concentration in normal adults was 30,2 μg/10⁸ leucocytes, and the lowest figure was 20,3 μg/10⁸ leucocytes. The figures in the normal children were very similar, the mean being 29,9 μg ascorbic acid per 10⁸ leucocytes, with the lowest figure of 22,5 μg/10⁸ leucocytes. In contrast, the highest figure in the siderotic subjects was 19,7 μg/10⁸ leucocytes, while the mean figure was only 9,0 μg/10⁸ leucocytes. The platelet ascorbic acid concentrations in the siderotic individuals were also low, which indicates that the low leucocyte concentrations were not due simply to dilution of the leucocytes by normoblasts in the Buffy layer. The mean platelet ascorbic acid concentration in the siderotic group was 26,0 μg/10⁸ platelets, compared with a mean figure of 81 μg/10⁸ platelets in 22 normal subjects.

White cell ascorbic acid concentrations were repeated on three of the siderotic subjects at intervals after they had been "saturated" with 250 mg of ascorbic acid three times daily for seven days. Within two months the levels had returned to approximately the same low figures as previously (Table 2).

The basal excretion of ascorbic acid was measured in two thalassaemic subjects, in two subjects with idiopathic haemochromatosis, and in two normal control individuals. It was lower than normal in the siderotic individuals. The oral administration of ascorbic acid was followed by the appearance of considerable amounts of ascorbic acid in the urine of the control subjects, with virtually the entire daily dose appearing in the 24-hour urine after three days. The increase in urinary ascorbic acid excretion was much less marked in the siderotic individuals, and even after nine days only 20% at most of the administered dose was recovered in the urine.

There was little difference in the basal urinary oxalic acid content of two control subjects and two individuals with thalassaemia major. The oral administration of ascorbic acid did not affect the urinary oxalic acid excretion of the normal subjects, but an increase occurred after several days in the thalassaemic individuals.

Discussion

The present investigation has established that subjects with idiopathic haemochromatosis and with transfu-
Table 2: Follow-up levels of ascorbic acid in three siderotic subjects after administration of ascorbic acid (750 mg daily) for seven days

| Values before ascorbic acid administration | Subsequent values |  |
|-------------------------------------------|-------------------|--|  |
| | White cell ascorbic acid concentrations (µg/10⁸ leucocytes) | After one week on ascorbic acid | One month later | Two months later |
| 7,1 | 43,9 | 20,3 | 11,6 |
| 8,9 | 44,5 | 23,5 | 9,6 |
| 4,1 | 38,9 | 23,4 | 8,7 |

Jesional siderosis exhibit the same type of abnormal ascorbic acid metabolism as do severely siderotic South African Bantu subjects. Leucocyte and platelet ascorbic acid concentrations were found to be consistently lower than normal, and the oral administration of ascorbic acid resulted in only a slight increase in the urinary ascorbic acid excretion compared with control subjects. In addition, the administration of ascorbic acid to two patients with thalassaemia led to an increased excretion of the oxidation product, oxalic acid, in the urine. These various findings, when taken together, suggest that iron overload of several different types can lead to the irreversible oxidation of a proportion of the available ascorbic acid. At the same time it is apparent that the deviations from normal are not as severe as those previously noted in severely siderotic Bantu (Lynch, et al., 1967a). The leucocyte ascorbic acid concentrations were not as low, and none showed clinical evidence of scurvy. This can almost certainly be ascribed to the good diet consumed by all these subjects. In contrast, the dietary ascorbic acid intake in most heavily siderotic Bantu subjects is low, and in late winter and early spring it is even lower than at other times of the year. This is the season when clinical scurvy is most commonly seen in these subjects (Seftel, et al., 1966). It is therefore possible that irreversible oxidative catabolism of ascorbic acid by the ferric iron deposits in these individuals is only partly responsible for the development of scurvy, and that dietary deficiency is a contributing factor.

The present findings may possibly have a wider relevance. Among the Bantu severe siderosis and ascorbic acid deficiency have been shown to be associated with an unusual variety of osteoporosis (Gursin and Samuel, 1957; Seftel, et al., 1966; Lynch, et al., 1967a). This osteoporosis occurs in middle-aged manual labourers, and commonly presents with vertebral body collapse. Some affected individuals exhibit clinical scurvy, but in others the ascorbic acid deficiency has been manifested only by decreased leucocyte ascorbic acid concentrations. It has been suggested, though not proved, that the severe siderosis contributes to the development of the ascorbic acid deficiency, which in turn leads to diminished bone formation. While this may prove not to be the correct explanation there is no doubt that a close clinical association exists in the Bantu between severe siderosis, ascorbic acid deficiency, and osteoporosis. In this context it may be noteworthy that osteoporosis has been found in white subjects with haemochromatosis (Delbarre, 1960). In addition, it is possible that the skeletal changes noted in thalassaemia (Caffey, 1957) may not be the result only of an expanded bone marrow. The subject is therefore one which warrants further study.

References

The effects of ascorbic acid supplementation on the absorption of iron in maize, wheat and soya

MH Sayers, SR Lynch, P Jacobs, RW Charlton, TH Bothwell, RB Walker and F Mayet


Summary

The absorption of iron from three staple vegetables was measured by the red cell utilization method in iron deficient subjects. The food iron had been labelled with $^{55}$Fe by the hydroponic cultivation method. In addition, $^{59}$Fe was added with or without carrier iron in the form of ferric ammonium citrate, prior to cooking. The constant relationship reported by others between the absorption of the two isotopes was confirmed, suggesting that the extrinsic iron and the food iron were absorbed from a common pool. The addition of ascorbic acid to maize porridge before cooking significantly enhanced the absorption of both the intrinsic and the added iron. However, no effect was noted with soya biscuits or with whole wheat bread (100% extraction). Evidence was obtained that these differences were due to the oxidative destruction of the ascorbic acid by the high temperatures required for baking. If, therefore, a feasible method were found for supplementing vegetable foodstuffs with ascorbic acid and inorganic iron, nutritional benefit would only be anticipated with uncooked or boiled foods.

Iron deficiency anaemia remains a public health problem in large areas of the world, and is particularly common in countries where cereals provide the bulk of the calories (WHO, 1970). While this is partly due to the fact that hookworm infestations is endemic in some of these countries, poor absorption of the iron in cereals such as wheat, corn and rice is a major factor (Hussain, et al., 1965; Layrisse, et al., 1969). Attempts to improve iron nutrition by adding iron salts to cereals have proved disappointing (Elwood, 1968), suggesting that the factors present in cereals which inhibit the absorption of intrinsic iron also affect the supplemental iron. In addition, some iron salts interfere with the storage properties of a cereal such as flour (Farrand, et al., 1968).

The present study was done to find out whether a different approach might be more rewarding. Chemical analyses suggest that the quantity of iron present in many cereal diets is adequate (Ramalingaswami & Patwardhan, 1949); it is its availability for absorption that is poor. If such iron could be rendered more absorbable, a significant improvement in iron nutrition would be anticipated. There is previous evidence that ascorbic acid can enhance the absorption of iron in certain foods, including eggs (Moore & Dubach, 1951; Callender, et al., 1970) and cereals (Steinkamp, et al., 1955; Callender & Warner, 1968; Elwood, et al., 1968; Kuhn, et al., 1968). However, in these studies the ascorbic acid was taken as orange juice or added after cooking, and it is not known whether it would be effective if introduced before the preparation of the food. Since a successful dietary supplement would have to be present in some standard dietary ingredient such as salt, the present investigation was designed to examine the effect of ascorbic acid added to staple foods prior to cooking. The absorption of both the intrinsic food iron and an iron salt added to the meal was examined under these conditions.

Subjects and methods

Sixty-six volunteers took part in the investigation; 64 were women. Sixty-two were Indians, two were Bantu and two were White. The mean age was 32 yr (range 15-71). All except one were considered to be iron deficient on the
basis of the absence of stainable iron from marrow aspirates (Rath & Finch, 1948). In addition, the majority were anaemic, had low plasma iron levels and a low percentage saturation of circulating transferrin. In order to establish the capacity of individual subjects to absorb iron, "reference" absorption studies were carried out on all subjects 2 weeks after the first investigation. In these studies a small dose of ferrous iron was fed in the fasting state; the degree to which it was absorbed was then taken as a measure of the ability of each individual to absorb iron (Cook, et al., 1969; Layrisse, et al., 1969).

Preparation and administration of test meals

The absorption of the iron in three different vegetable foods was measured; maize (Zea mays, var. Ida hybrid) was prepared as a porridge, soya beans (Glycine soja, var. Hawkeye) as biscuits and wheat (Triticum vulgare) as bread (100% extraction). They had been hydroponically labelled with iron (55Fe) in the Department of Botany, University of Washington, by methods previously described (Hussain, et al., 1965; Layrisse, et al., 1969) and 10-20 μCi were administered to each subject. In addition to the intrinsic 55Fe label, 1.5 μCi extrinsic 59Fe tracer were added to the water prior to cooking in all studies; in some experiments it was given together with small amounts of iron (2-5 mg) as ferric ammonium citrate. Ascorbic acid in doses varying between 50 and 250 mg was added prior to cooking in certain studies. Aliquots of porridge, biscuits or bread were taken for the preparation of isotopic standards and for determining the total iron and ascorbic acid contents.

Maize porridge
The 55Fe-labelled maize was thoroughly blended with carrier maize to provide 50 g dry maize per subject, and cooked with four times its weight of water for 20-25 min at 190°F to make a porridge. The final weight of porridge eaten by each subject was approximately 200 g and the pH of different aliquots lay between 5.9 and 6.4.

Soya biscuits
The 55Fe-labelled soya was blended with carrier soya to provide 20 g dry soya per subject, and water was added to form a thick paste. This was separated into approximately equal parts and baked into biscuits in an oven for 45 min at 400°F.

Whole wheat bread
Because there were no facilities for milling the radioactive wheat, it was necessary to use 100% extraction flour. The labelled wheat was thoroughly blended with carrier whole wheat to provide a final weight of 450 g 100% extraction flour. The baked loaf weighed 550-600 g and each subject ate approximately 80 g.

Administration of meals
After fasting overnight, the volunteers were weighed and blood samples were taken for serum iron, unsaturated iron-binding capacity and haemoglobin estimations. Precisely weighed quantities of maize porridge, soya biscuits or whole wheat bread were then eaten. Each subject was given 250 ml water to drink during the meal and, in addition, sugar was allowed with the porridge. No further food or drink was permitted for 4 hr. Fourteen days later the volunteers were again assembled after an overnight fast, and blood samples were taken for the measurement of 55Fe and 59Fe activities. Immediately thereafter a freshly prepared solution containing 3 mg iron as ferrous sulphate, 30 mg ascorbic acid and 3μCi 59Fe tracer in 160 ml distilled water was given to each subject. The absorption of the iron in this reference salt was calculated from the corrected radioactivity present in a blood sample taken after a further 14 days.

Isotopic and chemical methods
Aliquots of food (1 g) and blood samples (10 ml) were prepared for differential radioactive counting using the method of Katz, et al., (1964). The quantities of 55Fe and 59Fe in the processed samples were determined with a liquid scintillation system. The Packard Tri-Carb (model 3002) spectrometer which was used had a 3-4% counting efficiency for 55Fe at optimal gain and window settings. 4 ml blood samples collected at the beginning and at the end of the "reference salt" study were counted in a Packard Auto-gamma Tri-Carb (model 3001) spectrometer against suitable standards. All figures for percentage absorption were calculated on the assumption that 100% of the absorbed radioactivity was circulating in the blood and that the blood volume of each subject was 65 ml/kg.

Plasma iron concentrations were measured by a modification (Bothwell & Finch, 1962, p 18) of the method of Bothwell & Mallett (1955) in which sulphonated bathophenanthroline was used as the colour reagent. The unsaturated iron-binding capacity was determined by the method of Herbert, et al., (1967). The iron content of digested samples of food was estimated by a modification (Bothwell & Finch, 1962, p 26) of the method of
Lorber (1927). Measurement of the reduced ascorbic acid content of the meals was performed using the modified method of Tillmans, et al., (1932) as described by Roe (1967, p 31). The content of total ascorbic acid (including dehydroascorbic and diketogulonic acids) was estimated by the method of Roe & Kuether (1943) as described by Roe (1967, p 35).

Results

Comparison between absorptions of extrinsically and intrinsically labelled iron

Since vegetable foodstuffs which had been labelled hydroponically with $^{55}$Fe were used, it was possible to find out the degree to which the absorption of $^{59}$Fe-labelled extrinsic iron added during cooking reflected the absorption of the iron contained within the foodstuff. If the $^{55}$Fe-labelled intrinsic iron and the $^{59}$Fe-labelled extrinsic iron formed a single pool, then it would be anticipated that the ratio of the absorption of each isotope would be unity. The chronological order of the studies was not the same as the order in which they are set out in the Tables. This was due to the fact that use had to be made of hydroponically labelled material as it became available. The first three studies were A and B in Table 1 and J in Table 3. In each of these studies difficulty was experienced in preparing the blood samples for measurement of the $^{55}$Fe content, and experiments indicated that not much more than 50% was being recovered. This is reflected in the mean ratios of extrinsic $^{59}$Fe to intrinsic $^{55}$Fe labels, which were 1.85, 2.42 and 1.85 respectively. Minor modifications in the methods of sample preparation were then instituted and no further difficulties were encountered. The mean ratios of extrinsic to intrinsic labels in the remaining studies were 1.15, 1.13, 1.12, 1.02, 1.08, 1.11, 1.14 and 1.15. The mean ratio for the 42 individuals in which the absorption was greater than 1% was 1.12 (SD ± 0.08).

Effect of ascorbic acid on the absorption of iron in maize porridge (Table 1)

The mean absorption of the reference salt was 55.8% in the control group (A), and this was higher than it was in all the other groups with the exception of E (65.2%). Furthermore, the mean haemoglobin concentration of 7.3 g/100 ml in group A was more than 2 g/100 ml lower than that of any other group. On this basis it was felt that the increased iron absorption in the groups given ascorbic acid was not ascribable to a greater degree of iron deficiency. There are several points of interest in the results. Firstly, it is apparent that ascorbic acid in a dosage of 50 mg increased the mean absorption by between two and four times (compare studies B and C with study A). Comparable mean absorptions were obtained with a 100 mg dose of ascorbic acid (studies D and E). The addition of between 2.5 and 5.0 mg iron to the porridge appeared to have little, if any, effect on the percentage absorption. Further insight into the potential benefit to iron nutrition which might derive from ascorbic acid supplementation was obtained by calculating the actual quantities of iron absorbed in the various experiments. These quantities were derived from direct measurements of the iron content of aliquots of the fed cereal. (The quantities obtained were invariably higher than expected, probably because of variable iron contamination of the carrier maize and/or iron contamination during cooking). The mean figures for absorption were as follows: maize porridge alone, 0.12 mg; supplement 50 mg ascorbic acid, 0.31 mg; supplements 50 mg ascorbic acid and 2.5 mg iron, 0.96 mg; supplements 100 mg ascorbic acid and 2.5 mg iron, 1.36 mg; supplements 100 mg ascorbic acid and 5 mg iron, 1.60 mg. In a final experiment porridge containing a large amount of ascorbic acid (250 mg) and 2.5 mg iron was fed together with a soft boiled egg. The mean absorption remained high (25%).

Effect of ascorbic acid on the absorption of iron in soya biscuits (Table 2)

The mean absorption of iron in soya biscuits containing 2 mg supplemental iron was good, being approximately 20% in five individuals. In a further five subjects, supplementation with 100 mg ascorbic acid had no effect. Indeed, the mean absorption of 15% was somewhat lower than in the basal study. This may have been due to the fact that mean reference absorption in the second group was less than in the first, the figures being 47.1% and 72.8% respectively.

Effect of ascorbic acid on the absorption of iron in bread (Table 3)

The three groups of subjects which were studied were reasonably well matched in terms of the degree of iron deficiency. The mean percentage absorption in the five subjects given unsupplemented bread was similar to that noted for maize porridge (9.0% as compared with 6.8%). In the second experiment enough ascorbic acid was added to the dough to provide 50 mg per person, but the mean absorption of the bread iron (10.0%) was not enhanced. In a final experiment, a 2.5 mg iron supplement was present in addition to the ascorbic acid. The mean percentage
Table 1: Absorption of iron in maize porridge with special reference to the effects of varying doses of ascorbic acid added prior to cooking

<table>
<thead>
<tr>
<th>Study</th>
<th>Hb (g/100 ml)</th>
<th>Plasma iron (µg/100 ml)</th>
<th>% Saturation</th>
<th>% Iron absorption</th>
<th>Reference salt</th>
<th>δFe (Instrinsic)</th>
<th>δFe (Extrinsic)</th>
<th>Ratio</th>
<th>Intrinsic</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4.6</td>
<td>28</td>
<td>4.8</td>
<td>18.2</td>
<td>0.8</td>
<td>0.5</td>
<td>0.01</td>
<td>0.63</td>
<td>1.70</td>
</tr>
<tr>
<td>No added</td>
<td>4.1</td>
<td>53</td>
<td>9.5</td>
<td>29.9</td>
<td>0.9</td>
<td>2.3</td>
<td>7.0</td>
<td>1.67</td>
<td>2.09</td>
</tr>
<tr>
<td>Ascorbic acid (final iron content 1.8mg)</td>
<td>7.8</td>
<td>42</td>
<td>7.6</td>
<td>56.1</td>
<td>4.2</td>
<td>5.0</td>
<td>9.8</td>
<td>1.96</td>
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<tr>
<td>8.2</td>
<td>29</td>
<td>6.0</td>
<td>65.1</td>
<td>6.4</td>
<td>13.4</td>
<td>2.09</td>
<td>1.83</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.4</td>
<td>37</td>
<td>6.6</td>
<td>97.6</td>
<td>7.0</td>
<td>12.8</td>
<td>1.83</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>7.3</td>
<td>38</td>
<td>7.1</td>
<td>55.8</td>
<td>3.8</td>
<td>6.6</td>
<td>1.88</td>
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<td>Study B</td>
<td>12.8</td>
<td>72</td>
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<td>15.2</td>
<td>1.0</td>
<td>6.5</td>
<td>6.50</td>
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<tr>
<td>50 mg ascorbic acid (final iron content 1.3mg)</td>
<td>10.0</td>
<td>58</td>
<td>33.8</td>
<td>46.2</td>
<td>7.2</td>
<td>21.2</td>
<td>1.94</td>
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<tr>
<td>9.0</td>
<td>41</td>
<td>8.8</td>
<td>42.6</td>
<td>13.6</td>
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<tr>
<td>7.4</td>
<td>47</td>
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<td>50.4</td>
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<td>24.0</td>
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<tr>
<td>9.6</td>
<td>54</td>
<td>16.2</td>
<td>24.2</td>
<td>18.3</td>
<td>24.0</td>
<td>1.31</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.6</td>
<td>75</td>
<td>14.3</td>
<td>88.4</td>
<td>23.7</td>
<td>28.1</td>
<td>1.19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>9.5</td>
<td>54</td>
<td>13.8</td>
<td>48.9</td>
<td>14.9</td>
<td>23.7</td>
<td>2.42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study C</td>
<td>12.0</td>
<td>43</td>
<td>12.4</td>
<td>13.4</td>
<td>4.1</td>
<td>5.4</td>
<td>1.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 mg ascorbic acid + 2.5 mg added iron (final iron content 7.4mg)</td>
<td>13.6</td>
<td>152</td>
<td>38.7</td>
<td>14.8</td>
<td>8.8</td>
<td>9.2</td>
<td>1.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.0</td>
<td>43</td>
<td>7.4</td>
<td>50.7</td>
<td>9.8</td>
<td>11.2</td>
<td>1.14</td>
<td></td>
<td></td>
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<tr>
<td>9.4</td>
<td>29</td>
<td>5.1</td>
<td>35.4</td>
<td>10.5</td>
<td>12.6</td>
<td>1.20</td>
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<tr>
<td>10.6</td>
<td>75</td>
<td>14.3</td>
<td>88.4</td>
<td>23.7</td>
<td>28.1</td>
<td>1.19</td>
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<td></td>
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</tr>
<tr>
<td>Mean</td>
<td>9.5</td>
<td>54</td>
<td>13.8</td>
<td>48.9</td>
<td>14.9</td>
<td>23.7</td>
<td>2.42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study D</td>
<td>12.1</td>
<td>86</td>
<td>17.7</td>
<td>66.6</td>
<td>17.9</td>
<td>20.0</td>
<td>1.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100mg ascorbic acid + 2.5 mg added iron (final iron content 5.5 mg)</td>
<td>8.6</td>
<td>37</td>
<td>6.5</td>
<td>39.7</td>
<td>17.9</td>
<td>23.1</td>
<td>1.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.8</td>
<td>44</td>
<td>8.6</td>
<td>35.6</td>
<td>23.6</td>
<td>25.6</td>
<td>1.09</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.8</td>
<td>35</td>
<td>6.0</td>
<td>25.1</td>
<td>27.9</td>
<td>1.11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>11.3</td>
<td>76</td>
<td>17.1</td>
<td>33.4</td>
<td>12.1</td>
<td>1.15</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Study E</td>
<td>7.2</td>
<td>24</td>
<td>4.7</td>
<td>59.6</td>
<td>9.8</td>
<td>11.1</td>
<td>1.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 mg ascorbic acid + 5 mg added iron (final iron content 8.4 mg)</td>
<td>10.8</td>
<td>87</td>
<td>17.6</td>
<td>75.4</td>
<td>11.6</td>
<td>13.4</td>
<td>1.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.4</td>
<td>117</td>
<td>21.5</td>
<td>72.5</td>
<td>14.8</td>
<td>16.3</td>
<td>1.10</td>
<td></td>
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<tr>
<td>13.2</td>
<td>148</td>
<td>35.4</td>
<td>52.0</td>
<td>20.0</td>
<td>22.6</td>
<td>1.13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>10.3</td>
<td>82</td>
<td>17.1</td>
<td>65.2</td>
<td>15.5</td>
<td>17.4</td>
<td>1.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study F</td>
<td>10.6</td>
<td>67</td>
<td>22.6</td>
<td>9.6</td>
<td>2.4</td>
<td>0.1</td>
<td>0.04*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>250mg ascorbic acid + 2.5 mg added iron + 1 egg (final iron content 6.1 mg)</td>
<td>6.4</td>
<td>21</td>
<td>3.2</td>
<td>122.6</td>
<td>21.0</td>
<td>20.9</td>
<td>0.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.5</td>
<td>140</td>
<td>33.7</td>
<td>27.6</td>
<td>23.8</td>
<td>24.4</td>
<td>1.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.4</td>
<td>42</td>
<td>8.9</td>
<td>33.4</td>
<td>36.5</td>
<td>37.2</td>
<td>1.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.3</td>
<td>37</td>
<td>7.2</td>
<td>55.6</td>
<td>41.3</td>
<td>43.4</td>
<td>1.05</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Mean</td>
<td>10.0</td>
<td>61</td>
<td>15.1</td>
<td>49.8</td>
<td>24.8</td>
<td>25.2</td>
<td>1.03</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Results in which the absorption of food iron was less than 1.0% were excluded from calculations of the mean.

The stability of ascorbic acid during cooking (Table 4)

The fact that ascorbic acid enhanced the absorption of iron in maize porridge, but not in soya biscuits or wheat bread, suggested that it might have been oxidized to different degrees by the various cooking procedures. This was
Table 2: Absorption of iron in soya biscuits with special reference to the effects of ascorbic acid added prior to baking

<table>
<thead>
<tr>
<th></th>
<th>Hb (g/100 ml)</th>
<th>Plasma iron (µg/100 ml)</th>
<th>% Saturation</th>
<th>% Iron absorption</th>
<th>Ratio Extrinsic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study G</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 mg added iron but no ascorbic acid</td>
<td>11.6</td>
<td>64</td>
<td>18.5</td>
<td>93.4</td>
<td>7.9</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>31</td>
<td>6.8</td>
<td>56.7</td>
<td>8.1</td>
</tr>
<tr>
<td></td>
<td>10.3</td>
<td>80</td>
<td>14.2</td>
<td>102.0</td>
<td>16.3</td>
</tr>
<tr>
<td></td>
<td>10.4</td>
<td>38</td>
<td>8.7</td>
<td>11.5</td>
<td>18.6</td>
</tr>
<tr>
<td></td>
<td>11.5</td>
<td>56</td>
<td>11.2</td>
<td>99.7</td>
<td>46.0</td>
</tr>
<tr>
<td>Mean</td>
<td>10.8</td>
<td>54</td>
<td>11.9</td>
<td>72.8</td>
<td>19.8</td>
</tr>
<tr>
<td>Study H</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 mg added iron plus 100mg ascorbic acid</td>
<td>12.6</td>
<td>142</td>
<td>45.5</td>
<td>8.7</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>10.7</td>
<td>68</td>
<td>16.6</td>
<td>38.1</td>
<td>11.9</td>
</tr>
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<td>11.4</td>
<td>75</td>
<td>18.4</td>
<td>63.5</td>
<td>15.6</td>
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<tr>
<td></td>
<td>8.0</td>
<td>71</td>
<td>13.9</td>
<td>51.6</td>
<td>20.5</td>
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<tr>
<td></td>
<td>8.0</td>
<td>113</td>
<td>21.2</td>
<td>73.6</td>
<td>23.9</td>
</tr>
<tr>
<td>Mean</td>
<td>10.1</td>
<td>94</td>
<td>23.1</td>
<td>47.1</td>
<td>14.6</td>
</tr>
</tbody>
</table>

Table 3: Iron absorption from wheat bread (100% extraction) with special reference to the effects of ascorbic acid added prior to baking

<table>
<thead>
<tr>
<th></th>
<th>Hb (g/100 ml)</th>
<th>Plasma iron (µg/100 ml)</th>
<th>% Saturation</th>
<th>% Iron absorption</th>
<th>Ratio Extrinsic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No added ascorbic acid</td>
<td>8.4</td>
<td>18</td>
<td>3.7</td>
<td>69.7</td>
<td>0.6</td>
</tr>
<tr>
<td>(final iron content 3.7mg)</td>
<td>6.0</td>
<td>41</td>
<td>9.0</td>
<td>115.9</td>
<td>5.2</td>
</tr>
<tr>
<td>Mean</td>
<td>8.1</td>
<td>34</td>
<td>6.4</td>
<td>74.2</td>
<td>7.9</td>
</tr>
<tr>
<td>Study J</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 mg ascorbic acid (final iron content 3.1mg)</td>
<td>8.0</td>
<td>37</td>
<td>8.7</td>
<td>90.0</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>11.9</td>
<td>72</td>
<td>25.7</td>
<td>27.7</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>9.1</td>
<td>23</td>
<td>4.2</td>
<td>13.2</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>8.4</td>
<td>25</td>
<td>4.8</td>
<td>11.7</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>7.4</td>
<td>28</td>
<td>6.6</td>
<td>43.4</td>
<td>9.4</td>
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<td>19</td>
<td>4.9</td>
<td>101.0</td>
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</tr>
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<td></td>
<td>9.0</td>
<td>34</td>
<td>6.7</td>
<td>112.0</td>
<td>16.0</td>
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<tr>
<td>Mean</td>
<td>8.9</td>
<td>34</td>
<td>8.8</td>
<td>53.4</td>
<td>6.6</td>
</tr>
<tr>
<td>Study K</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 mg ascorbic acid + 2.5 mg added iron (final iron content 4.7mg)</td>
<td>9.0</td>
<td>20</td>
<td>3.2</td>
<td>63.6</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>25</td>
<td>4.9</td>
<td>36.9</td>
<td>3.3</td>
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<td>15</td>
<td>2.7</td>
<td>8.5</td>
<td>3.4</td>
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<tr>
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<td>10.7</td>
<td>78.5</td>
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<td>9.3</td>
<td>32</td>
<td>5.6</td>
<td>56.3</td>
<td>12.4</td>
</tr>
<tr>
<td>Mean</td>
<td>8.6</td>
<td>30</td>
<td>5.4</td>
<td>48.8</td>
<td>5.3</td>
</tr>
</tbody>
</table>

tested by measuring the concentrations of total and of reduced ascorbic acid before and after preparation. Most of the ascorbic acid added to maize porridge was still in the reduced form after cooking, namely 68% of 50 mg and 71% of 100 mg. The concentrations of "total" ascorbic acid, which includes dehydro-ascorbic acid and diketogulonic acid, were virtually unchanged by cooking.

By comparison, only 15% of 100 mg added ascorbic acid was present in the reduced form in soya biscuits after baking for 45 min. The concentration of "total" ascorbic acid at this time was 37%.
Table 4: Recovery of ascorbic acid added to white and brown dough (600 g) before and after baking into loaves (540 g)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ascorbic acid added (mg)</th>
<th>&quot;Total&quot;**</th>
<th>Reduced</th>
</tr>
</thead>
<tbody>
<tr>
<td>White dough</td>
<td>250*</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>8</td>
<td>37</td>
</tr>
<tr>
<td>Brown dough</td>
<td>250*</td>
<td>10</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>10</td>
<td>63</td>
</tr>
<tr>
<td>White bread</td>
<td>250*</td>
<td>52</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>34</td>
<td>89</td>
</tr>
<tr>
<td>Brown bread</td>
<td>250*</td>
<td>54</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>45</td>
<td>86</td>
</tr>
</tbody>
</table>

* Indicates level of ascorbic acid added in *in vivo* bread studies.

** "Total" ascorbic acid refers to ascorbic acid, dehydro-ascorbic acid and diketogulonic acid.

In the case of bread, losses in reduced ascorbic acid were revealed, not only after but also prior to baking (Table 4). For example, at the concentration of ascorbic acid used in the *in vivo* studies, 35% of the reduced ascorbic acid had disappeared even before baking. After baking there was virtually no reduced ascorbic acid left in either the white (72% extraction) or brown bread (80% extraction), and the amount of "total" ascorbic acid was about half the original figure.

**Discussion**

Some of the present findings confirm the observations of Cook, *et al.*, (1972) and of Bjöörn-Rasmussen & Hallberg (1972) that when inorganic iron and non-haem food iron are eaten together, they are absorbed from a common pool. With the exception of our initial experiments in which there were technical difficulties, the ratio of the absorption of the extrinsic to intrinsic radioactive tags was close to unity (1.12, SD ± 0.08). These figures were obtained in a consecutive series of 42 tests in which the absorption varied between 1% and 50% and the quantity of extrinsic iron ranged from tracer amounts up to 5 mg.

All the patients studied were sufficiently iron deficient to absorb large percentages of the 3 mg reference dose of ferrous iron, and yet the absorption of similar amounts of food iron was poor. Comparable mean figures were as follows: maize porridge 6.8% as compared with 55.8%; soya biscuits 19.8% as compared with 72.8%; and wheat bread 7.9% as compared with 74.2%. The iron in soya beans was the best absorbed of the three vegetables. This is in keeping with the earlier observations made by Layrisse, *et al.*, (1969). It should be noted, however, that in later investigations by Layrisse & Martinez-Torres (1971), the percentage absorption of iron from soya beans was considerably lower. This discrepancy may be the result of different cooking methods or differences in the cultivation of the soya beans.

The most striking observation in the present study was the pronounced enhancement of iron absorption on adding ascorbic acid to maize porridge prior to cooking. When the iron content of the meal was increased by supplementation with ferric ammonium citrate, the quantity of iron absorbed was greater than with ascorbic acid alone, although the percentage absorption fell. Doubling the ascorbic acid supplement led to a further significant increase in the actual amount of iron absorption. The nutritional advantage in quantitative terms was considerable: the mean calculated absorption from unsupplemented porridge was 0.31 mg, whereas it rose to 1.60 mg when 100 mg ascorbic acid and 5 mg inorganic iron had been added to the porridge. It was also possible to show that the consumption of an egg, which is known to inhibit iron absorption (Chodos, *et al.*, 1957; Elwood, *et al.*, 1968), failed to reduce absorption of iron in porridge to which a large amount of ascorbic acid had been added prior to cooking.

In contrast to its effect on the absorption of iron from maize porridge, the addition of ascorbic acid to soya biscuits and to bread made no difference. This appeared to be due to the fact that ascorbic acid was destroyed during the baking process, whereas only minimal oxidative degradation occurred during the preparation of the porridge. Presumably this can be ascribed to the much higher temperatures reached during baking, which are several fold greater than during boiling. Any potential value that ascorbic acid supplementation might have would therefore appear to be confined to those foodstuffs that are normally prepared by boiling. This would not necessarily exclude its use, since staples such as potato, rice and maize are usually cooked in this way. In addition, there is only 13.4% loss of reduced ascorbic acid.
during 2 hr cooking of soup (Sayer, 1972). Of more practical importance is the cost of supplementing diets with large quantities of ascorbic acid. It remains, however, possible that small amounts together with iron supplements may improve iron nutrition to a sufficient degree; alternatively, there may be cheaper substances that are as effective.

One final point deserves comment. In the present study the correlation between the percentage absorption of the iron in the reference salt and the iron in the vegetable was not statistically significant. Relevant figures were as follows: maize without ascorbic acid supplementation, \( r = +0.71, P > 0.05 \); maize with ascorbic acid supplementation, \( r = +0.26, P > 0.1 \); soya bean, \( r = +0.45, P > 0.1 \); wheat, \( r = +0.44, P > 0.05 \). These results are different from those reported by Layrisse, et al., (1969), who noted a significant correlation between the reference salt absorption and that of the iron in a variety of vegetable foodstuffs. There are several possible reasons for this discrepancy. The numbers in the present study were small, while the degree of ascorbic acid and of iron supplementation varied. In addition, all the subjects were iron deficient, and the groups were therefore more homogeneous in terms of their absorptive behaviour than were the heterogeneous subjects studied by Layrisse, et al., (1969).

Acknowledgements

We wish to thank Mrs Shirley Lichtigfeld for her valuable technical assistance. This work was supported by grants from the International Atomic Energy Agency, the South African Atomic Energy Board and the Wellcome Trust, Great Britain.

References

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Is transferrin normal in idiopathic haemochromatosis?

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Summary

Family studies were done to ascertain whether there is linkage between the transferrin locus and the HLA loci on chromosome 6. The findings in four families in which there was variation at the transferrin locus did not demonstrate any linkage, with 17 of the 30 offspring of heterozygous parents being recombinants and 13 non-recombinants. These results indicate that the HLA linked defect responsible for increased iron absorption in idiopathic haemochromatosis is not a consequence of any abnormality in the primary structure of transferrin.

The tendency to absorb iron excessively in idiopathic haemochromatosis is inherited in an autosomal recessive fashion, with homozygotes usually developing severe iron overload and heterozygotes sometimes exhibiting minor derangements in iron metabolism (Simon, et al., 1980). The locus is linked to the HLA loci on chromosome 6 (Simon, et al., 1980) but the nature of the metabolic defect responsible for the condition is unknown. It could theoretically be located in the mucosal cells of the upper gut or in the protein, transferrin, which transports iron through the plasma. If the latter, it might be predicted that the locus responsible for idiopathic haemochromatosis is the transferrin locus and linkage between it and the HLA loci between the transferrin locus would exist.

Methods and results

Individuals with variation at the transferrin locus were ascertained by screening healthy blood donors, or else by testing individuals who participated in a study to determine the prevalence of familial hypercholesterolaemia in the Afrikaans population. The individual and his family were then asked to participate in the linkage study, and when informed consent had been obtained, fresh blood samples, including defibrinated blood, were collected from them all.

Transferrin variation was detected by means of starch gel electrophoresis in a tris/EDTA/borate discontinuous buffer system, pH 8.6 (Smithies & Hiller, 1959), and typing for HLA-A, -B and -C locus antigens carried out by standard methods. The variant transferrin was TfD, in the two large "coloured", or racially-mixed, families and in one of the Caucasoid families, while in the other Caucasoid family it was TFB. The lod score method as described by Maynard-Smith, et al., (1961) was used.

The two three- and one four-generation families gave results as follows: (a) through males, seven non-recombinant and six recombinants; (b) through females, four non-recombinants and 10 recombinants. A single two-generation family in which the mother was the heterozygote, provided three children, one of whom was a recombinant.

The lod scores of the Tf:HLA linkage relationship are presented in Table 1, showing separately the scores when the mother and the father is the heterozygous parent. In the two-generation family a z score was used. It will be seen that linkage up to a recombination fraction of 0.30 has been virtually excluded, if sex differences are ignored.
### Table 1: Lod scores of Tf:HLA relationships in four families

<table>
<thead>
<tr>
<th>Offspring</th>
<th>0.05</th>
<th>0.10</th>
<th>0.20</th>
<th>0.30</th>
<th>0.35</th>
<th>0.40</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>r*</td>
<td>n-r</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>Mother heterozygous</td>
<td>17</td>
<td>11</td>
<td>6</td>
<td>-9.605</td>
<td>-6.414</td>
<td>-3.358</td>
</tr>
<tr>
<td>Father heterozygous</td>
<td>13</td>
<td>6</td>
<td>7</td>
<td>-4.047</td>
<td>-4.047</td>
<td>-0.960</td>
</tr>
<tr>
<td>Combined</td>
<td>30</td>
<td>17</td>
<td>13</td>
<td>-13.752</td>
<td>-8.823</td>
<td>-4.318</td>
</tr>
</tbody>
</table>

* r = recombinants; n-r = non-combinants.

### Discussion

In previous in vitro and in vivo experiments no abnormality in the behaviour of transferrin was noted in subjects with idiopathic haemochromatosis (Bothwell, et al., 1962). However, the methods used were relatively insensitive, and because the concept that the disorder might result from transferrin having an increased affinity for iron remained an attractive one, the problem was reinvestigated using a different approach. For such a relationship to exist it would be necessary to demonstrate linkage between the transferrin locus and the HLA loci, since linkage has already been shown between the HLA loci and the locus associated with increased iron absorption in idiopathic haemochromatosis (Simon, et al., 1980). The present findings were, however, negative. These results are compatible with other work suggesting that the Tf locus is not situated on chromosome 6. Although there is good evidence that the Tf and E, (pseudocholinesterase) loci are linked (Robson, et al., 1966) the claim that Tf and E are linked to the Rh locus (which has been confidently assigned to chromosome 1) is based on poor evidence (Chautard-Freiemaie, 1966). In this connection, WF Bodmer recently presented evidence at the 6th International Congress of Human Genetics, Jerusalem, September 1981, that his group had, by means of a somatic cell hybridization technique and a monoclonal antibody, assigned the Tf locus to chromosome 3.

### Acknowledgements

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### References

Elliott, Guy Abercrombie


(* Wynberg, CC, 5.11.1905 - Cape Town, 23.9.1975), academic, physician, and teacher, was the third of the four children of Henry Horne Elliott and his wife Clair Rushton. The great-grandfather, the Reverend William Elliott, came out to this country with the 1820 Settlers although he was not one of them, and his grandfather, Sir Charles Bletterman Elliott,* was General Manager of the Cape Railways. E. came from a distinguished family: Charles Caldwell Elliott, *the beloved physician*, and Frank Field Elliott, the biochemist, were uncles; and Frank Elliott, the American neurologist, was a cousin. Mr FC Kolbe* was the son of a great-aunt, and there were many other connections with prominent people.

E. was educated at the South African College and later at the University of Cape Town, where he graduated MBChB with first-class honours in 1929. After resident posts at the new Somerset Hospital in Cape town, he then (from 1931) followed a path of postgraduate training unique for that period, since he spent an extended period overseas, obtaining specialised clinical and research experience in cardiology (National Heart Hospital, London), neurology (National Hospital for Nervous Diseases, London), and haematology (John Hopkins Hospital, Baltimore). After four years’ practice as a private and honorary physician in Cape Town he returned to London in 1938 to carry out further research on anaemia in pregnancy at the British Post-Graduate Medical School. This work subsequently formed the subject of his doctoral thesis (awarded with first-class honours), the first doctorate in medicine to be conferred by the University of Cape Town, in 1941. During the Munich crisis (September 1938) he was one of a distinguished British team of three to study problems relating to the storage of blood for blood transfusion.

He served with distinction in the South African Medical Corps for six years (1940-45) during the Second World War (1939-45), and when on active service in North Africa and Italy was in charge of the medical divisions of military hospitals, rising to the rank of lieutenant-colonel. He was awarded the OBE (military division) in recognition of outstanding work in the treatment and prevention of trench foot, a serious hazard to Allied troops in the fierce ground fighting in the Apennines during the winter of 1944-45.

In 1946, after a short spell in Cape Town as a consultant physician in private practice and head of a medical unit at Groote Schuur Hospital, he became the first full-time professor of medicine at the University of the Witwatersrand, Johannesburg, and chief physician to the Johannesburg Hospital. He held this dual post until his retirement twenty-one years later. During this time he built up a formidable reputation as an inspiring teacher, a highly-skilled physician, and an able administrator who encouraged research, though he himself did not excel at original research. He was particularly conscious of the psychosomatic aspect of disease, and studied methods of teaching psychiatry and integrating this subject with other medical disciplines. In 1950 he was appointed director of the Cardio-Pulmonary Research Unit, one of the first research units set up by the Council for Scientific and Industrial Research (CSIR). He was awarded three World Health Organization Fellowships: one in 1953 to study teaching methods in psychiatry, another for nuclear medicine in 1958 and the third, in 1964, for the production, statutory control, safety and efficacy of drugs.

He wrote more than sixty articles on medical and educational subjects and of these "A physician looks down the microscope" in *Medical Proceedings* (22.8.1964) contains important biographical information. In 1954 his book *Medical Ethics* was published. Contributing in other ways to his profession, he served for twenty-four years on the South African Medical and Dental Council (for most of this time as chairman of its Specialist Committee and Educational Committee); he was the first president of the College of Physicians, Surgeons and Gynaecologists of South Africa (now known as the College of Medicine of South Africa), and in 1968 was awarded an honourary Fellowship of this College - the first holder of a South African medical qualification to be honoured in this way; a foundation member of the Medicines Control Council, he was on its executive committee and chairman of its Safety of Medicines Committee; when he settled in Cape Town after his retirement (1967), he was attached to the medical teaching institutions of both Cape Town and Stellenbosch.

E. was elected a Fellow of the Royal College of Physicians of London in 1943 and of the Royal Society of South Africa in 1950. In 1968 the University of the Witwatersrand conferred a doctorate of medicine (*honoris causa*) on him, and in 1972 the University of Cape Town made him a doctor of science (*honoris causa*) for his outstanding contributions to clinical medicine in South Africa.

Punctiliously courteous and friendly to people of all stations, E. was nevertheless of a reserved and retiring nature. A bachelor, he was the most self-sufficient of individuals. The range of his interests was wide: he had a fine collection of Africana, including all the books relating to his friend and patient, Field-Marshall JC Smuts*; he was extremely knowledgeable about silver, music, South African painting, and furniture; he could play the organ and flute and repair sports cars - indeed, he was one of the few South Africans to travel overland from London to South Africa. Above all, he delighted in the flora and fauna of Table Mountain and after he had retired, and until shortly before his death, he climbed it every week.


TH Bothwell, Mary Lucas


36
Anaemia, iron deficiency and exercise: extended studies in human subjects

RW Charlton, D Derman, B Skikne, JD Torrance, SR Lynch, MH Sayers, S Zwi, HI Goldman, A van As, G Margo, JT Schneider and TH Bothwell

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Summary

1. Ventilation and cardiac frequency were measured during repeated treadmill exercise in three healthy subjects over 36 weeks, before, during and after iron-deficiency anaemia was produced and after iron treatment. The haemoglobin and 2,3-diphosphoglycerate concentrations and the oxygen-binding ($P_{50}$) were measured.

2. There was an inverse relationship between the haemoglobin concentrations and the 2,3-diphosphoglycerate concentrations and the $P_{50}$ values.

3. The mean cardiac frequencies during the fourth to tenth minute of exercise showed a negative correlation with the haemoglobin concentrations in all three subjects, and the mean minute ventilations in two of them.

KEY WORDS: Anaemia, 2,3-diphosphoglycerate, exercise, iron deficiency.

Introduction

The recent proposals to increase iron fortification of flour in the United States have refocussed attention on the possible ill-effects of iron deficiency. In spite of numerous investigations there is still no general agreement that mild or even moderate anaemia affects exercise tolerance, although it is accepted that maximal work performance is limited by severe anaemia. For example, Wranne & Woodson (1973) found that the maximal work capacity of rats was directly proportional to the haemoglobin concentration right up to the normal range, and Viteri & Torun (1974) reported a similar relationship between the haemoglobin concentration and the Harvard step test score in Guatemalan agricultural labourers. On the other hand, Davies, Chukwemeka & van Haaren (1973) could detect a decrease in the maximal aerobic capacity only if the haemoglobin concentration in iron-deficient subjects was less than 12 g/dl, and Cotes, Dabbs, Elwood, Hall, McDonald & Saunders (1972) concluded that the ventilation and cardiac frequency during submaximal exercise were independent of the haemoglobin concentration in the range 8-15 g/dl. We have therefore studied in three of us the relationship between ventilation and cardiac frequency during treadmill exercise and haemoglobin concentration varied by repeated venesections over 36 weeks.
Materials and methods

Subjects

The three volunteers were healthy males aged 46 (RWC), 30 (BS) and 32 years (DD), who led essentially sedentary lives, although DD regularly played non-competitive squash racquets. Initially, DD smoked five cheroots per day, but he stopped altogether during the study. The other two were non-smokers. The study was performed in Johannesburg (mean altitude 1750 m). Each subject fully understood the nature, purpose, and risk of all the procedures used, and the study was approved by the Committee for Research on Human Subjects of the Faculty of Medicine of the University of the Witwatersrand.

Venesections

Each week 600 ml of blood was removed until the haemoglobin concentration, determined by the cyanmethaemoglobin method (Dacie & Lewis, 1968), had fallen to 9-11 g/dl. Depletion of the body iron reserve was demonstrated by the failure of the haemoglobin concentration to rise when venesections were stopped, by a mean cell volume below 75 fl and a mean cell haemoglobin below 1.55 fmol, a serum ferritin concentration (Miles, Lipschitz, Bieber & Cook, 1974) less than 23 pmol/l, a plasma iron concentration (International Committee for Standardization in Hematology, 1971) less than 9 pmol/l and a transferrin saturation (Herbert, Gottlieb, Lau, Gevirtz, Shamey & Cook, 1974) less than 23 pmol/l, a plasma iron concentration (International Committee for Standardization in Hematology, 1971) less than 9 pmol/l and a transferrin saturation (Herbert, Gottlieb, Lau, Gevirtz, Shamey & Cook, 1974) less than 23 pmol/l. The "iron-deficient phase" was arbitrarily taken to start 2 weeks after the last venesection, and during this phase only enough blood was removed to keep the haemoglobin concentration in the vicinity of 10 g/dl (this being about 60 ml/week). After 8 more weeks ferrous gluconate or ferrous sulphate tablets (not less than 1.8 mmol of iron every 6 h) were taken before meals and further venesections (600 ml) were performed at least every 4 days to prevent the haemoglobin concentration from rising. The period during which iron tablets were being taken plus the first week thereafter was termed the "iron-replete phase". It was followed by a second iron-deficient phase, which started 2 weeks after the last 600 ml venesections had been performed, and then iron tablets were again taken to produce a second iron-replete phase.

During treadmill exercise the cardiac frequency (fc), respiratory rate (fr) and the minute ventilation (Ve), were recorded, fc from precordial electrodes and a transmitter-receiver (Avionics Research Products Metretel models 2620 and 2800), and Ve with a Parkinson-Cowan gas meter. Starting 4 weeks before the first venesection, each subject exercised once a week throughout the study, but not less than 48 h after the most recent venesection. On each occasion the subject rested for at least 10 min in a chair before the observation period. Measurements were made for each of 3 min when sitting, for 2 min on standing and then when walking for 10 min on the treadmill, holding the handrail, at an incline of 17° and a speed of 3.22 km/h (2 miles/h). After resting for 20-30 min treadmill exercise was repeated at an incline and 15° and 6.44 km/h (4 miles/h). Immediately before and after each period of exercise, and again 5 min afterwards, capillary blood samples were taken from the warmed ear lobe for measurements of P02, Pco2, pH, standard bicarbonate and base excess. Venous blood was also collected (not on the day of exercise) for the measurement of the erythrocyte 2,3-diphosphoglycerate concentration by the automated enzymatic method of Prins & Loos (1969), and the oxygen tension at 50% oxygen saturation (Po2) was obtained from the oxygen dissociation curve plotted during oxygenation of deoxygenated blood (Duvelleroy, Buckles, Rosenkaimer, Tung & Laver, 1970).

Results

In the initial phase of repeated venesection the mean fc during the fourth to tenth minutes of exercise was inversely related to the haemoglobin concentration, as was Ve in the fourth to tenth minutes of exercise at the slower treadmill speed in RWC and BS (Table 1). During exercise at the higher speed in RWC fc and Ve also increased markedly, but when his haemoglobin concentration had fallen to about 12 g/dl he was unable to exercise for 10 min. DD stopped smoking on day 51 of the study, and his fc and Ve values fell thereafter. At the end of the study when his haemoglobin concentration had returned to its original value Ve and fc on exercise were lower than they had been at the beginning: for the last two exercises at 6.44 km/h Ve was 56.0 and 61.2 1/min, compared with the mean for the first five exercises of 69.9 (range 64.0-79.2) 1/min, and fc was 136 and 135 beats/min compared with 150 (146-153) beats/min.

In RWC and BS fc and Ve during the subsequent iron-deficient phase (which lasted 8 and 10 weeks respectively) were higher than at similar haemoglobin concentrations during the initial phase (Figure 1a and 1b). On iron repletion there were inconstant reductions in Ve and fc, but in RWC the haemoglobin concentration rose significantly despite the repeated venesections, which were intended to keep it constant (Table 2).

Because of this, and as only a few observations could be made at haemoglobin concentrations ([Hb]) in the vicinity of 10 g/dl before iron deficiency superimposed, the possibility that the relationships were curvilinear rather than linear was investigated by using all the data. The regressions of the fc and Ve with [Hb]2, [Hb] + [Hb]2, 1/[Hb], 1/[Hb]2 and
Table 1: Relationships between haemoglobin concentration, ventilation ($V_e$) and cardiac frequency ($f_c$)

Mean values of $V_e$ and $f_c$ at the fourth to tenth minutes during weekly treadmill exercises are shown. \([Hb] = \) haemoglobin concentration (g/dl).

<table>
<thead>
<tr>
<th>Subject</th>
<th>Treadmill speed (km/h)</th>
<th>No. of exercise studies</th>
<th>$V_e$ (l/min) = $a + b \times [Hb]$</th>
<th>$f_c$ (beats/min) = $a \times b \times [Hb]$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>$a$</td>
<td>$b$</td>
</tr>
<tr>
<td>Before onset of iron deficiency</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RWC</td>
<td>3.22</td>
<td>18</td>
<td>59.3</td>
<td>-.114</td>
</tr>
<tr>
<td>BS</td>
<td>3.22</td>
<td>11</td>
<td>41.6</td>
<td>-.122</td>
</tr>
<tr>
<td>6.44</td>
<td>68.2</td>
<td>-1.80</td>
<td>-.570</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>DD</td>
<td>3.22</td>
<td>15</td>
<td>37.4</td>
<td>-.080</td>
</tr>
<tr>
<td>6.44</td>
<td>69.6</td>
<td>-.17</td>
<td>-.099</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td>All results</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RWC</td>
<td>3.22</td>
<td>41</td>
<td>66.2</td>
<td>-.164</td>
</tr>
<tr>
<td>BS</td>
<td>3.22</td>
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</tr>
<tr>
<td>6.44</td>
<td>97.1</td>
<td>-.381</td>
<td>-.763</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

$1/\sqrt{[Hb]}$ did not yield significantly higher values for correlation coefficients than those with \([Hb]$, which are given in Table 1.

In all three subjects the erythrocyte 2,3-diphosphoglycerate concentrations rose as the haemoglobin concentrations fell, and in two this was accompanied by a rise in $P_x$ (Table 3).

The correlation between the erythrocyte 2,3-diphosphoglycerate concentration and the $P_x$ was not significant in DD, but from day 51 of the study $P_x$ followed the 2,3-diphosphoglycerate concentration closely as in the other two subjects.

In all three subjects there were no further changes in either 2,3-diphosphoglycerate or $P_x$ during the iron-deficient or iron-replete phases. Both returned to the ranges observed at the start of the study when the haemoglobin concentration was eventually allowed to rise.

Neither anaemia nor iron deficiency nor repletion affected the $pH$, $P_{O_2}$, $P_{CO_2}$, standard bicarbonate or base excess of capillary blood either before or after exercise. The relationship between $V_e$ and tidal volume was also unaltered by venesection and iron repletion.

Figure 1: Mean value over 4-10 min of exercise of (a) ventilation ($V_e$) and (b) cardiac frequency ($f_c$) during 3.22 km/h treadmill exercises at various haemoglobin concentrations in subject RWC. The regressions for the values obtained from the start of the study to the onset of iron deficiency (•) are shown [ventilation (l/min) = 59.3 - 1.14[Hb] (g/dl), $r = -0.721, P < 0.01$; cardiac frequency (beats/min) = 139.8 - 1.88[Hb] (g/dl), $r = -0.720, P < 0.01$]. •, First iron-deficient phase; □, first iron-replete phase.
Table 2: Effects of iron deficiency and iron repletion on ventilation (Ve) and cardiac frequency (fc).

Mean values ±SD of Ve and fc at the fourth and tenth minutes on weekly treadmill exercising during iron-deficient (D) and iron-replete (R) phases are shown. * \( P < 0.05 \); ** \( P < 0.01 \); unmarked results, \( P > 0.05 \) (Student's t-test for unpaired observation).

<table>
<thead>
<tr>
<th>Subject</th>
<th>Iron status</th>
<th>First or second phase</th>
<th>Treadmill speed (km/h)</th>
<th>No. of exercise studies</th>
<th>Mean haemoglobin (g/dl of blood)</th>
<th>Ve (l/min)</th>
<th>fc (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RWC</td>
<td>D</td>
<td>1</td>
<td>3.22</td>
<td>8</td>
<td>10.6 **</td>
<td>53.7 ± 2.2</td>
<td>128 ± 5.0</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>1</td>
<td>3.22</td>
<td>4</td>
<td>11.8 **</td>
<td>46.2 ± 2.2</td>
<td>127 ± 4.8</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>2</td>
<td>3.22</td>
<td>8</td>
<td>10.9 *</td>
<td>46.8 ± 3.4</td>
<td>123 ± 5.0</td>
</tr>
<tr>
<td>BS</td>
<td>D</td>
<td>1</td>
<td>3.22</td>
<td>4</td>
<td>10.7 **</td>
<td>33.8 ± 3.1</td>
<td>129 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>1</td>
<td>3.22</td>
<td>6</td>
<td>10.7 *</td>
<td>29.6 ± 2.8</td>
<td>119 ± 6.0</td>
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<tr>
<td></td>
<td>R</td>
<td>2</td>
<td>3.22</td>
<td>7</td>
<td>9.6 *</td>
<td>34.0 ± 2.4</td>
<td>133 ± 6.1</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>1</td>
<td>6.44</td>
<td>4</td>
<td>10.7 *</td>
<td>56.5 ± 5.8</td>
<td>156 ± 4.7</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>2</td>
<td>6.44</td>
<td>6</td>
<td>10.7 *</td>
<td>56.1 ± 1.4</td>
<td>154 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>2</td>
<td>6.44</td>
<td>5</td>
<td>9.5 *</td>
<td>66.5 ± 3.5</td>
<td>166 ± 2.8</td>
</tr>
</tbody>
</table>

Table 3: Effect of anaemia on erythrocyte 2,3-diphosphoglycerate concentration and oxygen-binding \( (P_{50}) \)

\[ [2,3-DPG] = 2,3\text{-diphosphoglycerate concentration}; \ [Hb] = \text{haemoglobin concentration (g/dl). Results show the relationship between [2,3-DPG] and [Hb], and between } P_{50} \text{ values and [2,3-DPG], before the onset of iron deficiency.} \]

<table>
<thead>
<tr>
<th>Subject</th>
<th>No observations</th>
<th>([2,3-DPG] \text{ (\mu mol/g of Hb) = } a + b \times [Hb])</th>
<th>(P_{50} \text{ (kPa) = } a + b \times [2,3-DPG] \text{ (\mu mol/g of Hb)})</th>
</tr>
</thead>
<tbody>
<tr>
<td>RWC</td>
<td>18</td>
<td>(a = 28.7, b = -0.90, r = 0.909, P &lt; 0.001)</td>
<td>(a = 2.14, b = 0.109, r = 0.827, P &lt; 0.001)</td>
</tr>
<tr>
<td>BS</td>
<td>11</td>
<td>(a = 27.7, b = -0.82, r = 0.987, P &lt; 0.001)</td>
<td>(a = 1.52, b = 0.137, r = 0.838, P &lt; 0.001)</td>
</tr>
<tr>
<td>DD</td>
<td>14</td>
<td>(a = 22.3, b = -0.54, r = 0.880, P &lt; 0.001)</td>
<td>(a = 2.98, b = 0.035, r = 0.227, P &gt; 0.1)</td>
</tr>
</tbody>
</table>

Discussion

The erythrocyte 2,3-diphosphoglycerate concentration rose as the haemoglobin concentration fell, and there was a corresponding change in \( P_{50} \) (Table 3). In spite of this adaptation of anaemia (Torrance, Jacobs, Restrepo, Eschbach, Lenfant & Finch, 1970), the cardiac frequency (fc) during the exercise increased in all three subjects, and the ventilation (Ve) was also higher in two (Table 1). The increases were, however, small: for example, in RWC fc at 3.22 km/h increased by only 18 beats/min (16%) and his Ve value by 11 litres/min (26%) as his haemoglobin concentration fell from 16 g/dl to 10 g/dl. Moreover, there was an appreciable variation (Figure 1), even under the conditions of the present study in which the same subjects exercised regularly for many weeks. Others have studied groups of anaemic and non-anaemic individuals, or anaemic subjects who exercised once before and once after treatment, so that changes in cardiac frequency or ventilation have sometimes remained undetected. It is also conceivable that the ventilation does not always increase; that of DD certainly did not do so (Table 1), although this may have resulted from his stopping smoking.

These results indicate that mild iron-deficiency anaemia limits work capacity, but this is unlikely to be of any importance for most people. Even manual labourers seldom work at anything approaching their maximal capacity, and the average sedentary individual would not be expected to be at any disadvantage. The presence of significant respiratory or cardiovascular diseases, however, compromises the compensatory mechanisms, and the repair of iron deficiency in such subjects may lead to a worthwhile improvement in exercise tolerance.

Although we conclude that moderate iron-deficiency anaemia produces little disability in otherwise healthy individuals, the relationships between the haemoglobin concentration, fc and Ve during exercise suggest that the fc and Ve during the iron-deficient phase tended to be higher than would be expected from the haemoglobin concen-
There were, however, too few observations at low haemoglobin values before the onset of iron deficiency (Figure 1) for this interpretation to be accepted without reserve, and the results of iron repletion were also inconclusive (Table 2). Iron deficiency may affect the response to exercise by some mechanisms other than the reduction in haemoglobin concentration, as iron-deficient rats were unable to run on a treadmill for nearly as long as iron-replete rats with similar haemoglobin concentrations (Finch, Miller, Inamdar, Person, Seiler & Mackler, 1976). However, we cannot exclude the possibility that \( f_c \) and \( V_e \) are related to haemoglobin concentrations curvilinearly, this accounting for the higher values of \( f_c \) and \( V_e \) at the lower haemoglobin values.

Acknowledgement

The authors gratefully acknowledge support from the South African Atomic Energy Board.

References

Thoughts on the future of medical research in South Africa

TH Bothwell. Professor and Head, Dept. of Medicine, University of the Witwatersrand, Chief Physician, Johannesburg Hospital.

Paper delivered at a Symposium "Science in South Africa", held at the University of the Witwatersrand, July 1972.

Reprinted from *The Leech*, 1973; 43: 3-5

In this paper I shall confine my comments to medical research since it is the only field I have any competence to discuss. At the outset I should like to make it clear that the ideas I express are my own and do not necessarily reflect those of the various bodies to which I belong or with which I am associated. I must admit to some diffidence in putting forward a personal viewpoint on this particular subject as it is so far removed from my own everyday life. The decisions I am expected to make are mundane and practical. They relate to patient care, to undergraduate and postgraduate teaching programmes, to a wide variety of administrative matters and lastly to the day to day management of an ongoing research programme. When my colleagues and I discuss future plans, our objectives are modest and defined. We are concerned with what is feasible and practical; all this within the framework of the money and skilled personnel available. In contrast, the present discussion is centred on much broader issues. How, in fact, do we go about planning a utopia for medical research in this country? Can this be done by building on the infrastructure that already exists or should we re-define our objectives and start from scratch? To answer this question we have to know something of the present place of medical research in this country and of how it reached this position.

Prior to the second world war virtually no research of any sort was going on in the Medical Schools and what was being done was confined to the basic science departments. This pattern was, I may add, not peculiar to South Africa, but was characteristic of most Medical Schools in the Western world. The late forties were associated with radical changes, both here and overseas. Clinical medicine finally developed teeth in the form of drugs that actually worked and investigative methods that allowed for the measurements of deranged bodily functions in precise quantitative terms. What had been an imprecise and indefinable art was burgeoning forth as a young but formidable science. Within a relatively short period of time a host of new techniques became available. Clinically orientated doctors were becoming familiar with electrophoresis, immunoassays and chromatography, they were employing radioisotopes in a variety of metabolic studies, and they were using, as if to the manner born, increasingly sophisticated electronic equipment.

In South Africa the growth of medical research was given further impetus by the creation of full-time clinical Chairs and by the establishment of the CSIR. It is difficult to overestimate the role that this latter organisation played in developing the medical research potential of South Africa during the fifties and sixties. It made its dispensations with a benign flexibility and wisdom that earned the admiration and gratitude of generations of aspiring research workers. Over these years medical research came of age in South Africa. Now we have a fully fledged Medical Research Council which supports individuals, Groups and Units in the Universities and elsewhere. In addition, the MRC runs two research institutes, the one concerned with nutritional diseases and the other with occupational diseases. In the main, however, the Council's major intention is to emphasize the strengthening of medical research at existing Institutions, particularly Universities and Teaching Hospitals.

That then is the current position, with active research programmes going on in most Departments, clinical as well as pre-clinical, of the Medical Schools. In this endeavour, the distinction between the work going on in clinical as opposed to pre-clinical disciplines has become progressively more blurred. We now recognize, as has been eloquently
pointed out by Professor Henry Miller that the hierarchial distinction between pure and applied science is an anachronism. "The pragmatic activities of the clinician often pay unexpected dividends in basic knowledge. The renal physician has taught us more about the function of the human kidney than the physiologist; the stereotaxic neurosurgeon more about the corpus striatum of man than those whose observations are made on the cat. Without the activities of the transplant teams modern immunology would hardly have got off the ground, and the neuroradiologists' new routine investigations have opened wide a field of extracranial vascular pathology that had entirely escaped the attention of the pathologist."

These few examples indicate that the dividing line between pure and applied research is a subtle one. It does not depend on the subject studied or the method of study. It rather reflects the intent of the investigator. A chemist who tackles a chemical problem that he has set himself is doing pure research. The same person doing the same work in order to solve some problem that goes beyond chemistry is doing applied research. This brings us to a most important and contentious point. Who nominates the problems? In the past, both here and overseas, most projects undertaken by individuals or units have been strongly influenced by local circumstances. Anyone, for example, who has looked after patients belonging to the different ethnic groups in South Africa cannot but be impressed by the striking differences in the incidence of certain diseases. If one happens to be a cardiologist and notes the very low incidence of coronary thrombosis in Africans it is natural to seek for the reasons, since their activities of the transplant teams modem immunology would hardly have got off the ground; and the neuroradiologists' new routine investigations have opened wide a field of extracranial vascular pathology that had entirely escaped the attention of the pathologist.

During the last twenty five year I have watched at close quarters the tremendous growth of biomedical research,
both here and overseas. I have watched it grow in experimental laboratories and in the clinical environment. I have also seen the tangible results - an increasing array of powerful drugs, intensive care units with their respirators and cardiac monitors, worthwhile advances in the treatment of malignant disease, successful transplantation programmes for patients with end-stage kidney disease and so on. Every effort is being expended to improve and enlarge the scope of patient care. As this occurs the intellectual challenge of each field continues to grow and the young graduate of exceptional talent is perforce drawn into one or other of the moving fronts of medicine. In making this choice he is strongly influenced by several factors. Of these, perhaps the most important, is the fact that the student is exposed throughout his undergraduate course to a series of enthusiastic and dedicated teachers, most of them based in one or other of the major disciplines, and each selling his speciality to the best of his ability. Students identify with their teachers and in their early post-graduate careers many of the brightest zero in on either well established basic scientific fields or on one of the patient orientated super-specialities. We therefore continue to produce the sort of people we ourselves are. As a result, the standard of applied clinical research in South Africa goes from strength to strength. In each of the Medical Schools, there are numbers of Units that have gained world-wide recognition and young graduates who have trained in these Units and settle overseas are accepted with open arms wherever they go - adequate testimony to the high standards maintained in our local Units. But I repeat - is this enough?

Anyone who lives in South Africa cannot but be vividly aware of the magnitude of our public health problems. Diseases such as tuberculosis, bilharzia and malnutrition each pose their own particular challenge. It has been argued that the eradication of many of the health problems endemic in South Africa only requires the application of knowledge that is already available. There is, of course, some truth in this but it over-simplifies what seems to me a complex problem. When I mentioned my misgivings about directed research, it was not because I underestimated the size of the gaps in our current research endeavour. It was simply a belief that this was not an effective way of tackling these problems. To my mind the major pathology may well lie in the Universities themselves. The heaviest emphasis of undergraduate training is laid on the care of patients in hospital. With the highly specialised Units that exist at the Medical Schools, there is virtually no limit to what can be offered in terms of sophisticated methods of diagnosis and management. Salvage operations, both medical and surgical, of the greatest virtuosity are the order of the day. As a result the student when he has qualified as a young doctor tends to direct his own research endeavours in the same direction; his efforts revolve around the sick patient. In South Africa and for that matter in any country this is just not good enough. The majority of our best medical research workers are executing exquisite miniatures. They ignore the challenge of the large canvases in spite of the fact that there are so many of them waiting to be painted. A few examples come immediately to mind. How should health care be delivered to communities as diverse as those that exist in South Africa? Certainly the present hospital which functions in splendid isolation from the community as a whole is not the answer. How do you eliminate tuberculosis? How do you supplement ill-balanced diets at source? What are the practical possibilities in terms of population control? How do you go about promoting positive health and not just eliminating disease? To believe that any of these problems will be effectively solved within the framework of the health services as they presently exist is to my mind naive. Each of them requires just as much disciplined and controlled study as does the laboratory experiment. But the disciplines that are required are different. They range from epidemiology and statistics on the one had to sociology and psychology on the other. At present, however, not one amongst our brightest medical students would give even a passing thought to training himself for research in any of these fields. There are two reasons. Firstly his exposure to these broader concepts during his undergraduate course is minimal, and, secondly, he is all too often taught by people who have done little in the way of research in these specific fields - indeed many of them seem unaware of the exciting possibilities for worthwhile original work that exist in South Africa.

In speaking to senior medical students I often raise the points I have been discussing. I tell them of my own personal regret that I was not aware of the wider potential of medicine when I was a student. My remarks are accepted politely but with scepticism. It is assumed that they are the ramblings of someone who is aging rather rapidly! I can understand why they think so. The undergraduate curriculum as it presently exists is just not designed to explore the wider issues. No undergraduate qualifying today has any reason to believe that the application of medical knowledge to a society made up vastly differing population groups represents a glittering challenge for fruitful applied research. If we can correct our undergraduate curricula I believe there will be no need for so-called directed research. Provided they get the requisite stimulation and opportunities, we will produce young people of talent who will have the enthusiasm and competence to do productive research in the fields I have mentioned. At the same time I have no illusions about the difficulties inherent in bringing about the changes I envisage. Suffice it to say that it would involve not only radical changes in the content of the medical curriculum but also in its intent. This would mean a swing away from hospital based teaching to a course which involved the student throughout his undergraduate curriculum in all the many facets of health care as they are encountered in the community at large. I do not believe that such a change in emphasis will affect the care of individual patients deleteriously. There will always be those who derive their emotional and professional satisfaction in this way.

All this boils down to a simple issue - one, in fact, that I was first made aware of almost twenty years ago when crossing the United States by air. I was seated next to a senior official from the Boeing Corporation. He talked in a general way of the future plans of his company and mentioned in passing how easy it is for large corpo-
rations to lose their sense of direction. He cited the example of the railways in the United States which were at that time in dire straits. The reason was simple. The people in charge of them had always assumed they were in the railways business. They were wrong. They were in the transportation business and should have planned accordingly. The time has come for us in medicine to re-define our goals. Our job is not just tending to the needs of sick people. We are in the health business. It is only when we accept this fact that we will be able to plan meaningfully for the future.

References
The recent publication of a book on Professor WH (Don) Craib by Professor EB (Barry) Adams, which is reviewed elsewhere in this issue (p. 115), provides not only a vivid portrait of a remarkable man but also important insights into the development of academic medicine in South Africa. At a time when academic medicine is threatened on several fronts, it is salutary to be reminded of how recently the traditions we accept as normal were introduced into South Africa and how much we owe to those who helped establish them.

While departments of medicine were set up at the University of Cape Town in 1920 and at the University of the Witwatersrand in 1922, the initial emphasis was on clinical service and undergraduate teaching, with most of the duties being performed by honourary members of the part-time staff, some of whom obtained small university stipends for their teaching commitments. However, from the outset there were important differences between the two medical schools. At Cape Town the heads of the clinical departments, including medicine, were full-time professors, with the right to some limited private practice, while in Johannesburg they were appointed on a part-time basis. The differences between the departments of medicine at the two schools became even more marked in 1938 when two full-time professors, JF (Jack) Brock and Frank Forman were appointed in Cape Town. Brock returned to his alma mater after a number of years at Cambridge, where he had been the assistant-director of clinical research under the eminent Professor John Ryle. Forman, on the other hand, had already been full-time assistant to the previous professor (Arthur Falconer) for a number of years, having first joined the department in 1923. Over the decades that followed it was to prove a felicitous partnership. Forman, a superb clinician, held the chair of clinical medicine, while Brock was the administrative head and directed the research programme. In this latter capacity he played a dominant role in the establishment of academic medicine in South Africa. His own research programme capitalised on the effects of dietary habits on disease patterns in the different ethnic groups in South Africa and his department rapidly achieved an international reputation for excellence, which it has maintained ever since.

The road was a much rockier one for the department of medicine at the University of the Witwatersrand during its early days. When Craib took over the department from Professor OK Williamson in 1932 he found that he was expected to care for patients, teach the students, perhaps undertake some research, and maintain a private practice as the university salary was only £750 per annum. To fulfil these duties proved virtually impossible and after some pressure one full-time assistant was appointed. He was Dr JH (Jock) Gear, who was to spend the rest of his professional life making a major and selfless contribution to the building up of standards in the department. Craib felt, with justification, that the medical school was the Cinderella of the university and throughout the 14 stormy years he spent there he exerted intense and unrelenting pressure on the authorities to institute full-time clinical chairs. When, however, such chairs were finally introduced in 1946 he and the other clinical professors (IW (Breb) Brebner and James Black) were told that they would have to reapply for their posts and none of them was prepared to do so.

Set down in stark outline, the story of Craib's time at the University of the Witwatersrand seems unremarkable and it is certainly true that little or no research was done in the department during his tenure. However, the bare facts give no flavour of the Craib era nor of the man himself. Craib emerges from Barry Adams' portrait as the complete Renaissance man - soldier, scientist, clinician, teacher, administrator, philosopher and wit.

As students we knew nothing of his bravery as a trench mortar officer on the Western Front during World War 1 (twice mentioned in dispatches; Military Cross and Bar) nor of the controversy surrounding his seminal discoveries on the electrophysiology of heart muscle. It was work that had brought him into conflict with the major authorities of the day - Professor Willem Einthoven, Lord Adrian and Sir Thomas Lewis - and which had driven him out of scientific research. Craib felt, with justification, that the medical school was the Cinderella of the university and throughout the 14 stormy years he spent there he exerted intense and unrelenting pressure on the authorities to institute full-time clinical chairs. When, however, such chairs were finally introduced in 1946 he and the other clinical professors (IW (Breb) Brebner and James Black) were told that they would have to reapply for their posts and none of them was prepared to do so.

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While there is no way of assessing Craib's impact in tangible terms, generations of his students believe that the attitudes he instilled in them have guided them throughout their professional lives. When Don Craib left the university, Guy Elliott replaced him as the first full-time professor of medicine and a proper departmental infrastructure was also created. Not long afterwards the calibre of the work being done in the department was recognised - in 1950 when the South African Council for Scientific and Industrial Research (CSIR) created a cardiopulmonary research unit. Prominent in its activities were four of Craib's erstwhile students - Jock Gear, Bernard van Lingen, Maurice McGregor and Margot Becklake.

After Craib left the medical school he spent 15 years in specialist practice in Port Elizabeth. He was then asked by the president of the CSIR to serve initially as associate medical advisor and later as a vice-president. The very successful years he spent there (1963 - 1968) covered the crucial gestational period of the South African Medical Research Council, which held its inaugural meeting in 1969.

In addition, Craib had the satisfaction of receiving not only belated recognition but acclamation for his pioneering work in the 1920s in which he elucidated the electrophysiological basis for interpreting the ECG. Despite the rewards and relative tranquillity of his later life, some of his writings reflect self-doubt and a sense of failure. Indeed, he made two major decisions in his life that can be seriously questioned. The first was to abandon his research career at an early age and the second was not to apply for the full-time chair of medicine at the University of the Witwatersrand. However, given the circumstances of the times in which he lived it is difficult to make meaningful judgements on these decisions.

Perhaps the best answer to Craib's sense of failure was provided in a letter written to him by his sister Ismay, who was the wife of another distinguished South African, Sir Basil Schonland. She wrote: "Your own life has been so noble and so full that it stands entirely justified in its own right. So do not regret your decisions in the past. You would, of course, have been a very great figure in the medical research world if you had stayed in research. But you were a very great figure anyway and have at any rate served South Africa very well - with the training of medicals, the organisation of research and the actual patients to whom you gave life and courage."

I believe that Craib's most lasting legacy to those he taught was embodied in the inscription on a bowl presented to him when he left the CSIR. It reads: "We heed your teaching: in rebus scientiarum auctoritatem dubitate" [in matters of science doubt authority].

TH Bothwell

References

Iron circulates in blood plasma as a complex with β-globulin (Schade & Caroline, 1946) which is neither dialysable nor ultrafiltrable (Barkan, 1927). Most methods for estimating this iron involve three stages, (i) incubation of the plasma or serum with dilute hydrochloric acid for periods of 15 min to several hours to separate the iron from its protein, (ii) precipitation of plasma proteins with trichloroacetic acid, (iii) formation of a coloured complex with reagents such as 8-phenanthroline or 2:2'-dipyridyl (Hemmeler, 1951). Recently, Ramsay (1953) has described a method in which the plasma proteins are coagulated in the presence of a buffered solution containing a reducing agent and 2:2'-dipyridyl. In this way a coloured complex is formed under conditions which render reversibility of the reaction unlikely. Ramsay's results, however, are 30-60 μg/100 ml higher than those previously published, and he has suggested that the lower values found by other workers may be explained by a subsequent chemical or physical reaction between a proportion of the dissolved iron and the precipitated proteins.

In view of the discrepancies between Ramsay's findings and those of others, it seemed important to find out whether all the iron is liberated from its protein by hydrochloric acid, and also whether any of it is subsequently lost by entrainment with the protein precipitated by trichloroacetic acid. These two points have been investigated using radioactive iron. The results of these experiments made it possible to design a method for plasma-iron estimation which incorporates the basic features of other methods but which is more simple and rapid in use.

Experimental

Determination of iron in blood plasma

Glassware
All-glass syringes with stainless-steel needles were used for taking blood. The syringes and other glassware were thoroughly washed and then immersed in chromic acid for several hours. They were then rinsed with hot and cold tap water, distilled water and finally with glass-distilled water.

Reagents
The following reagents were used (all were AR grade and were made up in glass-distilled water): 2n-HCl, 20% (w/v) trichloroacetic acid (TCA), thioglycollic acid, 0.4% (w/v) 2:2'-dipyridyl (0.4 g dissolved in 5 ml glacial acetic acid and made up to 100 ml with glass-distilled water), saturated solution of sodium acetate, standard solution of iron (100 mg of pure iron wire dissolved in dilute H₂SO₄ and oxidized with KMnO₄; the volume made up to 100 ml with glass-distilled water and diluted 1 in 100 g to give a solution for use containing 10 μg/ml).

Procedure
2 ml 2n-HCl are added to 4 ml plasma or serum and the mixture is stirred with a glass rod. TCA (2 ml) is then added and the final mixture stirred vigorously for at least 45 sec. After centrifuging for 20 min at 2500 rev/min the supernatant fluid is decanted. The supernatant fluid (5 ml) is then added to a tube containing 2 drops of thioglycollic...
Table 1: Accuracy of 2,2'-dipyridyl in determining iron in standard solutions

<table>
<thead>
<tr>
<th>Fe in standard solutions (µg/100 ml)</th>
<th>Coefficient of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>7.0</td>
</tr>
<tr>
<td>80</td>
<td>2.4</td>
</tr>
<tr>
<td>160</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Comment on procedure

2,2'-Dipyridyl, first used in the estimation of iron in biological material by Hill (1930), forms a stable coloured complex with iron within the pH range 3-9 (Moss & Mellon, 1942). In the method described above, thioglycollic acid, which rapidly reduces iron from ferric to the ferrous state at pH between 4.5 and 4.9 (Laurell, 1952), was used as the reducing agent and the solution buffered with a saturated solution of sodium acetate. Experiments were carried out to find out the importance of the various factors in the colour reaction and the accuracy of the method.

Accuracy of the method (Table 1)
The error in measurement was relatively greater with low concentrations of iron but was small enough to allow for moderate accuracy over a wide range of iron concentrations.

Clarity of the centrifuged solution

Hemmeler (1951) has reported difficulty in obtaining a clear supernatant after centrifuging. However, in the present method it was found that vigorous stirring of the mixture before centrifuging yields a clear supernatant that gave readings in the colorimeter no higher than a blank made up with the same reagents and glass-distilled water.

Importance of pH

The mean pH of the final solution was 4.7 (range 4.54-4.77) in forty-eight estimations carried out with a glass electrode. By varying the experimental conditions it was also shown that the final pH was not critical enough to necessitate the titration of each sample as is described in some methods (Table 2).

Table 2: Effect of variations in pH on the recovery of iron from standard solutions

<table>
<thead>
<tr>
<th>Range of pH</th>
<th>No. of estimations</th>
<th>Fe recovered (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.7-4.5</td>
<td>18</td>
<td>98.9 ± 2.07</td>
</tr>
<tr>
<td>4.5-5.2</td>
<td>20</td>
<td>100.6 ± 1.50</td>
</tr>
</tbody>
</table>
Table 3: Efficiency of thioglycollic acid as a reducing agent

Each estimation was carried out in duplicate on two samples, of which one was treated by the present method and the other either with Na₂S₀₃ or with HN₀₃ and KCNS (for details see text). The iron content of the samples varied from 30 to 224 µg/100 ml (mean 106 µg/100 ml).

<table>
<thead>
<tr>
<th>Alternative treatment of iron in supernatant</th>
<th>No. of duplicate estimations</th>
<th>Mean difference between each pair of estimations (µg Fe/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduced with 0.1 M Na₂S₀₃</td>
<td>10</td>
<td>3.4 ± 2.4</td>
</tr>
<tr>
<td>Oxidized by boiling with HN₀₃ and converted into thiocyanate</td>
<td>10</td>
<td>5.6 ± 3.2</td>
</tr>
</tbody>
</table>

Efficiency of thioglycollic acid as a reducing agent
The present method was compared with procedures in which the colour was developed by different techniques. The supernatant fluid was treated in one of two ways. In the first, 0.1 ml 0.1 M Na₂S₀₃ was used as a reducing agent and the solution buffered to pH 4.7 with saturated solution of sodium acetate. In the second, the iron was oxidized by boiling for 30 min with 0.1 ml HN₀₃. By the addition of KCNS the iron was converted into thiocyanate and extracted with amyl alcohol. In both sets of experiments there was good agreement with results obtained by the present method (Table 3).

Time taken for development of final colour
In thirty samples and the reagent blank the intensity of colour was read immediately after preparation, then the samples were covered with cellophan, incubated at 37° for 1 hr, and the reading repeated. The mean difference in plasma iron concentration after re-reading was only 0.9 µg/100 ml (SD ± 1.2 µg/100 ml).

Reproducibility and effect of anticoagulants
The method gave reproducible results unaffected by the use of anti-coagulants (Table 4).

Table 4: Reproductibility of results obtained on heparinized and oxalated plasma and serum

The iron concentration of the samples varied from 18 to 234 µg/100 ml (mean 106 µg/100 ml).

<table>
<thead>
<tr>
<th></th>
<th>No. of estimations</th>
<th>Mean difference between each pair of estimations (µg Fe/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duplicate samples of heparinized plasma</td>
<td>30</td>
<td>2.6 ± 3.8</td>
</tr>
<tr>
<td>Comparison between heparinized plasma and oxalated plasma</td>
<td>15</td>
<td>3.2 ± 3.9</td>
</tr>
<tr>
<td>Comparison between heparinized plasma and serum</td>
<td>15</td>
<td>2.0 ± 1.9</td>
</tr>
</tbody>
</table>
The determination of iron in plasma or serum

TH Bothwell and Barbara Mallett


Iron circulates in blood plasma as a complex with ß-globulin (Schade & Caroline, 1946) which is neither dialysable nor ultrafiltrable (Barkan, 1927). Most methods for estimating this iron involve three stages, (i) incubation of the plasma or serum with dilute hydrochloric acid for periods of 15 min to several hours to separate the iron from its protein, (ii) precipitation of plasma proteins with trichloroacetic acid, (iii) formation of a coloured complex with reagents such as ð-phenanthrolone or 2:2'-dipyridyl (Hemmeler, 1951). Recently, Ramsay (1953) has described a method in which the plasma proteins are coagulated in the presence of a buffered solution containing a reducing agent and 2:2'-dipyridyl. In this way a coloured complex is formed under conditions which render reversibility of the reaction unlikely. Ramsay's results, however, are 30-60 µg/100 ml higher than those previously published, and he has suggested that the lower values found by other workers may be explained by a subsequent chemical or physical reaction between a proportion of the dissolved iron and the precipitated proteins.

In view of the discrepancies between Ramsay's findings and those of others, it seemed important to find out whether all the iron is liberated from its protein by hydrochloric acid, and also whether any of it is subsequently lost by entrainment with the protein precipitated by trichloroacetic acid. These two points have been investigated using radioactive iron. The results of these experiments made it possible to design a method for plasma-iron estimation which incorporates the basic features of other methods but which is more simple and rapid in use.

Experimental

**Determination of iron in blood plasma**

**Glassware**

All-glass syringes with stainless-steel needles were used for taking blood. The syringes and other glassware were thoroughly washed and then immersed in chromic acid for several hours. They were then rinsed with hot and cold tap water, distilled water and finally with glass-distilled water.

**Reagents**

The following reagents were used (all were AR grade and were made up in glass-distilled water): 2N-HCl, 20% (w/v) trichloroacetic acid (TCA), thioglycollic acid, 0.4% (w/v) 2:2'-dipyridyl (0.4 g dissolved in 5 ml glacial acetic acid and made up to 100 ml with glass-distilled water), saturated solution of sodium acetate, standard solution of iron (100 mg of pure iron wire dissolved in dilute H₂SO₄ and oxidized with KMnO₄; the volume made up to 100 ml with glass-distilled water and diluted 1 in 100 g to give a solution for use containing 10 µg/ml).

**Procedure**

2 ml 2N-HCl are added to 4 ml plasma or serum and the mixture is stirred with a glass rod. TCA (2 ml) is then added and the final mixture stirred vigorously for at least 45 sec. After centrifuging for 20 min at 2500 rev/min the supernatant fluid is decanted. The supernatant fluid (5 ml) is then added to a tube containing 2 drops of thioglycollic
Table 1: Accuracy of 2:2'-dipyridyl in determining iron in standard solutions

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<th>Fe in standard solutions (µg/100 ml)</th>
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acid, 0.5 ml 0.4% 2:2'-dipyridyl and 2.5 ml saturated solution of sodium acetate and the mixture shaken thoroughly. The colour intensity is measured in a Hilger New Biochem absorptiometer with a 520 m/ filter. This is calibrated by means of solutions of iron containing from 2 to 10 µg Fe together with 1.25 ml of 2n-HCl and 1.25 ml 20% TCA; the volume is made up to 5 ml and the mixture treated as above. A reagent blank is also measured.

Comment on procedure

2:2'-Dipyridyl, first used in the estimation of iron in biological material by Hill (1930), forms a stable coloured complex with iron within the pH range 3-9 (Moss & Mellon, 1942). In the method described above, thioglycollic acid, which rapidly reduces iron from ferric to the ferrous state at pH between 4.5 and 4.9 (Laurell, 1952), was used as the reducing agent and the solution buffered with a saturated solution of sodium acetate. Experiments were carried out to find out the importance of the various factors in the colour reaction and the accuracy of the method.

Accuracy of the method (Table 1)
The error in measurement was relatively greater with low concentrations of iron but was small enough to allow for moderate accuracy over a wide range of iron concentrations.

Clarity of the centrifuged solution
Hemmeler (1951) has reported difficulty in obtaining a clear supernatant after centrifuging. However, in the present method it was found that vigorous stirring of the mixture before centrifuging yields a clear supernatant that gave readings in the colorimeter no higher than a blank made up with the same reagents and glass-distilled water.

Importance of pH
The mean pH of the final solution was 4.7 (range 4.54-4.77) in forty-eight estimations carried out with a glass electrode. By varying the experimental conditions it was also shown that the final pH was not critical enough to necessitate the titration of each sample as is described in some methods (Table 2).

Table 2: Effect of variations in pH on the recovery of iron from standard solutions

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### Table 5: Recovery of iron added to plasma samples

<table>
<thead>
<tr>
<th>Form in which iron was added to 3 ml plasma samples</th>
<th>Quantity of added iron (μg/100 ml)</th>
<th>No. of estimations</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard ferric sulphate solutions</td>
<td>2 - 6</td>
<td>25</td>
<td>98.6 ± 4.2</td>
</tr>
<tr>
<td>Ferric sulphate solution buffered to pH 6.4 with sodium citrate and bound to β-globulin</td>
<td>2</td>
<td>12</td>
<td>99.0 ± 4.2</td>
</tr>
</tbody>
</table>

**Recovery of added iron**

It was shown that iron added to plasma either as a salt or bound to β-globulin (supplied by Cutter Laboratories, USA) could be recovered quantitatively (Table 5).

**Effect of haemolysis**

Red cells of known haemoglobin content were washed 3 times with 0.9% NaCl and were then haemolysed with water. Their approximate iron content was calculated on the assumption that iron forms 0.34% of the haemoglobin molecule by weight (Bernhart & Skeggs, 1943). Small volumes of haemolysed solution with iron content varying from approximately 60 to 600 μg/100 ml were then added to twenty plasma samples and the mixtures were analysed for iron content. The mean difference in plasma iron concentration after the addition of haemolysed erythrocytes was only 3.5 μg/100 ml (SD ± 4.5 μg/100 ml).

**Comparison with other methods**

There was good agreement with the results obtained by two other methods for plasma iron determination, namely that of Ramsay (1954) and that of Barkan & Walker (1940), which is similar in principle to the present method but which has longer time intervals and involves the use of hydrazine sulphate as reducing agent and α-phenanthroline for the development of the colour (Table 6).

**Experiments with 59Fe**

Experiments were carried out to determine whether all the iron was detached from the metal-binding protein by precipitating the proteins with acids.

### Table 6: Comparison of results obtained by present method with those of two published methods

The iron concentration of the plasma samples varied between 30 and 224 μg/100 ml (mean 103 μg/100 ml). Ten duplicate estimations were carried out for each comparison.

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean difference between each pair of estimations (μg Fe/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present method compared with Barkan &amp; Walker’s (1940) method</td>
<td>3.0 ± 2.6</td>
</tr>
<tr>
<td>Present method compared with Ramsay’s (1954) modified techniques</td>
<td>4.6 ± 2.0</td>
</tr>
</tbody>
</table>
Table 7: Recoveries of $^{59}$Fe obtained from plasma treated with 2N-HCl and 20% trichloroacetic acid

(A) Samples were mixed with HCl and allowed to stand for 10-60 min. Trichloroacetic acid was then added and the samples were mixed thoroughly and allowed to stand for a further 5-60 min.
(B) Samples were treated in the same way as in (A) except that they were not left to stand after the addition of HCl and trichloroacetic acid.

<table>
<thead>
<tr>
<th>Method of administration of $^{59}$Fe</th>
<th>Treatment of plasma samples</th>
<th>No. of estimations</th>
<th>% recoveries of radioiron from supernatant fluid after centrifuging</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{59}$Fe bound to $\beta_1$-globulin and injected intravenously (six subjects)</td>
<td>A</td>
<td>28</td>
<td>101.2 ± 3.7</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>16</td>
<td>100.8 ± 5.2</td>
</tr>
<tr>
<td>$^{59}$Fe incubated with plasma and injected intravenously (three subjects)</td>
<td>A</td>
<td>16</td>
<td>97.8 ± 6.2</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>15</td>
<td>101.0 ± 7.1</td>
</tr>
<tr>
<td>$^{59}$Fe fed by mouth (three subjects)</td>
<td>A</td>
<td>7</td>
<td>107.1 ± 7.6</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>19</td>
<td>104.7 ± 6.6</td>
</tr>
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Administration of $^{59}$Fe
$^{59}$FeCl$_3$ of high specific activity (1 µc/0.5 µg Fe), obtained from an American source through AERE, Harwell, was given in tracer doses of 10-20 µc to a number of subjects in three ways:

(a) Injected intravenously after having been added in vitro to a solution of $\beta_1$-globulin as described by Huff, et al., (1951).

(b) Injected intravenously after preliminary incubation with the patient’s plasma as described by Huff, et al., (1950).

(c) Fed by mouth together with approximately 5 mg of unlabelled ferrous ascorbate.

By taking blood samples at periods of 10-60 min after the administration of $^{59}$Fe it was possible to obtain plasma labelled with $^{59}$Fe.

Separation of iron from protein
The radioactivity in plasma was compared with that present in the supernatant fluid from samples which had been treated in various ways with HCl and TCA (Table 7). The counting was done in a scintillation counter (type 1186 on loan from AERE, Harwell) by the technique of Badenoch & Callender (1954). With the quantities of $^{59}$Fe used in these studies the maximum counting error was ± 5%.

Results and discussion
Table 7 shows that complete recoveries were obtained in 101 estimations. There was no apparent difference between the results obtained after allowing the plasma to stand for varying periods with HCl and trichloroacetic acid and those after immediate mixing, nor did the initial manner of binding the iron to the $\beta_1$-globulin affect the results. However, in four estimations in which the plasma proteins were precipitated with trichloroacetic acid without having been treated with HCl, recoveries of only about 65% were obtained.

By giving tracer doses of $^{59}$Fe of high specific activity parenterally or orally, it was possible to obtain plasma samples in which the transport iron attached to $\beta_1$-globulin was labelled with radioactivity. It was shown that this iron was rapidly and completely separated from its protein attachment after thorough mixing with dilute HCl and trichloroacetic acid. As there is no reason to believe that radioactive iron behaves differently from ordinary iron it seems valid to assume that the many methods which use such a technique for the separation of iron from protein should give reliable results.

The present technique for iron estimation in plasma or serum, although it involves no new principles, has the
advantage of simplicity and speed. The results of duplicate analyses have shown good agreement, and the occurrence of haemolysis has not affected the results significantly. A comparison of the results obtained by this method with those obtained by Barkin & Walker (1940) showed satisfactory agreement. In addition, it was also found that the present method gave results no lower than those obtained with Ramsay's modified technique (1954).

Summary

1. A simple and rapid modification of previous techniques for the estimation of iron in plasma is described.
2. Recovery experiments with $^{59}$Fe showed that iron is effectively separated from $\beta_1$-globulin in plasma by treatment with dilute hydrochloric acid and trichloroacetic acid.

Thanks are due to Mr JRP O'Brien, Dr Sheila T Callender and Dr GH Spray for helpful criticisms and to Professor LJ Witts for advice and encouragement.

References

Iron overload in Africans

HC Seftel, MB BSc Dip Med; RW Charlton, BSc MD FRCPE and TH Bothwell, MD DSc FRCP

Reprinted from The Leech, 1968; 38: 85-87

Prevalence

Nearly 40 years have passed since it was first noticed that siderosis was common in the African population of South Africa. These findings have been confirmed in a number of subsequent studies, and iron overload has been shown to occur also in the Africans of Rhodesia, Botswana, Malawi, Mozambique, Tanzania and Ghana. In all these areas the condition is seen almost exclusively among Africans; it is not found in White subjects and is rare in Indians.

Siderosis in Africans first becomes manifest in late adolescence, and both the incidence and the severity are greatest between the ages of 40 and 60 years. It is commoner and more severe in males, but a small proportion of African females also accumulate large iron stores. In one study the incidence in the Johannesburg area was defined by estimating the chemical concentrations of iron in the livers of several hundred African males dying in hospital. As many as 20% had hepatic iron concentrations as large as those described in idiopathic haemochromatosis, i.e. more than 2% dry weight.

Aetiology

Although it was always apparent that the iron overload must be the result of increased absorption from the gut, there has been controversy in the past as to why this should occur. Originally the Gillmans postulated that chronic malnutrition induced an alteration in the behaviour of the bowel, so that the physiological control of iron absorption was deranged. However, Walker and his colleagues later produced compelling evidence that the African diet contains abnormally large amounts of iron, and it is now generally accepted that this is the cause of the siderosis. Only a small percentage of the excessive dietary iron is absorbed, but this is sufficient to cause the slow accumulation of massive iron stores over the years. Some of the dietary iron is derived from the utensils used in cooking, but a more important source is the drums and tins used in the preparation of home-brewed alcoholic drinks. In one study in which several hundred samples of various types of drinks were analysed, it was found that the mean iron concentration was several milligrams per 100 ml. Since large volumes are consumed, it has been calculated that many African males ingest between 50 and 100 mg of iron daily in beer alone. This may be compared with the 10 to 20 mg of iron, only some of which is available for absorption, which is present in the average daily Western diet.

More than 80% of the iron present in the drinks consumed by Africans is in an ionized form. Isotopic studies carried out on radioactively tagged Bantu beer have demonstrated that it is absorbed to the same degree as a simple ferric salt. Africans absorb at least 2 to 3 mg per day of the 100 mg or so ingested in beer, and this produces the positive iron balance which accounts for the siderosis found in middle age. The high iron intake in Africans has been confirmed by measuring the iron concentrations in faeces. The mean concentration of iron in stools collected from African males on Monday mornings was found to be several times the figure on Thursday mornings, a variation which probably reflects the weekend drinking habits of urbanized Africans.
Pathology

The pathological findings in Africans with siderosis are fairly constant. In the earliest phases of iron overload, haemosiderin granules can be detected in the liver, in parenchymal cells and Kupffer cells. With concentrations of 5 to 10 times normal, increasing amounts of haemosiderin are present in these sites, and at this stage the portal tracts also show involvement. When the concentrations are 20 times normal, heavy deposits are present throughout the liver. Splenic deposits are also present from an early stage, and with increasing levels of iron, the concentration in the spleen is often higher than in the liver. Deposits in the reticulum cells of the bone marrow are also a feature, and in vivo isotopic studies indicate that as much as 10 g of iron may be stored in this site. Iron deposits elsewhere in the body are relatively scant, and in the majority of subjects the major impact is thus on the parenchymal cells of the liver and on the reticulo-endothelial system.

Siderosis and tissue damage

In attempts to assess the relationship between iron overload in Africans and tissue damage, most attention has been directed to the liver, since a major portion of the excess iron is located in this organ. The results of several studies have shown a close correlation between the degree of siderosis and the presence of significant portal fibrosis or cirrhosis. The relationship between siderosis and portal cirrhosis was further clarified by another investigation in which measurements were made of iron concentrations in the livers of a consecutive series of African subjects exhibiting the features of portal cirrhosis at necropsy. It was found that severe siderosis was almost invariably present. In contrast, there was no correlation between the presence of post-necrotic cirrhosis (the other common form of cirrhosis in Africans) and the degree of iron overload. These observations are therefore compatible with the thesis that there is a close relationship between the presence of heavy hepatic deposits of iron and the development of a portal fibrous reaction in the liver.

This is not, however, the only point of interest that has emerged from these studies. It was noted that the presence of portal cirrhosis was associated with a change in the distribution of the iron deposits; in addition to the usual sites, storage iron was also present in a number of parenchymal organs. The deposits were especially prominent in the pancreas, but appreciable quantities were also found in the thyroid, adrenals, pituitary and myocardium. This distribution resembles that found in idiopathic haemochromatosis, and a proportion of adult Africans - about 3% of subjects in the necropsy study just cited - therefore exhibit pathological findings similar to those found in the idiopathic disease. However, it nevertheless remains possible to distinguish between idiopathic haemochromatosis and the haemochromatosis which occurs in Africans on morphological criteria alone, since far more iron is present in the reticulo-endothelial cells of the liver and the spleen in African subjects.

The similarity between the pathological appearances of African and idiopathic haemochromatosis extends also to the clinical manifestations. Analysis of clinical records has shown that more than 20% of Africans with pathological evidence of haemochromatosis at necropsy were diabetic before death while in a survey of living diabetics attending the out-patient department approximately 7% were found to be suffering from fully developed haemochromatosis. The clinical picture in these African haemochromatotic diabetics was fairly characteristic. The male to female ratio was 2:1, and the majority of affected individuals was between the ages of 40 and 60 years. All gave a history of excessive consumption of home-brewed alcoholic beverages. They were almost invariably under-weight, firm hepatomegaly was always present, and insulin was often needed for control of diabetes. These features served to distinguish the condition from the common form of late onset diabetes found in urban Africans, since this usually occurs in middle-aged, obese females, and can often be controlled by diet or by oral hypoglycaemic agents. The prognosis in Africans subjects with haemochromatosis is poor, and death usually results from liver failure and/or portal hypertension.

Although these various findings support the concept that long-term oral iron overload in Africans can cause severe damage to body tissues, it is important to stress that there are still puzzling discrepancies. While it is clear that the majority of subjects with marked hepatic siderosis develop portal fibrosis or cirrhosis, an appreciable percentage do not do so. For example, in one study 30% of subjects with hepatic iron concentrations above 2% dry weight showed no significant portal fibrosis. Such a finding raises the possibility that iron is only a low grade fibrogenic agent and that other potentiating factors are often present. It is perhaps noteworthy that the major source of iron in Africans is an alcoholic drink which is frequently adulterated with a variety of noxious agents. There is, therefore, the added insult of alcohol on the liver together with the possible harmful effects of adulterants and associated malnutrition. A concept such as this would fit in with Golberg's experimental observation that the siderotic liver is particularly vulnerable to toxic, metabolic and nutritional hazards.

Siderosis, scurvy and osteoporosis

As already indicated, the more severe grades of siderosis occur most commonly in middle-aged African males who consume large amounts of home brews. Two other conditions are frequently seen in such subjects, namely scurvy...
and spinal osteoporosis, and there is now considerable evidence that the three disorders are causally linked. In the presentation which follows this evidence is summarized, and a possible explanation for the association is suggested.

**Association between siderosis and scurvy**

In Johannesburg Africans scurvy is almost exclusively a disease of middle-aged male subjects whose livers are invariably heavily siderotic on biopsy or at necropsy. Despite the high incidence of nutritional disorders such as kwashiorkor, marasmus and rickets in African infants, scurvy is extremely rare; in contrast, it is common in siderotic adults. Indeed, ascorbic acid deficiency short of frank clinical scurvy is virtually universal in such individuals. In one study the leucocyte ascorbic acid concentration, which is generally regarded as a good index of ascorbic acid nutrition, was found to be low in asymptomatic manual labourers with raised serum iron concentrations. It was also observed that the lowest white blood cell ascorbic acid levels tended to occur in the heaviest consumers of beer. That these findings are not simply a reflection of alcoholism and dietary inadequacy but are related in some way to siderosis, was shown in another study in which low white cell ascorbic acid levels were found in well nourished White subjects suffering from iron overload associated with blood transfusions and idiopathic haemochromatosis. There seems little doubt that siderosis directly influences ascorbic acid metabolism. In vitro ferric iron accelerates the oxidative catabolism of the vitamin C, and Schultz and Swanepoel were the first to suggest that the massive ferric deposits in severely siderotic Africans might act in a similar way. These workers demonstrated that the urinary excretion of ascorbic acid in two scorbutic African patients with severe siderosis remained very low in spite of prolonged administration of large doses of the vitamin. These observations have been confirmed and extended by showing that ascorbic acid loading is associated with not only a low urinary excretion of the vitamin, but also with an increased output of the oxidation end product, oxalic acid. This was demonstrated in severely siderotic Africans whether they were clinically scorbutic or not, and also in Whites with other varieties of siderosis. In addition, the plasma clearance of ascorbic acid was shown to be accelerated. When these findings are taken in conjunction with the fact that severely siderotic Africans generally subsist on a diet low in vitamin C, it is readily understandable that they should commonly develop a state of chronic and severe ascorbic acid depletion. Moreover, when the dietary ascorbic acid content reaches its seasonal low (in late winter and early spring), frank clinical scurvy is seen.

**Association between siderosis and osteoporosis**

Spinal osteoporosis, presenting clinically with vertebral collapse and backache, is a common and disabling condition in middle-aged African males. Many of those affected are manual labourers, and osteoporosis in such individuals is virtually unknown in other population groups. It has been firmly established that the thinning of the bones is indeed osteoporosis, and not any other condition such as osteomalacia. The usual variety of osteoporosis, that seen in elderly women, is rare in Africans.

In one study all the sufferers were found to be heavy drinkers, and firm hepatomegaly was present in 70% of the control subjects. In the osteoporotic subjects, the mean serum iron level and percentage saturation of the iron-binding capacity were significantly higher than those of matched controls. On biopsy or at necropsy their livers were almost invariably severely siderotic, whereas in non-osteoporotic controls the prevalence of comparably severe degrees of iron overload was less than 50%. In a necropsy study hepatic iron concentrations were found to be inversely correlated with mineral bone density. There can therefore be little doubt that the association between osteoporosis and severe siderosis in Africans is real. Such an association has been described previously in other circumstances. For example, beef cattle in an area of New Zealand where the iron content of the water is exceptionally high (up to 20 mg per 100 ml) develop a form of siderosis similar to that seen in Africans, and in many of the severely affected animals osteoporosis of the vertebrae, sternum and ribs is a prominent feature. Osteoporosis has also been reported in French subjects with haemochromatosis.

**Association between osteoporosis and scurvy**

That there is a significant association between osteoporosis and scurvy in Africans was originally noted by Grusin and Samuel. They found that 69% of osteoporotic subjects in their series were scorbutic, or had been so in the past. Conversely, it was established that 19% of patients presenting with classical scurvy exhibited severe osteoporosis. In addition, it was shown that the closeness of the association varied with the season. Seventy-one percent of osteoporotic patients presenting during the "scurvy season" were scorbutic, while only 22% of these admitted during the reminder of the year suffered from scurvy. In another study the levels of white blood cell ascorbic acid were found to be extremely low in osteoporotic subjects, even though clinical scurvy was not present.
A possible explanation

When all the evidence is considered there can be little doubt that severe siderosis, scurvy and osteoporosis are causally linked and constitute a triangular syndrome. There are a number of possible explanations, but the most likely one at the present time seems to be that osteoporosis is the result of chronic ascorbic acid deficiency, and that this deficiency is in turn a function of severe siderosis.

The association between osteoporosis and scurvy has been well documented in both children and experimental animals, and probably relates to the role of ascorbic acid in the formation of collagen and hence new bone. Although it has not been documented in adult humans, there seems no theoretical reason why it should not occur in them as well.

References

5. Bothwell TH and Bradlow BA. *Arch Path*, 1960; 70: 279.
The Department of Medicine at Wits: Its past, present and uncertain future

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The first head of the Department of Medicine was Professor OL Williamson (1922-1931). His was a part-time appointment and Professor WH Craib who succeeded him (1931-1946) also held the post in a similar capacity. GA Elliott was appointed as the first full-time Professor in 1946 and with the takeover of hospital services by the Transvaal Provincial Administration in 1946 more realistic full-time structures were created at the Johannesburg hospital. This was true also at Coronation hospital, which opened in 1946 and at Baragwanath hospital which opened in 1947. The next 20 years were marked by a steady rise in academic standards in the various teaching hospitals and as a result there was a concomitant increase in the output of worthwhile research. In this regard, particularly valuable contributions were made in the delineation of the unique patterns of disease occurring in the local black population. In addition, the wide diversities in nutritional and social conditions in the different population groups made it possible to carry out many worthwhile, clinical, physiological, pathological and epidemiologic studies. All in all, it was a golden era for clinical investigation in the Department of Medicine. Hand in hand with research commitment there was an equally impressive expansion in the range of clinical services offered by the Department. Neurology and Dermatology had always functioned autonomously and by 1950 Cardiology and Pulmonology were also launched as separate sub-specialities. With the passage of time others were added and final recognition of these developments was provided in the late sixties and early seventies when full time staff establishments were created in virtually all branches of Medicine.

The Departments of Medicine in the various academic hospitals provided a dual service. On the one hand they were responsible for outpatient and inpatient services for general medical patients while on the other a number of specialized services were offered. The Department's commitments were further increased in 1967 when it took over the running of the Department of Medicine at the JG Strijdom hospital. These few historical facts are provided in order to give some idea of the current size of the Academic Department of Medicine and the range of activities that have been built up over the years. The final product is one of which we are all proud. In-so-far as its research output, its clinical commitments and its standards of undergraduate and postgraduate teaching are concerned the Department of Medicine can hold its own with similar fine Departments here and overseas.

What are the special qualities that are required if standards of academic excellence are to be maintained? The environment should be such that it attracts the most talented and highly motivated doctors. The standards of patient care should be better than those available elsewhere and medical personnel should have sufficient time to develop their specialities, to teach undergraduates and postgraduates and to pursue research. Intrinsic to this system is peer review. In the hierarchial structure of the teaching department the students learn from teachers who themselves practise under the critical eyes of pupils and colleagues. The range of interests within such a department is peer review. In the hierarchial structure of the teaching department the students learn from teachers who themselves practise under the critical eyes of pupils and colleagues. The range of interests within such a department should be very wide and should extend from primary care in rural and urban settings to sophisticated "state of the art" tertiary care. Finally, the department must be sensitive to the needs of all the communities it serves and must adapt its priorities and interests accordingly.

The unique strength of the Department of Medicine and paradoxically its unique vulnerability lie in its enormous size. There is no other department with as large a "clinical laboratory", including as it does disease profiles varying from those associated with the affluent West to those occurring in the deprived Third World.
However, the cost of these clinical riches is a heavy one. The Department of Medicine has the ongoing commitment to staff the five academic hospitals with suitably qualified doctors so that its major service commitments can be met. This it has done with enthusiasm over the years, since it has accepted its social responsibility to make available its skills and expertise to as many of the local population as it can. At the same time it must be accepted that meeting its formidable service commitments is exerting pressures. But this is only one of a number of interweaving factors which are currently exerting adverse effects on the wellbeing of not only the Department of Medicine but other clinical departments as well. Several merit brief mention.

Fragmentation of the health care delivery services has bedeviled the development of Academic Medicine in the past and threatens to continue to do so in the future. While the allocation of the medical schools to "General Affairs" is to be welcomed, it in no way mitigates against the problems created by the division of health care into a number of "Own Affairs" departments. This division threatens in particular the ability of clinical departments in the Medical School to extend their range of interests and commitments into rural communities. A further form of fragmentation may be expected to occur with the introduction of privatisation, since a wide range of essential clinical material will be excluded from the teaching hospitals.

Another pressing problem is segregation in hospitals. Restrictions in the free access of students, doctors and patients to academic hospitals poses an ongoing and major threat to the future of Academic Medicine in our Medical School. Recent qualified concessions have only served to highlight the current inequalities in the delivery of health care in the five academic hospitals, with black patients being denied free access to the superior facilities at the white hospitals.

The funding of academic hospitals is also a matter for concern. It had been hoped that such hospitals would have played a key and central role in a unitary national health service but all the current evidence indicates that the Government is moving in the opposite direction of "privatisation". To what degree health services will be affected by privatisation is not yet clear but it is becoming increasingly apparent that academic hospitals are going to have to generate substantial funds if they are to remain viable. There are many possible options but the most attractive is one in which departments are allowed to charge private patients for their services. The funds so generated could then be equitably dispersed for the supplementation of salaries, the purchase of research equipment and the support of research. It is only when this is done that an academic environment can be created in the clinical departments which will hopefully be attractive to the best graduates from here and elsewhere.

For the Department of Medicine to survive in its present form it has to be able to recruit doctors of quality to fill the more than 400 posts in the five academic hospitals. This task is becoming increasingly difficult and a major reason is the ongoing emigration overseas of graduates from this Medical School. It is a phenomenon that has existed for a long time and there is no doubt that it has adversely affected the development of Academic Medicine at our Medical School. Some idea of the extent of the problem can be gauged from a few statistics that have been collected over the years. In 1977 an analysis was carried out on the 204 graduates who had acquired the FCP of the College of Medicine of South Africa. Seventy three of them had emigrated. What has happened since then is not known. Another analysis was done in 1984 on graduates who had qualified at this Medical School between 1960 and 1973. The names of 42% of them no longer appeared on the medical register. Perhaps the most disturbing results were those obtained by a Sunday Tribune survey reported on July 19th, 1987. The reporter, Sarah Sussens, sent telegrams to all members of the class of 1983 asking them to phone her. She then found out from them where they were working and what they were doing. She also enquired about their friends. In this way, she obtained firm data on 149 members out of a class of 180. Forty nine of them had already left the country and another third were seriously considering leaving.

Reasons for emigrating vary. The political situation may well be the dominant factor but it is an oversimplification to believe that all doctors emigrate because they find the political situation intolerable. Many emigrate because they wish to advance their own careers in less volatile surroundings. The perceived better academic opportunities elsewhere include better provision for academic pursuits, the encouragement of participation in congresses and meetings and more attractive salaries.

The road ahead will not be easy and if the Department of Medicine is to reach its full potential each of the problems that has been identified will have to be solved. The Medical School will, I know, continue to strive for the opening of the Academic hospitals to all races and for a more unified health care system in which academic departments can play a key role in the training of a wide range of health care personnel. Meanwhile there is a large Academic Department which must be staffed so that the patients in our five hospitals can be cared for, our students can be taught, worthwhile relevant research can be done and the challenges of the future can be met. The fact that it is becoming increasingly difficult to fulfil our commitments can be ascribed to the large scale emigration of our own graduates. This escalating problem has been with us for years but has never been squarely addressed. If emigration is to take a lesser toll of our graduates then the political situation will have to improve, which seems unlikely at present, or ways and means will have to be found for ensuring that most graduates stay in the country. This could be done by restrictive legislation, which is undesirable but may be necessary, by significant improvements in conditions of service for hospital personnel, or by drastic changes in selection procedures. Whatever options are followed action must be taken now, since a continuation of the present situation poses a very real threat to the continued wellbeing of a Department which has maintained high academic standards over the years and which has a proud record of service to the communities of Johannesburg.
The quantitative estimation of total iron stores in human bone marrow

Edward Gale, John Torrance and Thomas Bothwell


Summary

Total bone-marrow iron stores were estimated in 61 human subjects by use of an isotopic dilution technique. The mean value in 24 white males was 288 mg as compared with 99 mg in 17 white females. The high incidence of iron overload in Bantu adults was reflected in the finding of a mean value of 1 629 mg in 15 Bantu males. Chemical analyses of iron concentrations in the marrows and livers of 199 white and Bantu necropsy subjects revealed a close correlation over a wide range of iron concentrations. There was moderately good agreement between histological assessments of bone-marrow iron stores and chemical estimations of iron concentrations.

In normal adult males, about 25% of the body iron content is in storage depots. This iron exists in two forms: as a diffuse soluble fraction called ferritin, in which the molecules are dispersed, and as insoluble aggregates of hemosiderin, which can be visualized by conventional microscopy. Although the liver is regarded as the chief storage organ, chemical analyses suggest that it normally contains up to 300 mg, which is only between one-quarter and one-third of what can be mobilized from total stores when healthy young males are repeatedly phlebotomized. Although little is known of the quantities present in other organs, hemosiderin can be seen in the reticuloendothelial cells of the bone marrow and spleen, and there is some chemical evidence to indicate that significant amounts of storage iron may be present in skeletal muscles.

The present study was undertaken to find out how much iron is normally stored in the reticuloendothelial cells of the bone marrow and to define the extent to which these stores can expand when iron overload is present. In addition, a comparison was made between the concentrations of iron present in the bone marrows and livers of subjects with varying quantities of storage iron.

Materials and methods

Clinical material

The total iron stores present in bone marrow were estimated in 61 adult subjects undergoing thoracotomy. Forty-one were white and the remainder were Bantu. The individual diagnoses are shown in Table 1.

The chemical concentrations of storage iron were estimated in the livers and bone marrows of adult Bantu and white subjects dying in the hospital. The Bantu specimens (58 males and 44 females) were obtained from Baragwanath Hospital, Johannesburg, and the white specimens (25 males and 28 females) from the General Hospital, Johannesburg.

The use of radioiron as a marrow label

When a tracer quantity of radioiron is injected intravenously, most of it is taken up by the red-cell precursors of the bone marrow. This iron is subsequently released over the next few days as part of the hemoglobin of new red cells,
Table 1: Clinical diagnosis in 61 patients subjected to thoracotomy

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>White</th>
<th></th>
<th>Bantu</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>Mitral stenosis</td>
<td>7</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Intrathoracic neoplasms</td>
<td>8</td>
<td>0</td>
<td>1</td>
<td>0</td>
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<tr>
<td>Pulmonary tuberculosis</td>
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<td>8</td>
<td>3</td>
</tr>
<tr>
<td>Pulmonary sepsis</td>
<td>0</td>
<td>4</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Hiatus hernia</td>
<td>6</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>17</td>
<td>15</td>
<td>5</td>
</tr>
</tbody>
</table>

and the percentage present in circulation at 10 to 14 days has been used as a measure of the fraction initially taken up by the bone marrow. If a specimen of bone marrow is therefore removed between 18 and 24 hours after the injection of the radioiron (i.e. at a time when marrow activity is maximal, and circulating activity is negligible), it is possible to use the Fe\textsuperscript{59} as a label for relating values in the same to the total marrow. This principle, which has previously been employed for estimating the total number of cell precursors, was used in the present study as a means of calculating the total amount of storage iron present in the bone marrow. To do this, we must assume that the reticulum cells containing storage iron are fairly evenly dispersed throughout the erythroid marrow.

**Isotopic techniques**

Subjects undergoing thoracotomy were given between 5 and 10 $\mu$g high specific activity Fe\textsuperscript{59} citrate intravenously between 18 and 24 hours before surgery. A weighed sample of this solution was set aside as a standard. Blood samples were collected over the next few hours, and plasma volumes were then calculated from the line of clearance of radioiron as described previously. The total blood volume was obtained from the plasma volume and packed cell volume corrected for the body: venous hematocrit ratio at an altitude of 5,900 feet. With this approach, the figure for blood volume in the group was 68.2 ml per kg (SD 10.7).

The rib removed at operation was squeezed dry, and the marrow so obtained was weighed and counted for radioactivity in a well-type counter. The amount of storage iron present was then measured chemically (see Chemical methods).

Blood samples were collected from all the subjects 10 to 14 days after the injection of radioiron. The total percentage of injected radioactivity in circulation was then calculated by using the estimation of blood volume obtained from radioiron data on the first day of the study. The mean figure for the group was 83% (SD 10.7). On the assumption that the radioiron present in circulation at this time was in the marrow 18 to 24 hours after the original injection, it was then possible to obtain a measure of the total iron stores present in bone marrow. Marrow iron stores = (fraction of injected Fe\textsuperscript{59} in circulation at 10 to 14 days x counts injected/counts in sample) x nonheme iron in marrow sample.

**Chemical methods**

The quantity of nonheme iron present in specimens of marrow was estimated by a modification of the method of Brückmann and Zondek. Four ml saturated sodium pyrophosphate was added to the marrow and sufficient 20% trichloroacetic acid to give a final volume of 10 ml. (In this calculation the specific gravity of the marrow was assumed to be 1.0). After thorough mixing, the tube was heated in a water bath and was maintained at a temperature of 80° C for 10 minutes. During this time the solution was stirred frequently. After cooling, the mixture was filtered and an iron estimation was carried out on a sample of the filtrate by use of the thioglycolic acid method.

The validity of the whole procedure as a means of separating heme from nonheme iron was established in preliminary studies with solutions of hemoglobin and ferritin and suspensions of hemosiderin granules. Ferritin was prepared by the method of Mazur and Shorr, and hemosiderin by the method of Shoden and Sturgeon. Only 5% (range 3 to 8%) of heme iron was present in the filtrate. Calculations based on the quantities of hemoglobin present in marrow samples indicated that the error that could be introduced by hemoglobin iron was not more than 10 $\mu$g per g marrow. The mean recovery of ferritin iron in concentrations varying between 100 and 2,000 $\mu$g per ml was 104% (range 100 to 106%), whereas that of hemosiderin iron in concentrations varying between 70 and 10,000 $\mu$g per ml was 103% (range 97 to 111%).

In necropsy studies, specimens of rib and samples of liver were collected. Marrow was obtained from the ribs.
by squeezing them dry, and the concentrations of nonheme iron were estimated with the method described in the previous paragraphs. As the validity of results obtained from the concentrations of storage iron in marrow depended on there being a moderately constant relationship between storage iron and the weight of marrow samples, some preliminary studies were done in which the concentrations of storage iron were estimated in samples of marrow obtained from different sites. Specimens were taken from two sites in subjects with concentrations of marrow storage iron varying between 15 to 4,476 μg per g. The average difference from the mean of each pair of observations was 5.9% (range 0 to 23%) in 16 cases. It was felt that this degree of agreement was sufficiently close for meaningful information to be obtained from single samples.

The concentration of total iron present in each formalin-treated sample of liver was estimated as described previously. In addition, the concentration of heme iron was estimated on a separate sample of liver, and the concentration of storage iron was then obtained by subtracting this result from the figure for total iron. This proved somewhat difficult as there are no simple, described techniques for the quantitative estimation of hemoglobin iron in formalin-treated tissues. The method eventually devised was as follows. Approximately 1 g formalin-treated liver was blotted and weighed. The sample was then cut into slices and placed in a 50 ml volumetric flask. One-half ml of the detergent sodium alkyl sulfate (marketed as Teepol by the Shell Chemical Co, London, England) was added, and the flask was placed in an oven at 56°C for 18 hours. After cooling, the solution was filtered, and the optical density of the alkali-heme was determined by using an Evelyn colorimeter with a 540 filter. The concentration of hemoglobin iron present was then read from a calibration curve constructed with hemoglobin solutions of known iron content which had been treated in the same way. With this technique, reproducible calibration curves were obtained on solutions of hemoglobin that had been stored in formalin for as long as 3 months.

In an attempt to establish the specificity of the method, estimations of hemoglobin iron were carried out on deeply jaundiced livers obtained from rats in which the bile duct had been tied several days previously. The results were no higher than in normal rats. In addition, the fact that concentrations of heme iron were in the same range as those obtained by other methods on fresh liver specimens further supports the validity of the present technique.

** Histological methods **

In the majority of studies, samples of marrow specimens were squashed onto slides that were then stained for iron by Dry's method. The arbitrary histological gradings of storage iron in marrow particles were as follows: grade 0 - no visible iron under oil immersion (magnification x 720); grade 1+ - small iron particles just visible in reticulum cells under oil immersion (magnification x 720); grade 2+ - small, sparsely distributed iron particles usually visible under low power (magnification x 80); grade 3+ - numerous small particles present in reticulum cells throughout the marrow particles; grade 4+ - larger particles throughout the marrow with tendency to aggregate into clumps; grade 5+ - dense, large clumps of iron throughout the marrow; and grade 6+ - very large deposits of iron, both intra- and extracellular, which obscure cellular detail in the marrow particles.

** Results **

**Total iron stores in the marrow (Figure 1)**

The mean value in 17 white females was 99 mg (range 19 to 237 mg), and in 24 white males, 288 mg (range 10 to 833 mg). Only 3 of the males had values below 100 mg, and 2 of these subjects gave a history of previous gastro-
Figure 2: Correlation between storage iron concentrations in the marrows and livers of 155 necropsy subjects. The regression line is indicated ($r = +0.88$; $p < 0.001$).

intestinal hemorrhage. The 3 males with figures above 500 mg were all suffering from bronchial carcinoma. Only 5 Bantu females were studied. Results showed a wide scatter, with values ranging between 56 and 781 mg. The mean value in 15 Bantu males was 1 629 mg (range 304 to 4 820 mg).

Correlation between concentrations of storage iron in marrow and liver (Figure 2)

The results in 155 subjects show that the concentrations of storage iron present in the two organs were approximately the same over a very wide range.

Correlation between chemical and histological assessment of storage iron in the marrow

The results in 199 subjects are shown in Figure 3. Although there was some degree of overlap, moderately good agreement was obtained between the histological gradings and the chemical concentrations of storage iron in the marrow. The mean figures for each grade were as follows: 0 = 43 μg per g (SD 23); 1+ = 130 μg per g (SD 50); 2+ = 223 μg per g (SD 75); 3+ = 406 μg per g (SD 131); 4+ = 762 μg per g (SD 247); 5+ = 1 618 μg per g (SD 464); and 6+ = 3 681 μg per g (SD 1 400).

Discussion

Estimates of the amounts of iron present in stores have been made in different ways. When normal subjects are repeatedly phlebotomized, the red-cell deficit is corrected by mobilizing iron from stores. Calculations from such data indicate that the total stores in healthy young males vary between 1 000 and 1 500 mg. Although data derived from an isotopic dilution technique have given a mean figure of only 600 mg in man,24 these discrepancies are not surprising, since the response to repeated phlebotomies is a measure of the total mobilizable stores, whereas the isotopic technique is only a measure of miscible stores.

The major sites of storage iron in the body have been demonstrated by using both histological and chemical methods. The liver normally contains up to 300 mg, and significant quantities are present in the reticuloendothelial cells of the bone marrow7,25 and spleen. In addition, there is some chemical evidence to suggest that significant amounts of storage iron may be present in skeletal muscles.9,10 There is, however, little quantitative information on the total amount of iron present in organs other than the liver and spleen.

In the present study, an attempt was made to estimate the total amount of storage iron in the bone marrow. This was done by adapting an isotopic dilution technique which has been previously used for gauging the number of cell precursors in bone marrow.13-15 The data obtained with this technique indicate a mean figure for marrow storage iron of 288 mg in white males and 99 mg in white females. Some of the individual values must certainly have been modified by the diseases from which the subjects were suffering. For example, the highest figures in males were obtained in subjects with cancer, while the lowest results were in subjects with hiatus hernia and a history of
previous gastrointestinal hemorrhage. In spite of these qualifications, it is felt that the results obtained are probably a fair reflection of the findings in the general population, as the mean values in the 7 males and 9 females suffering from uncomplicated mitral stenosis, with no signs of rheumatic activity, were very similar to those in the whole group, i.e. 256 mg and 70 mg, respectively. All these subjects had hemoglobin levels above 13 g per 100 ml, and only two, both of whom were females, had ever donated blood (2 and 5 pints, respectively). The well-marked sex difference, which is probably a reflection of the increased losses of iron via menstruation and pregnancies in females, has also been noted previously in histological assessments of bone-marrow iron and in chemical estimations of liver iron concentrations.

Figures for storage iron in Bantu subjects were a good deal higher than in white subjects. The mean figure of 1 629 mg in Bantu males was approximately 6 times the value in the white subjects, and the highest figure obtained was 17 times greater. Although the majority of these subjects were suffering from sepsis, the relative hemosiderosis that is so often a feature in chronic infection would not be expected to raise reticuloendothelial stores to more than about twice normal, and the very high values obtained therefore represent an absolute increase in the body iron content. This fits in with several previous studies that have demonstrated a high incidence of varying degrees of iron overload in the adult Bantu population of Southern Africa. The excess iron in these subjects is largely derived from the containers used for the preparation of alcoholic beverages. In most individuals, the iron is almost exclusively stored in the liver and in the reticuloendothelial system of the body.

In an extension of the initial investigation, specimens of bone marrow and liver were obtained at necropsy from white and Bantu subjects in order to compare the concentrations of storage iron present in the two organs. It was of considerable interest to find that the concentrations of iron were very similar in subjects with depleted stores, normal stores, and all degrees of iron overload. Although it is not possible to translate the necropsy data into accurate figures for the absolute amounts of storage iron present in bone marrow and liver, some idea of the relative capacities of the two organs can be obtained from the isotopic data. Calculations from these results gave a mean figure for the total marrow weight of about 1 200 g, which is in the same range as the normal liver weight. This suggests that the liver and marrow have a capacity to store iron that is of the same order. Although the relationship probably holds true when iron stores are normal or moderately increased, there is usually significant hepatomegaly when gross degrees of iron overload are present, and the liver’s capacity is therefore increased. In quantitative terms, the highest marrow iron concentration obtained in the autopsy study was 8 647 μg per g. If it is presumed that the total marrow weight in this subject was 1 200 g, then the total marrow iron stores must have been approximately 10 g. This is about half the amount usually present in the liver in idiopathic hemochromatosis.

One final point is worthy of comment. In the past, the histological assessment of hemosiderin in the reticulum cells of marrow particles has been widely used as possibly the best method for assessing the status of iron stores in an individual. In the present investigation, a comparison was made between histological gradings and chemical estimations of storage iron in the marrow. The good agreement obtained over a wide range of iron concentrations further confirms the validity of the histological assessment of marrow particles as a useful means of gauging the size of reticuloendothelial stores.
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