

VASCULAR RESISTANCE IN JAUNDICE
AND HYPERLIPIDAEMIA

D.S. BLOOM

A thesis submitted to the Faculty of Science,
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Doctor of Philosophy

Johannesburg,
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This is to certify that this thesis is my
own work and has not been presented for any
degree of another University.

A handwritten signature consisting of several overlapping, stylized horizontal strokes.

TO MAUREEN, NORMAN, JONATHAN AND RAFAEL.

ABSTRACT

This thesis deals with the changes seen in vascular resistance following bile duct ligation. The work is presented in five sections.

In the first section the outline and scope of the thesis is presented. The relationship between liver disease and renal dysfunction is then reviewed. It is pointed out that in various conditions such as cirrhosis of the liver alterations in renal perfusion are seen which precede the onset of frank renal failure. It is also mentioned that in obstructive jaundice there is a high incidence of post-operative renal failure and the possible aetiology of this is reviewed.

The second section deals with the effects of obstructive jaundice on renal blood flow. In this section the renal vascular anatomy is described. The regulation of renal blood flow as well as methods for its measurement are reviewed. The results of experiments in which renal blood flow was measured during obstructive jaundice is then presented. Here the ^{133}Xe technique was used. It was found that there are alterations in renal perfusion similar to that seen in cirrhosis and other liver diseases such as portal hypertension. It was found that there is a shift of blood flow distribution from the outer cortical areas of the kidney to the inner cortical - outer medullary areas. It was also found that there is an increased sensitivity of the kidney vasculature to the effects of noradrenaline, and it is postulated that this may be one of the reasons for the high incidence of post-operative renal failure seen following bile duct surgery.

In the third section an attempt is made to see whether there is a general hypersensitivity to the effects of noradrenaline in obstructive jaundice. The cerebral circulation is used for this. The control of the cerebral vasculature is reviewed and the results of experiments in which noradrenaline was infused into the cerebral circulation of animals in which the bile duct had been ligated is presented. In these experiments it was

found that infusions of noradrenaline produced vasoconstriction, while in normal animals, vaso-dilatation was seen. In addition it was found that following bile duct ligation there is also an attenuated response to increasing arterial pCO_2 .

In the next section an isolated organ preparation is used in an attempt to elucidate the aetiology of the increased sensitivity to noradrenaline found in the previous experiments. It is shown that jaundiced plasma is capable of causing a potentiated response of an isolated artery or kidney to the effects of noradrenaline. This is mediated by a decreased equilibrium coefficient and an increased maximal response of the preparation. In this section because of the increase in plasma cholesterol seen in obstructive jaundice, the results of experiments in which hyperlipidaemic plasma, pure cholesterol and low density lipoprotein were infused into an isolated artery is presented. Here it was found that hyperlipidaemic plasma and low density lipoproteins produced similar effects seen during infusion of jaundiced plasma, whereas pure cholesterol did not. Other constituents of jaundiced plasma were infused into the artery preparation but these did not potentiate the response of the artery to the pressor effects of noradrenaline. It is concluded that an increase in plasma cholesterol attached to its apoprotein may be one of the factors responsible for the above mentioned changes.

In the last section pure low density lipoprotein is infused into the cerebral circulation. The responses of the cerebral vasculature to noradrenaline was measured during the above lipoprotein infusion. It was found that a similar vasoconstriction, as that seen during jaundice, occurred. It is concluded that an increased plasma cholesterol in obstructive jaundice may be one of the factors responsible for the alteration in vascular resistance seen in that condition. It is postulated that

cholesterol may be producing this effect by altering smooth muscle uptake mechanisms involved in the metabolism of noradrenaline. It is also pointed out that cholesterol may be having other intra-cellular effects which may account for the alterations in vascular resistance found in the present study.

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INDEX

PAGE

SECTION I

Introduction and clinical background of study.	
Outline and scope of thesis	1
The relationship between the liver and kidney	4
1.Pre-existing kidney disease associated with increased incidence of hepatic disease or dysfunction.	4
2.Toxins and systemic diseases involving both liver and kidney.	6
3.Renal damage secondary to a complication of primary liver disease.	8
4.Primary disease of the liver or biliary system affecting renal function.	8
The kidney and obstructive jaundice	13
Water and electrolyte metabolism in hepato-renal failure	18
Summary	20

SECTION II

Studies on the effects of obstructive jaundice on renal blood flow distribution in the baboon kidney	
The renal circulation	21
Regulation of intrarenal blood flow	28
Methods of measurement of renal blood flow	39
Renal blood flow distribution in obstructive jaundice	49
Summary	79

SECTION III

The effect of obstructive jaundice on cerebral blood flow	
Control of cerebral blood flow	82

	<u>PAGE</u>
Measurement of cerebral blood flow	86
The effect of obstructive jaundice on cerebral blood flow in baboons	93
Summary	115

SECTION IV

Experiments using an isolated perfused artery or kidney preparation	
The effect of jaundiced plasma on the responses of a perfused artery or kidney to the pressor effects of noradrenaline	118
Effects of hypercholesterolaemic plasma, cholesterol and low density lipoproteins on the vascular sensitivity to the effects of noradrenaline	139
Experiments with extracts from jaundiced plasma	157
Summary	171

SECTION V

Infusions of low density lipoproteins into the cerebral vasculature	
Final discussion and conclusions	
Infusions of lipoproteins into the cerebral vasculature	173
Final discussion and conclusions	188
References	200

LIST OF TABLES

<u>TABLE</u>	<u>PAGE</u>
1. Relationship between liver and kidney	5
2. Systemic disorders causing simultaneous renal and hepatic failure	7
3. Renal blood flow distribution - jaundice one week	62
4. Mean changes renal blood flow distribution	63
5. Renal blood flow distribution - jaundice two weeks	64
6. Renal blood flow distribution - sham operations	65
7. Mean percentage change C I and C II rates after infusion of noradrenaline	68
8. Effect of sympathetic blocking agents	70
9. Infusion of phenoxybenzamine	71
10. The biochemical data	72
11. Mean grey matter flow following infusions of noradrenaline	96
12. Mean grey matter cerebral blood flow; mean change in cerebral blood flow; and mean arterial blood pressure following infusions of noradrenaline	97
13. Percentage change cerebral blood flow	99
14. Mean cerebral vascular resistance	102
15. Mean percentage change cerebrovascular resistance	103
16. Biochemical data	106
17. Mean biochemical data	108
18. Response to P_{aCO_2}	110
19. Responses and perfusion pressure during jaundiced plasma infusion	124
20. Biochemical investigations	130
21. Values of R_{max} and $K_{0.5}$ for all experiments and controls	134

<u>TABLE</u>	<u>PAGE</u>
22. Comparison of Lineweaver-Burke plot	135
23. Values of interaction coefficient	138
24. Experimental protocol for examining the effects of cholesterol on the responses of an isolated perfused artery to the actions of noradrenaline	141
25. The perfusate pressure changes during hypercholesterolaemic plasma perfusion	145
26. The equilibrium coefficient and the maximal possible response for hypercholesterolaemic plasma experiments and normal controls	149
27. Perfusate pressures during low density lipoprotein perfusion	152
28. Values of equilibrium coefficient and maximal responses during lipoprotein infusions	153
29. Perfusate pressure changes during deproteinised jaundiced plasma perfusion	159
30. Changes in equilibrium coefficient and maximum response during lipoprotein perfusion from jaundiced plasma	162
31. Perfusate pressure changes during perfusion with jaundiced plasma from the low density lipoproteins had been removed	163
32. Perfusate pressure changes during bilirubin perfusion	165
33. Perfusate pressure changes during triglyceride perfusion	166
34. Perfusate pressure changes during perfusion with cholesterol oleate	167
35. Grey matter flow during doses of noradrenaline infusion	175
36. Mean values of grey matter flow and cerebrovascular resistance in the control period	177
37. Mean grey matter flow before and after lipoprotein perfusion	178

<u>TABLE</u>	<u>PAGE</u>
38. Mean percentage change in grey matter flow and cerebrovascular resistance after infusion of noradrenaline before and during lipoprotein perfusion	179
39. Cerebrovascular resistance during noradrenaline perfusion	182
40. Changes in lipoprotein fractions	185

LIST OF FIGURES

<u>FIGURE</u>		<u>PAGE</u>
1.	Injected baboon kidney	22
2.	Diagrammatic representation of the renal circulation	24
3.	Schematic representation of the experimental apparatus	51
4.	Washout curve of ^{133}Xe from the baboon kidney	56
5.	Changes in blood flow and percentage distribution after one week of jaundice	58
6.	Changes in blood flow and percentage distribution after two weeks of jaundice	59
7.	Computer derived analysis of a ^{133}Xe washout curve in the control period and after two weeks of jaundice	60
8.	Changes in C I flow rate after infusion of .0005 - .5 $\mu\text{g}/\text{min}$ noreadrenaline	66
9.	A fusion curve	69
10.	Section of kidney after two weeks of jaundice x 100	74
11.	Section of kidney after two weeks of jaundice x 400	75
12.	Changes in flow rate and percentage distribution during jaundice and during perfusion with noreadrenaline	80
13.	Diagrammatic representation of the technique for infusing ^{133}Xe via a catheter in the lingual artery	93
14.	Changes in grey matter cerebral blood flow with noreadrenaline	100
15.	Mean change (CWF)	101
16.	Changes in grey matter cerebrovascular resistance with noreadrenaline	104
17.	Mean percentage change (CVR)	105

<u>FIGURE</u>		<u>PAGE</u>
1.	CO ₂ response curves	111
	The isolated perfused artery preparation	120
	Responses of an isolated perfused kidney	123
21.	Response of an isolated perfused kidney during perfusion with jaundiced plasma	126
22.	Log/dose response curve from kidney experiment	127
23.	Responses of an isolated kidney during perfusion with normal plasma	128
24.	Log/dose response curve during perfusion with normal plasma	129
25.	Lineweaver-Burke plot of data from one kidney experiment	131
26.	Hofstee plot of data from one kidney experiment	133
27.	Perfusion with hypercholesterolaemic plasma	143
28.	Log/dose response curve during perfusion with Krebs and during hypercholesterolaemic plasma	146
29.	Log/dose response curve obtained during perfusion of normal plasma	147
30.	Double reciprocal plot from experiment comparing responses in Krebs solution to those with hypercholesterolaemic plasma	148
31.	Perfusion with low density lipoprotein	151
32.	Log/dose response curve during lipoprotein perfusion	154
33.	Perfusion with low density lipoproteins from jaundiced plasma	161
34.	Perfusion with bile salt	169
35.	Mean change in grey matter flow	180
36.	Mean percentage change in grey matter flow	181
37.	Mean change in cerebrovascular resistance	183
36.	Mean percentage change in cerebrovascular resistance	184

SECTION I

INTRODUCTION AND CLINICAL BACKGROUND OF STUDY.

INTRODUCTION

OUTLINE AND SCOPE OF THESIS

In 1863 Austin Flint¹ first called attention to the presence of oliguria in patients with cirrhosis of the liver and hydroperitoneum. Since that time it has become apparent that oliguria is common in patients with cirrhosis and ascites, and may progress to renal failure^{2,3,4}. Although in some patients this complication may be due to co-existing primary renal disease, in many others azotaemia and oliguria arise either spontaneously, or in response to minor changes in blood volume. The renal failure associated with hepatic disease was termed the "hepato-renal syndrome" by Boyce⁵ in 1935. Since this implies a specific relationship between the kidneys and liver which has not been established, most authorities have urged that the term be abandoned^{6,7}. Since there is good evidence that the renal failure in cirrhotic patients has a functional cause, Vesin⁸ introduced the term "functional renal failure in cirrhosis", and the name is generally preferred although "hepatorenal syndrome" has become common usage.

The syndrome characteristically occurs in patients with decompensated cirrhosis and refractory ascites. Reduced renal blood flow has consistently been demonstrated in these patients^{7,9,10} associated with increased vascular resistance^{7,9,10,11} and a redistribution of blood away from the cortex^{7,10,12}. It has also been shown by Kew¹² that there are alterations in renal circulation in cirrhotic patients without overt renal failure. He has shown in addition that there are changes in intrarenal distribution of blood in malignant disease of the liver,¹³ and in non-cirrhotic portal hypertension¹⁴. This indicates that the term "functional renal failure of cirrhosis" is a misnomer and that perhaps the original term "hepatorenal syndrome" may be more useful.

Page 2.

Although it has been shown by Dawson¹⁵ that there is a high incidence of oliguric failure in obstructive jaundice, there have been no studies on intrarenal blood flow distribution in this condition. In this study, therefore, the intra-renal blood flow distribution in obstructive jaundice was investigated in order to establish whether similar changes occur in this condition as those described for other hepatic diseases.

The aetiology of the renal circulatory changes is not known, although various theories have been suggested which will be reviewed in this thesis. It has, however, been suggested by Dawson¹⁶ that in obstructive jaundice the kidneys are more sensitive to ischaemia, and it has postulated by Aoyagi and Lowenstein¹⁷ that the sensitivity of the kidney to ischaemia is due to an increase in circulating bile salts. Baum¹⁸ has implicated conjugated bilirubin as the factor responsible for the increased sensitivity of the kidney to periods of hypotension. The second aim of this thesis is to attempt to establish an aetiology for the renal changes observed in obstructive jaundice (if any) and in addition to show whether any other vascular bed is affected by the disease.

The thesis will be presented in five sections. In the first, the outline of the thesis will be presented and a review of the relationship between the liver and the kidney will be given. In the second section, a description of the renal circulation and its control will be given and the methods of measurement will be discussed. The ¹³³Xenon technique will be discussed in detail. A study on renal blood flow in obstructive jaundice, using the ¹³³Xenon technique will be presented. This is followed by a section in which the effect of obstructive jaundice on cerebral blood flow will be studied also using the ¹³³Xe technique. There then follows a section in which the effect of jaundiced plasma on isolated arteries and

Page 3.

kidneys is studied and also in this section there is a description of the attempts made to isolate the factor or factors responsible. Then the effect of this isolated substance on the cerebral circulation in normal animals will be presented. Finally in this latter section the findings of the entire study will be summarised and its significance commented upon.

THE RELATIONSHIP BETWEEN THE LIVER AND KIDNEY

There are numerous associations, known or postulated between diseases or disordered functions of the liver and the kidney. These are summarised in Table I. Although this study is concerned mainly with the renal failure accompanying obstructive jaundice and its aetiology, the others will be discussed briefly in order to put the relationship between the liver and the kidney into perspective, and in addition to provide a suitable background against which this study was carried out.

1) PRE-EXISTING KIDNEY DISEASE ASSOCIATED WITH INCREASED INCIDENCE OF HEPATIC DISEASE OR DYSFUNCTION

Liebowitz¹⁹ found pre-existing primary renal disease in 13 out of 50 patients with cirrhosis of the liver, and felt that the two occurred together more commonly than could be explained by chance. Most authors^{7,20} believe the association to be quite fortuitous and it will not be discussed further.

The association between renal tumours and altered hepatic function is however more definitive. The syndrome of non-metastatic hepatomegaly and hepatic dysfunction in patients with hypernephroma has been described by many workers^{21,22}. In addition Summerskill²³ reported hepatic abnormalities in a patient with a sarcoma of the kidney which improved upon removal of the tumour. The liver dysfunction is manifest in these conditions by reduced prothrombin time, raised serum alkaline phosphatase and retention of bromsulphthalein. The liver is usually normal although occasionally there may be a non specific reactive hepatitis⁶.

The aetiology of the syndrome is obscure and it has postulated that some humoral factor is released from the diseased kidney²⁴, but Utz²⁵ was unable to produce liver

TABLE I

- 1) Pre-existing renal disease associated with increased incidence of hepatic dysfunction.
- 2) Toxins and systemic disease involving both liver and kidney.
- 3) Renal damage secondary to a complication of primary liver disease or its treatment.
- 4) Primary disease of the liver or biliary system affecting renal functions
 - a) Renal failure associated with cirrhosis
 - b) Renal changes in malignant disease of the liver.
 - c) Renal changes in non-cirrhotic portal hypertension.
 - d) Renal failure complicating surgery in patients with obstructive jaundice.

cell injury in mice by injecting extracts of a hypernephroma. Hori²⁶ has shown a relationship between the autonomic nervous system of the liver and the kidney, and it is possible that neural involvement of the kidney produces secondary circulatory changes in the liver, manifest by altered hepatic function. This is obviously highly speculative and the aetiology of the hepatic changes seen with renal tumours remains obscure.

2) TOXINS AND SYSTEMIC DISEASES INVOLVING BOTH LIVER AND KIDNEY

These diseases are associated with microscopic and macroscopic renal damage and are reviewed in order to differentiate them from the functional group in which no renal damage is manifest.

The systemic disorders which involve both liver and kidney are usually toxic or infective in origin, but a group of conditions usually manifest by circulatory disturbances may also present with concomitant renal and hepatic failure. The more common causes are listed in Table 2 (quoted from Kew²⁴).

In most of these conditions the renal and hepatic injury is caused by the primary disorder and there is no evidence that the hepatic failure produces the renal damage. However once the hepatorenal failure is established, the presence of uraemia aggravates the metabolic disturbance of liver failure. For example raised amounts of urea excreted into the bowel in uraemia, will produce elevated blood ammonia level²⁷. Also the excretion of conjugated bilirubin and other dialysable substances will be reduced in renal failure²⁷. Shear¹¹ has also postulated that the metabolic abnormalities found in liver failure aggravates the co-existing renal dysfunction.

The kidneys in these conditions usually show changes

TABLE 2

TOXINS

Carbon tetrachloride
Trichloroethylene
Toluene
Halothane
Tetracycline
Methyl alcohol
Methoxyflurane
Paracetamol
Tannic acid
Stilbamidine
Mercury
Phosphorus
Mushroom poisoning

INFECTIONS

Weill's disease
Yellow fever
Septicemia
Falciparum malaria

MISCELLANEOUS

Shock
Disseminated intravascular coagulation
Eclampsia
Heatstroke
Hypothermia

consistent with acute tubular necrosis, although tubular dilatation, areas of interstitial nephritis and so-called biliary nephrosis have also been described^{28,20}. The importance of this group rests in the fact that a primary condition may be identified which damages both the liver and kidney.

3) RENAL DAMAGE SECONDARY TO A COMPLICATION OF PRIMARY LIVER DISEASE OR ITS TREATMENT

Renal failure may commonly be seen in patients with cirrhosis consequent on a severe haemorrhage from oesophageal varices or from peptic ulceration²⁸. Renal failure may also occur following diuretic therapy²⁰, or from Neomycin used to sterilise the bowel^{29,30}. It has been shown that neomycin may be absorbed from the bowel in sufficient quantities to be nephrotoxic.³⁰ This nephrotoxicity is characterised by proteinuria and tubular necrosis. Septicaemia also commonly occurs in these patients and may terminate in circulatory and secondary renal failure²⁴.

4) PRIMARY DISEASE OF THE LIVER OR BILIARY SYSTEM AFFECTING RENAL FUNCTION

a) Functional renal failure in cirrhosis

This has emerged as an entity which is manifest as an elevation of the blood urea or serum creatinine in the absence of renal disease or clearly demonstrable secondary causes of renal failure. This will be described in detail to provide a suitable background to which the changes in obstructive jaundice may be compared.

Presentation

In the earliest stages renal dysfunction may manifest only with reversal of the normal rhythm of urinary excretion or an inability to excrete a water load²³. Sodium excretion

is reduced and a hyponatraemia is usual. Jaundice may or may not be present but hepatic function is deranged and ascites is almost invariable²⁰. Later the patient may experience nausea, thirst and vomiting and the biochemical abnormalities become more severe. These biochemical abnormalities will be discussed further below.

With further deterioration the neuro-psychiatric disorder, electrocardiography changes and elevated ammonia concentrations consistent with hepatic coma appear²³. It has already been mentioned that the uraemic contributes to the high serum ammonia levels²⁷. During the late phases oliguria and arterial hypotension appear thus contrasting with earlier concepts that azotaemia invariably had a pre-renal basis. At the height of the azotaemia the relative contribution of hepatic and renal failure to the clinical and biochemical changes are difficult to distinguish²⁴ but death is usually due to hepatic failure. The chances of survival depend on the degree of reversability of the hepatic disease as renal function improves only when the liver disease is controlled²⁰.

Histological changes in the kidney

Examination of the kidneys of cirrhotic patients usually reveals nothing abnormal and when lesions are found these are usually non specific^{23,24}. Recently, however, Salmon³¹ identified glomerular changes in renal biopsy tissue from all twenty-four patients studied with hepatic disease. In this series studies by electron microscopy revealed deposits in the capillary wall and the mesangium, thickening of the basement membranes and an increased mesangial matrix. These glomerular changes have, however, not been correlated with the abnormalities in renal function nor with the type, duration or severity of the hepatic disease. Furthermore Kew²⁴ feels that many of the changes observed such as widening of the mesangial matrix, thickening or splitting of the glomerular basement membrane or areas of interstitial

nephritis and bile staining of the tubular epithelium are of doubtful significance. He states that similar changes have been found in cirrhotic patients without renal failure.

Renal circulatory function.

Conventional tests of renal function show the reduced clearances of inulin, para-aminohippuric acid, creatinine and urea^{7,20}. This progressive deterioration in clearance values has been correlated with the severity of the disease⁷. Total renal plasma flow and the renal fraction of the cardiac output are reduced^{4,32,33}, although cardiac output, mean arterial blood pressure and systemic vascular resistance are usually within normal limits^{4,33}. No major contributions by reduced plasma colloid osmotic pressure and serum osmolality to the renal failure have been substantiated^{11,34}. Summerskill²⁰ also feels that renal hemodynamic function is unlikely to be impaired as a result of compression of renal venous outflow as the glomerular filtration rate and the effective renal plasma flow are little influenced by the disappearance of the ascites. This question of raised abdominal pressure producing reno-vascular changes will be discussed further when considering the aetiology of the condition. It would seem however, that the changes in renal haemodynamics is functional and reversible since Koppel³⁵ has successfully transplanted kidneys from cirrhotic patients dying with renal failure to patients without liver disease. These kidneys have regained their normal function. This functional abnormality in the kidneys in cirrhosis appears to be an increased renal resistance. Renal blood flow in cirrhosis can be improved by drugs which lower renal vascular resistance^{36,37}, which has been shown to be increased^{4,9}. The increase in renal resistance is probably due to an active vaso-constriction. Epstein¹⁰ showed a consistent reversal of all the vascular abnormalities in the kidneys in five cirrhotic patients at post-mortem angiography. He had shown that in these patients there was a decrease in both mean renal blood flow and the percentage of the blood flowing to the outer cortex of the kidney. This shift of blood flow distribution in cirrhosis has

Page 11.

also been shown by Kew¹² Schroeder³⁸ and others.^{4,10,12,11}

Pathogenesis

The aetiology of the syndrome is obscure and Conn³⁹ feels that there is most probably more than one cause. Tristani and Conn⁹ have shown that patients with functional renal failure secondary to cirrhosis have a decreased plasma volume which is associated with a decreased cardiac output and increased renal vasoconstriction. Expansion of plasma volume in their series improved renal blood flow. However most studies have shown that the plasma volume is increased in cirrhosis^{40,41}, and Conn³⁹ feels that there may be two types of haemodynamic pattern associated with oliguric hepato-renal failure; - one with a decreased plasma volume, reversible by volume expansion and the other a high output, increased plasma volume type.

Mashford⁴² found that patients with cirrhosis behaved as if they were depleted of tissue noradrenaline as his patients tended to be hypotensive, and had a diminished response to tyramine. This is compatible with a theory by Fisher⁴³ that inert amines such as phenylalanine or tyramine may act as false neuro transmitters and may replace noradrenaline or dopamine in hepatorenal failure. This, however, would not explain increased renal vascular resistance in these patients or why there is a redistribution of blood away from the cortex into the juxtamedullary regions.

It has also been postulated that some toxic substance is released by the liver which is vaso-active. Shorr⁴⁴ suggests that an equilibrium exists between a vaso-constrictor substance synthesised in the kidney and a vasodilatory substance produced and degraded by the liver. The former, which appears in blood in early shock, causes a protective vaso-constrictor response and opens arterio-venous anastomoses to maintain circulating volume; the latter, which he believes to be ferritin, causes vasodilation of capillaries and stagnant pooling of blood. In

cirrhotic animals he found diminished hepatic degradation of vaso-depressor material. This concept, which fits some of the features of functional renal failure, has however never been validated clinically, and no such vaso-depressor substance has ever been convincingly isolated from the liver. It has however been shown by Vargish and colleagues⁴⁵ that hepatic anoxia can cause an increased vascular resistance in the kidney. These authors suggest that this releases a vaso-dilator substance which opens up channels in the inner cortex of the kidney. In view of the connection shown by Hori²⁶ between hepatic sympathetic supply and that of the kidney, it may be, that the vaso-constrictions in hepatic anoxia is due to a sympathetic response reflected in both organs.

It has been mentioned above that increased abdominal pressure is also considered a possible cause since paracentesis can reverse the renal haemodynamic changes of cirrhosis⁴⁶. This is in contrast to the findings by Summerskill mentioned previously. In addition Mullane and Gliedman⁴⁷ found that the inferior vena cava pressure is increased in cirrhosis with or without ascites, although the latter was more common. They contend that the enlarged liver itself may obstruct the inferior vena cava. Conn,³⁹ however, points out that increments in renal venous pressure required to impair renal function far exceed those observed in cirrhosis.

Hyponatraemia is common in cirrhosis and this has been postulated as the cause of the condition³⁹, but it is probably dilutional in nature and will be discussed further when considering the biochemical abnormalities of the hepato-renal failure.

Alterations in oxygen saturation³⁹ and portal systemic shunting³⁹ have also been mentioned as being involved in these conditions, but basically the exact aetiology of the syndrome remains obscure. The only definitive finding is

that there is increased renal resistance in this condition. The aetiology of this increase is as stated as yet unidentified but is probably multi-factorial in nature.

b) Renal changes in malignant diseases of the liver and non-cirrhotic hypertension

Kew using the ¹³³xenon technique in both of these conditions, showed a reduction in outer cortical flow which was accompanied by a reduction in glomerular filtration rate.^{13,14} The pattern of renal failure changes was similar to that seen in renal dysfunction associated with hepatic cirrhosis. Kew suggests that since the blood flow distribution changes in non-cirrhotic portal hypertension are similar to those found in hepatic cirrhosis, the increased renal resistance found in the latter disease may be due to a raised portal pressure. This would further support the contention that raised abdominal pressure is responsible for the pathogenesis of the disease. This has been discussed above.

THE KIDNEY AND OBSTRUCTIVE JAUNDICE

In 1911 Clairmont and Von Haberer⁴⁸ drew attention to a definite hazard found in a jaundiced patient who undergoes surgery i.e. acute post-operative renal failure. They described five patients with obstructive jaundice all of whom died of oliguric renal failure soon after an operation for the removal of gall-stones. They emphasised that apart from the jaundice these patients were in good health with no previous renal disease. They postulated that obstructive jaundice predisposes to oliguric renal failure.

Heyd⁴⁹ in 1924 noted that following operations on the biliary tract for a variety of reasons, there was a high mortality even in patients who were not jaundiced. While many of these post-operative deaths were probably due to infection, inadequate pre-operative preparation or problems of anaesthesia, it would appear that post-operative renal failure accompanying obstructive jaundice is a distinct entity.

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Page 14.

The clinical findings by Zollinger and Williams⁵⁰ as well as Dawson¹⁵ that there is a much higher incidence of acute post-operative renal failure in obstructive jaundice would tend to confirm these earlier studies.

The aetiology of this post-operative oliguric renal failure is unknown. Boyce and McPetridge⁵ ligated the common bile duct in dogs and maintained the obstruction for eighteen days. They then released the obstruction and the deaths which followed this second operation occurred ten to fourteen days thereafter, and was accompanied by degenerative changes in the tubules of the kidney with preceding symptoms consistent with uraemia. These authors therefore contend that the release of the biliary obstruction is responsible for the fatal outcome, and postulate that a toxic substance is released into the circulation. Autopsy findings by Helwig and Schutz⁵¹ in traumatic damage to the liver, are similar to those described by Boyce and McPetridge and these authors also believed that some substance is released by the liver into the circulation resulting in kidney damage. As mentioned when discussing the functional renal failure of cirrhosis no such toxic substance has as yet been identified.

It has been suggested by Williams and Zollinger⁵² that in obstructive jaundice the kidneys are sensitive to periods of hypotension. They noted that in thirty-seven patients who died following operations, twenty-seven developed hypotension lasting for one and a half to nineteen hours prior to death. They also examined the effect on the kidney of hypotension in jaundiced and normal dogs and showed that urea clearance was always lower in the jaundiced dogs. These authors also showed that hypotension was induced with much less blood loss in jaundiced animals than in the normal animals. This suggestion that the kidneys are more sensitive to ischaemia in obstructive jaundice is further strengthened by the work of Dawson¹⁵ in rats. He showed that the damage to the renal parenchyma caused by

prolonged clamping of the renal artery was more severe in the jaundiced animals than in normal controls. Dawson also postulated that there is a reduction in renal blood flow in obstructive jaundice since a mannitol diuresis which maintains renal blood flow, largely diminished ischaemic renal damage both in dogs with obstructive jaundice as well as humans.

The possible substances causing this increased sensitivity to ischaemia are unknown but the two which are most obvious are conjugated bilirubin and bile salts, although there are numerous other biochemical abnormalities in obstructive jaundice which must also be considered.

The kidney does not excrete unconjugated bilirubin which is non-dialysable⁵³. Conjugated bilirubin (bilirubin diglucuronide) is however water soluble and most of its excretion is via glomerular filtration⁵³, although the tubular cells may also be exposed to bilirubin via the vasa rectae. There is evidence that bilirubin may be toxic to cells and this has been shown to uncouple oxidative phosphorylation in mitochondria⁵⁴ as well as having an inhibitory effect on electron transfer⁵⁴. It has also been shown that in the presence of anoxia or ischaemia that capillary permeability increases and the amount of conjugated bilirubin entering the cells rises thereby compounding the damage²⁴. Further evidence of the toxic effects of bilirubin on cellular function is shown by the work of Antoine and Nevenou⁵⁵ who demonstrated renal antigens in the urine of patients of obstructive jaundice that they believed were derived from damaged renal tubules.

Further evidence that conjugated bilirubin is involved in the development of renal damage in obstructive jaundice, is provided by the work of Baum¹⁸ on Gunn and Wistar rats. In Gunn rats a very little conjugated bilirubin is formed as they have a congenital absence of the enzyme uridine diphosphate glucuronyl transferase. Baum showed that these

rats were less sensitive to renal damage from ischaemia following ligation of the common bile duct, than Wistar rats who have normal amounts of conjugated bilirubin. This is also supported by the clinical finding that the renal failure in obstructive jaundice is related to the depth of the jaundice¹⁵ although Baldus⁷ and Spellberg⁵⁶ have noted that the development of renal failure in cirrhosis does not appear to be related to the level of the hyperbilirubinaemia.

Although unconjugated bilirubin is not significantly increased in obstructive jaundice it may compound the damage caused by other factors since it itself is toxic to cells. In *Kernicterus* Schmidt⁵⁷ has shown marked bilirubin staining of the inner medulla of the kidney, and in Gunn rats with cholestasis, Odell⁵⁸ shows that the concentration of bilirubin in the medulla was markedly increased. Diamond and Schmidt⁵⁹ have however, shown that the serum concentrations of unconjugated bilirubin in obstructive jaundice are insufficient to affect oxidative phosphorylation. In addition Aoyagi and Loenstein¹⁷ were also unable to induce renal insufficiency in rats by infusing unconjugated bilirubin.

It is possible that a bile salts may be responsible for the renal failure found with cholestasis. Aoyagi and Loenstein¹⁷ showed that rats in whom sodium cholate and taurocholates were infused into the kidney, developed renal failure after a thirty minute period of ischaemia whereas normal rats did not. They postulate that bile salts are responsible for the hyper-sensitivity of the kidney to periods of ischaemia. It is also possible that bile salts may potentiate the effect of bilirubin on the kidney. Conjugated bilirubin is less firmly bound to protein (albumen and beta globulin) in the presence of bile salts⁵³ and also becomes more easily dialysed. Thus bile salts may enhance the ability of the bilirubin to enter the cells thereby compounding the damage caused by it.

A further possible explanation for the increase in sensitivity of the kidneys to ischaemia, is that in experimental biliary obstruction the abdominal vena caval pressure is elevated. This has been discussed above when considering the aetiology of the changes due to cirrhosis of the liver. It was mentioned there that the abdominal pressures are not high enough to account for the changes in renal perfusion which are found with the disease³. However the rise in abdominal pressure could result in the release of renin which by forming angiotensin II could cause renal vascular constriction⁶⁰. This latter explanation is supported by Kew, who found changes in non-cirrhotic portal hypertension similar to those found in cirrhosis¹⁵.

It is also possible that the acute renal failure is secondary to circulatory failure from septicæmic shock. A high proportion of deaths in the Dawson¹⁵ series was caused by infection and cholangitis and recently Wardle and Wright⁶¹ have shown that the clearance of endotoxin in rats with cholestasis is delayed. Additional evidence for this theory is provided by the findings of Wilkinson et al⁶² that fourteen of twenty-two patients with fulminant hepatic failure had endotoxaemia as detected by an assay for Limulus lysate. There were statistically significant correlation between the occurrence of endotoxaemia and the development of renal failure. Also endotoxin injected into the baboon kidney causes a pronounced increased renal blood flow due to an increased renal resistance⁶³.

Thus it can be seen that the aetiology of the renal failure seen post-operatively in obstructive jaundice remains unidentified. It would seem however that there is a definite increase in sensitivity of the kidney to the effects of ischaemia in this condition and this will be investigated further in this thesis.

WATER AND ELECTROLYTE METABOLISM IN HEPATO-RENAL FAILURE

Total body water and total body exchangeable sodium are increased in patients with decompensated liver failure accompanied by the renal disturbances mentioned above.^{64,65} Hyponatraemia is however commonly seen in azotaemic patients with serum sodium concentrations usually falling below 130 mEq/litre^{64,65,20}. This is of the dilutional variety due to retention of excessive amounts of water by the kidneys. Reabsorption of sodium in this condition often exceeds 99.5% of the filtered load⁹ and is probably due to an excessive reabsorption from the proximal tubule⁶⁶. There is also an associated secondary hyper-aldosteronism which is both due to impaired degradation of aldosterone⁶⁷ as well as an increased production⁶⁸. The renin angiotensin system is a potent stimulus of aldosterone secretion, and elevated plasma renin activity has been found in cirrhosis^{38,69} but this is probably secondary to the kidney disturbances and not the cause of them since improving the blood flow to the kidney reduces the plasma renin levels. Also, aldosterone has a primary action on the distal tubule of the kidney and as mentioned above, the increased sodium retention is probably due to increased proximal reabsorption.

The defective renal tubular excretion of water by the kidney is characterised by reductions in the maximal urine flow and free water clearance²⁰. This abnormal pattern of water excretion is consistent with excess anti-diuretic hormone activity and probably accounts for the hyponatraemia and overhydration which precede or are associated with the development of azotemia^{65,70,71}, although the excessive reabsorption of sodium by the proximal tubule could also be expected to increase water reabsorption by passive diffusions.

The retention in salt and water could also be explained by the shift of blood flow from the cortex to the juxta-medullary area mentioned previously. Inner nephrons tend

to retain sodium whereas more peripheral nephrons tend to lose sodium⁷². Thus when renal blood is distributed to medullary and inner cortical areas sodium retention would occur. This however remains speculative and the relationship between the renal circulation and the renal handling of sodium has not been clarified.

Total body exchangeable potassium is usually low in liver failure with ascites⁷³, but Cassey et al⁷⁴ has claimed a correlation with the serum potassium level and the degree of hepatic dysfunction. This may be due to diuretic therapy or hyper-aldosteronism. Summerskill²⁰ notes that serum potassium may be high or low in liver failure but when azotaemia has developed hyperkalaemia may be seen. Thus it would appear that the question of serum potassium levels in the syndrome remains to be clarified.

Hyperchloraemia is usual in azotaemia but chloride deficiencies have not been successfully demonstrated in hepato-renal failure²⁰. Similarly disturbances in acid base balance occur in hepatic failure which are not characteristic of those seen in azotaemia²⁰.

It must be stressed that the above changes in water and electrolyte metabolism refer to those seen in hepato-cellular failure. Very little data is available on these parameters in the early stages of obstructive jaundice. Dawson¹⁵ mentions glomerular filtration rate is usually decreased, but this was post-operatively. It can only be assumed that the changes seen in the renal failure accompanying hepato cellular damage are similar to those found with obstructive jaundice.

Summary

In this section the scope and outline of the thesis was presented. The relationship between the liver and the kidney was outlined. It was shown that in diseases such as cirrhosis of the liver, portal hypertension and non metastatic malignancy of the liver, there are alterations in renal blood flow distribution. Various workers have shown that there is a shift in blood flow from the outer costal areas to the juxtamedullary areas of the kidney before the onset of overt renal failure. The aetiology of this change in distribution are also discussed. It was mentioned that in obstructive jaundice there is a high incidence of renal failure secondary to the decompensated phase of hepatic cirrhosis. It was also mentioned that in this condition the kidney appears hypersensitive to ischaemia. Whether there are changes in renal blood flow in obstructive jaundice, similar to that found in cirrhosis, and the aetiology of the increased sensitivity to ischaemia, has not been investigated. The remainder of this study will deal with these latter problems.

SECTION II

STUDIES ON THE EFFECTS OF OBSTRUCTIVE JAUNDICE ON RENAL
BLOOD FLOW DISTRIBUTION IN THE RABBIT KIDNEY.

THE RENAL CIRCULATION

The vasculature of the kidney is complex and highly specialized. There are also many variations in the basic pattern of blood vessels in different species, which depend on the functional capacity of the kidney, and which must be borne in mind when interpreting animal experiments. The difficulty in extrapolating results found in animals to the human situation is obvious, and the limitations of animal experiments are self evident. Nevertheless, in the absence of published data on the baboon kidney vasculature, I have assumed that the renal circulation in the baboon is essentially similar to that found in humans. A study on a baboon kidney injected with a plastic polymer revealed no obvious differences (Figure I). Furthermore Fourman and Moffat⁷⁵ in their study of the blood vessels of the kidney, state that the basic pattern of blood vessels is similar in all mammals, although there are slight variations in detail.

Arteries

The kidney is supplied by a single renal artery in most cases although Merkin and Michels⁷⁶ found in humans that in twenty-eight percent of cases two renal arteries were present. There was a single renal artery in all sixty baboons studied here. This artery arises from the aorta, and soon after entering the hilus of the kidney divides into anterior and posterior branches⁷⁵. Each of these in turn divides into further segmental arteries from which the interlobular arteries arise. (Figure I). Each of these segmental arteries is virtually an end artery since it supplies a definite segment of the kidney and does not anastomose with the arteries of adjacent segments, except via very small capsular and pelvic branches which are unable to establish a collateral circulation⁷⁵. There are five segments; apical, anterior, middle, lower and posterior⁷⁵.



FIGURE I

INJECTED BAROQUE KIDNEY.

Arteries shown in red, veins in blue.

A = Interlobar arteries

B = Arcuate arteries

C = Interlobular arteries

Gordier⁷⁷ however describes a further system of classification in which the kidney is divided into anterior and posterior mesorenal, and inferior polar territories. These segmental arteries then divide into two to form the interlobar arteries which pass in a direction parallel to the dorsal and ventral surface of the kidney. (Figure 2). At the junction of the cortex and the outer medulla the arcuate arteries are formed by a sub-division of the interlobar arteries. (Figure 2). From the arcuate arteries arise most of the interlobar arteries which pass towards the capsular surface of the kidney. (Figure 2). Barger and Herd⁷⁸ mention that there are numerous vessels which pass to the superficial cortex, branching in the subcapsular region and supplying a major portion of the outer cortex with terminal afferent arterioles, often passing in a retrograde direction. These vessels are unusually sensitive to sympathetic stimuli⁷⁸. Thus superficial nephrons seem in large part to be supplied by vessels which are anatomically and physiologically distinct from those supplying deeper nephrons. It follows therefore, that if a single trunk is strongly constricted, complete arterial deficiency of a region may be noted⁷⁸.

The glomerular afferent arterioles are given off mainly from the interlobar arteries, but some arise directly from the arcuate or even the interlobar arteries⁷⁵. The proximal afferent arterioles arise at an angle from the interlobar arteries so that their course is directed towards the arcuate vessel, while the more distal afferent arterioles are directed towards the surface of the kidneys, and the terminal branches are virtually a continuation of the line of the parent vessel⁷⁵. In addition glomerular branches are given off from the interlobar arteries both in the juxtamedullary region and in the outer cortical region. (Figure 2).

The efferent arteriole of cortical nephrons differs from those of juxtaglomerular nephrons^{75,79}. The efferent arterioles of cortical nephrons are short and fine, and rapidly break up into a capillary plexus around the tubules.

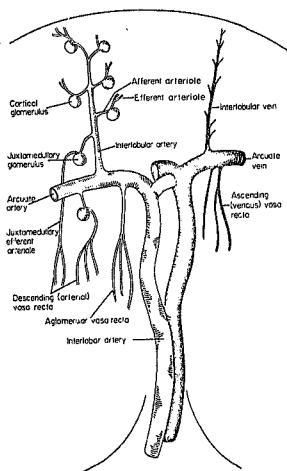


FIGURE 2

DIAGRAMATIC REPRESENTATION OF THE RENAL
CIRCULATION.

A few divide to supply the more elongated capillary network of the medullary rays. The juxtamedullary network consists of two patterns^{80,81}.

- 1) Thin walled efferent vessels that subdivide immediately into a capillary network.
- 2) Straight muscular efferents, usually larger than the afferents, which subdivide to provide multiple branches of larger calibre, which run to the medulla as vasa recta.

Many of the glomeruli which give rise to vasa recta, are not however juxtamedullary in position, since they lie in the middle zone of the cortex. These efferent arterioles therefore, have a long course giving off numerous capillary branches before they break up into vasa recta.

The efferent arterioles of the juxtamedullary glomeruli, are surrounded by "sphincters" of smooth muscle⁷⁵, and also there is smooth muscle present in the more distal parts of the vasa recta⁷⁶. Also Pourman⁷⁵ reports that non myelinated nerves are found in relation to all the vessels with smooth muscle. Therefore the anatomical basis for control and redistribution of intramedullary flow is present.

It can be seen from the above description that the cortical and juxtamedullary nephrons are anatomically distinct. Their blood supply differs, and in addition loops of Henle in the cortical nephrons are much shorter⁸². Also the glomerular arrangement is different and the juxtamedullary glomerulus is much bigger, and has a greater glomerular filtration rate⁷⁸. Thus the reported alteration in electrolyte and water metabolism in cirrhosis and other liver conditions which have been discussed previously can be explained by a shift in blood flow from outer cortical to juxtamedullary regions.

The parenchyma of the medullary papilla is supplied by long central branches of the vasa recta⁷⁸. Many of these vessels are relatively large in calibre and do not branch until they approach the tip of the papilla, which suggests that much of the peri-tubular capillary flow in this region

is countercurrent⁷⁸. Fourman and Moffat⁷⁵ state that the medulla may be supplied via the direct route to the capillary plexus at the tip of the papilla, or by an indirect route through the inner and outer peri-tubular capillary network. Rollhauser⁸¹, however suggests that the medulla is separated into three regions; i.e. an outer strip, an inner strip and a zone for the papilla. He feels that each of these zones has its own arterial and venous vasa recta, and that capillary anastomoses between the plexuses are of minor importance.

Shunts and anastomoses

Arterio-venous anastomoses were first reported in the kidney by Spanner⁸⁴ but Fourman and Moffat believe that direct arterio-venous anastomoses are not present⁷⁵. They do however state that the kidney contains numerous vessels of a distensible nature, which, from a physiological point of view can act as rapid transit carriers from arteries to veins. Attempts to find cortical shunts described by Trabucco and Marquez⁸³ or the juxtamedullary shunts described by Trueta⁸⁴ have also been unsuccessful. It would seem that short circuits which occur within the kidney are the result of rapid flow of blood through a low pressure system, composed of widely dilated normal channels⁷⁵. The venous channels draining the medulla provide such a system and thus bypasses have been described principally in the juxtamedullary regions. Also as described, sympathetic activity would reduce blood to the outer cortex and the resultant increase in blood flow to the juxtamedullary region, would appear as if shunting had occurred, whereas blood is simply flowing down the path of least resistance.

Veins

The arcuate and interlobar arteries are accompanied by single veins. (Figure 2). The arcuate veins anastomose with each other but these are infrequent⁷⁵. The interlobar veins and in the arcuate veins and receive the wide capillaries, which drain the cortical plexus, and some of the ascending vasa recta⁷⁵. The superficial area of the cortex is drained by stellate veins which also enter some of the interlobular veins.

The medulla is drained by ascending vasa recta which open into the arcuate veins directly, or by short collecting veins. Some drain into interlobular veins⁷⁸. The ascending vasa recta receive many tributaries from the surrounding peritubular capillary plexuses. Unlike the arteries large anastomotic channels interconnect major veins, so that venous drainage is unaffected by closure of sizable venous trunks⁷⁸.

Innervation

The renal nerves arise mainly from the coeliac plexus, but kidney also receives fibres from the thoracic and upper splanchnic nerves, the intermesenteric nerves and the hypogastric plexus⁸⁵. This rich supply of autonomic nerves is destined mainly for the blood vessels. The current evidence indicates that renal tubular structures are not innervated⁸⁶.

McKenna and Angelikos⁸⁷ observed that in the dog the nerves follow the course of the blood vessels. These nerves terminate near the interlobular arteries, the afferent arterioles of all nephrons and the efferent arterioles of the juxtaglomerular nephrons⁷⁵. This anatomical arrangement supports the contention of Bergrer⁷⁸ that in most species vascular resistance beyond the glomerulus of the outer cortical nephrons is regulated by local metabolic factors or circulatory hormones. The vasa recta are only supplied with venous innervation in the outer medullary region, and nerve fibres do not penetrate the inner medulla⁷⁸.

The majority of the nerves are adrenergic but cholinergic fibres are present and these have essentially the same distribution as the adrenergic nerves⁷⁸. McKenna and Angelikos⁸⁷ state that no cholinergic fibres are found in association with the glomerular efferent arteriole, veins or tubules but they are present along the vasa recta of the outer medulla. Although denervation leads to degeneration of the adrenergic fibres most of the cholinergic fibres remain, suggesting that they arise from

ganglion cells in the hilar region or renal parenchyma⁸⁶. Finally afferent nerves have been described as arising in the kidney which are subcapsular or situated in the renal vessels⁸⁶.

REGULATION OF INTRA-RENAL BLOOD FLOW

Within the kidney, despite serious restrictions imposed by inadequate methodology (to be reviewed below) a large amount of data from a wide variety of experiments using different techniques, have shown that the flow rates of blood into the different areas of the kidney are dissimilar, and are independently regulated.

Cortical flow is extremely high. In man and dogs values between 300 and 800 mls/100 G tissue are commonly found^{86,88,89}. The flow is slightly less in anaesthetised animals⁸⁶. There is also good correlation between cortical flow and total renal blood flow measured directly, which not only confirms the validity of the cortical measurements, but also permits the extrapolation to cortical flow, from the vast literature on total renal blood flow^{86,90,91}. Medullary flow is not clearly defined. Functionally it is taken to represent flow through the high resistance juxta-medullary nephrons and their complex post-glomerular appendages⁸⁶. Some methods however, measure flow in precise regions defined as outer medullary or inner medullary. Since measurement of medullary flow is inaccurate and considerable amounts of the medulla are supplied by vessels arising from nephrons which in fact lie in the cortex, sub-division of medulla flow is artificial.

The juxtamedullary circulation is a high resistance circuit compared with the outer cortex, although stimulation of the sympathetic system may increase the resistance of the outer cortex considerably, by constriction of the vessels passing directly to the cortex as described above⁷⁸. The flow rate given by various investigators of the medullary

Page 29.

circulation range between 30 and 300 ml^{88,89,90}.
Nashat⁸⁶ feels that this wide scatter is probably due to imprecise methodology and also to differences in total body haematocrit.

Cortical flow is "autoregulated", being little altered by mean perfusion pressures varying from 80 - 100 mmHg^{78,91,92,93}. In contrast medullary flow appears to be directly related to perfusion pressure⁹³, although Barger⁷⁸ and Auckland⁹² have both shown an increase in medullary resistance with a rise in pressure. Since no evidence has been presented to attribute the regulation in the juxtaglomerular region to the afferent arteriole, it is possible that this latter result is not due to true autoregulation, but due to nervous and endocrine influences on the post-glomerular vessels.

The kidney vasculature is capable of autoregulation but it would appear that normally the control of blood flow distribution is dependent on the renal nerves, circulating hormones or alterations in body electrolytes. Since the precise nature of autoregulation within the kidney has not been clearly elucidated, and because this study is concerned with hormonal influences on the kidney circulation, it will not be discussed further. The other factors influencing renal blood flow distribution will now be considered.

The role of the renal nerves

In 1883 Cohnheim and Roy⁹⁴ demonstrated that sectioning of the splanchnic nerves produced an increase in kidney volume which they attributed to vasodilatation. They also stimulated the peripheral nerve stumps and showed a reduction in volume. Bradford⁹⁵ in 1889 showed that renal vasoconstrictor fibres originated from the spinal segments T4 to L2 with the most marked changes induced by stimulation of the lower three dorsal nerves. Recently Takeuchi et al⁹⁶ have shown that stimulation of the lateral column, the

lateral border of the anterior column and the surface of the transitional zone between the anterior and posterior column from T5 to L₂, produced an increase in renal resistance. Evidence for supraspinal modification of renal nerve activity have been shown by Wise and Gannon⁹⁷, who showed that stimulation of areas in the medulla lateral to the midline, produced a fifty percent fall in glomerular filtration rate, thereby suggesting a fall in blood flow. Takeuchi⁹⁸ also demonstrated that renal vasoconstriction may be produced by stimulation of areas in the diencephalon. Also it has been shown by many workers that the cerebral cortex may influence renal blood flow. Hoff⁹⁹ demonstrated that direct stimulation of areas of cerebral cortex produced renal vasoconstriction and Smith¹⁰⁰ reported that fright or pain reduces renal blood flow by fifty percent. In addition it has been shown by Miller and Dicera¹⁰¹ that both increases or decreases in renal blood flow can be induced by conditioning experiments, presumably by neurogenically induced vasomotor changes.

In view of the above experimental evidence, it can be seen that renal nerve stimulation produces considerable reductions in total renal blood flow, but in addition it is becoming evident that the renal autonomic nerves may be responsible for distribution changes within the kidney. Trueta et al⁸⁴ on the basis of dye injection studies, reported that stimulation of the renal nerves shunted blood away from the cortex to the medulla. This has been confirmed by the work of Pomeranz et al¹⁰² using radioactive ⁸⁵krypton. These workers have shown that reducing the pressure in the carotid sinus, or directly stimulating the splanchnic nerves in the anesthetized dog, leads to a reduction in cortical flow and an increase in the outer medullary (juxtamedullary) flow. This distributional change was also confirmed by these workers using silicone rubber casts. The relationship of cortical flow to sympathetic activity was also found by

Auckland⁹¹ although he found that medullary flow was also reduced. Pomeranz however makes the point in his paper, that mild stimulation, which may not alter the total renal blood flow, produces distributional changes, and Auckland's results can probably be explained by the fact that he used more intense nervous stimulation.

Under normal conditions the cortex receives about ninety per cent of the total renal blood flow⁸⁶. Increased sympathetic activity to kidney reduces not only flow rate but also the percentage of blood distributed to the outer cortex¹⁰³. This is confirmed by several other techniques such as autoradiography studies, and renal casts which show closure and underfilling of regions in the cortex thereby giving it a patchy appearance⁷⁸. The medullary vasodilation seen in circumstances resulting from increased sympathetic activity is difficult to explain. It has been suggested above that this is a passive consequence of increased cortical resistance. Nevertheless there is much evidence that medullary vasodilation is active and mediated through neural or humoral pathways. McKenna and Angelakos⁸⁷ found cholinergic fibres in the outer medulla which Kashat⁸⁶ suggests are vasodilator. Also injections of acetylcholine increase medullary flow¹⁰⁴. Alterations in the activity of the rich sympathetic nerve supply to the efferent arterioles mentioned above, could also profoundly affect the volume of blood flowing through this region. Therefore it would appear from the above evidence that the increases in medullary blood flow seen under varying conditions may be explained either by passive dilation, or by active neural mechanisms.

Hormonal Control

(1) Noradrenaline. It would be expected that since noradrenaline is the main neuro-transmitter of the sympathetic nervous system⁸², that intra-renal perfusion of the hormone would have similar effects to generalised sympathetic activation. This may not be the case since an increase in

generalised sympathetic activity may alter renovascular resistance by an autoregulatory response to increased cortical blood pressure. It is therefore useful to consider the effects of noradrenaline on renal haemodynamics to elucidate the primary effect of the sympathetic system on the blood flow of the kidney.

Noradrenaline has both α and β adrenergic receptor stimulating properties and evidence has been obtained that the kidney possesses both of these adrenergic receptors. The intrarenal infusion of the beta agonist isoproterenol, produces a mild vasodilatation in the kidney¹⁰⁵. In contrast adrenergic stimulation appears to produce major physiological effects on renal haemodynamics. Noradrenaline which is primarily an α adrenergic agonist produces renal vasoconstriction even in small amounts^{102,103,106}. It also appears that it not only affects the afferent and efferent arterioles but the venules as well¹⁰⁷. It has also been shown that noradrenaline in addition to reducing the total renal blood flow, alters the regional distribution of blood within the kidney as well. Carriere¹⁰⁶ using the ⁸⁵Kr states that noradrenaline reduces volume of blood distributed to the cortex, with an increase in both the rate of blood flow and the volume of blood distributed to the medulla. Schrier¹⁰⁷ feels that this is due to noradrenaline exerting a preferential vasoconstrictor effect on the efferent arteriole, because the fall in glomerular filtration rate is less than the decrease in renal blood flow. He feels that in small doses, mild constriction of afferent arteriole may be counterbalanced by sufficient constriction of the efferent arteriole, thereby maintaining glomerular filtration rate constant although renal blood flow is decreased. The counterpart of this effect may occur during β adrenergic stimulation with isoproterenol in which preferential vasodilatation of the afferent arteriole, leads to a rise in renal blood flow while glomerular filtration rate remains constant⁷⁸.

In contrast to these results, some workers, using radioactive microsphere, have failed to demonstrate any effect on renal blood flow distribution of either renal nerve stimulation¹⁰⁸ or infusion of noradrenaline¹⁰⁹. Also, these investigators measured renal blood flow during haemorrhage and maintained that it is the hypotension produced by the haemorrhage that produces a decreased cortical blood flow and not a concomitant increase in circulating catecholamines. This explanation was based on the demonstration that the decrease of the fractional to the outer cortex could be produced by a decreased renal perfusion pressure, but not by noradrenaline or angiotensin infusion. Schrier¹⁰⁷ mentions that the baseline level of vascular tone may be important in determining renal vascular response to various stimuli. For example, in the presence of a high control level of renal vascular resistance, adrenergic stimulation may be less effective, but any vasodilatory response be accentuated. This latter explanation could be the reason for the conflicting results reported using radioactive microspheres.

Further evidence that adrenergic stimulation in the kidney causes a redistribution of the blood flow away from the cortex, is seen in patients with heart failure. In these there is a high concentration of endogenous catecholamines¹¹⁰ and measurements of intrarenal blood flow distribution showed a shift of blood away from the cortex¹¹¹. This increase in renal resistance was reversed with α adrenergic blocking agents in patients¹¹² and in experimental animals¹¹³ with heart failure. Therefore in considering all the evidence it would seem that α adrenergic stimulation by noradrenaline or by renal nerve activity causes a decreased renal blood flow with alterations in distributional flow. However, it must be recalled that stimulation of the sympathetic system also releases renin^{114,115}. This in turn forms angiotensin II which is a potent renal vasoconstrictor. This system will now be considered.

The Renin-Angiotensin System and Renal Haemodynamics

The Renin-angiotensin System and Renal Haemodynamics

There is considerable evidence that increased activity of the adrenergic nervous system provides a potent stimulus to increased renin secretion. Several manoeuvres are known to stimulate both sympathetic neural pathways and renin release including haemorrhage^{116,117}, carotid occlusion¹¹⁸, direct stimulation of the renal nerves^{114,115} infusions of catecholamines or ganglion stimulating agents¹¹⁷, stimulation of the mid brain¹¹⁹ and hypoglycaemic agents¹²⁰. It has been proposed that either circulating catecholamines or amines from the adrenal medulla or noradrenaline liberated at renal adrenergic nerve terminals or a combination thereof, may mediate these responses¹⁰⁷.

There are several suggested mechanisms whereby adrenergic stimulation may cause an increase in renal excretion of renin. It has been suggested that the renal vasoconstriction associated with adrenergic stimulation, could increase renin release by decreasing sodium delivery to the macula densa area¹²¹. It has also been suggested that alterations in pressure affect an intrarenal receptor site and thereby influence renin secretion¹²². Recent evidence would suggest that sympathetic stimulation increases renin release by a direct affect on the juxta-glomerular cells. The juxta-glomerular cells are richly innervated by post-ganglionic nerve fibres¹²³, and it appears that the mechanism for renin release is via a β receptor, since propranolol is able to prevent renin release by the stimuli mentioned above^{124,125}. In contrast to this Winor et al¹²⁵ have demonstrated that both propranolol and the α antagonist phentolamine prevents the ability of noradrenaline to increase renin secretion. Other workers however have shown that α blockade increases renin secretion¹¹⁵, so that although the exact mechanism of renin release remains as yet unidentified, it would seem that β adrenergic stimulation is certainly involved¹²⁶.

Within the kidney the renin-angiotensin system is itself involved in the distribution of the regional blood flow. Many workers have shown that angiotensin infused into the renal artery produces a dose dependent reduction in flow^{102,103,127}. Brech et al¹²⁸ have shown that during reduction of perfusion pressure below the autoregulatory range a redistribution of intrarenal perfusion could be observed from the outer cortical to the inner cortical-juxtamedullary compartments, which was more pronounced in renin depleted than in normal animals. Also in normal dogs reduction of perfusion pressure resulted in an increase in outer cortical perfusion rate as long as autoregulation was maintained, whereas in renin depleted animals, outer cortical perfusion rates decreased in proportion to pressure reduction. These workers conclude that the regulation of blood flow within the kidney involves two mechanisms; one located in the outer cortical area under the influence of the renin angiotensin system, and the other located in the juxtamedullary compartment and independent of the renin angiotensin system. This contention is supported by the finding that the greatest concentration of renin is found in the outer cortical area¹²⁹. In addition Hornych et al¹³⁰ have infused angiotensin II into the kidney and shown that it produced vasoconstriction of the glomeruli of the outer cortical region but that it had no effect on the juxtamedullary group of nephrons. In addition these changes were not prevented by an adrenergic blocking agent.

From the above description it can be seen that the sympathetic nervous system and the renin angiotensin system are intimately linked. Similar stimuli result in increased sympathetic activity and increased renin release. Furthermore sympathetic activity itself can release renin and within the kidney both angiotensin and noradrenaline have similar effects. Therefore when considering any changes of renal blood flow distribution one must take both systems into account.

(4) Adrenaline.

Adrenaline may also contribute to the vasoconstriction produced by sympathetic stimulation, particularly since it is also released by the adrenal medulla in response to such stimulation⁸². It has been found that intravenous injections of adrenaline decreased renal blood flow^{131,132}. This has been interpreted as being due to an efferent vasoconstriction¹³³. Occasionally small doses of adrenaline have been reported to induce renovasodilatation¹³⁴. Since adrenaline is both an alpha and beta stimulator this is not unexpected and probably the effects of adrenaline on renal perfusion is dose dependent; small doses producing vasodilatation while larger doses result in vasoconstriction.

(5) Prostaglandins.

These are 20 carbon-linid soluble unsaturated hydroxy-acids¹³⁵. Six primary prostaglandins are known to occur naturally, PGE₁, PGE₂, PGE₃, PGE₄, PGE₅ and PGE₆¹³⁵. The main renal prostaglandins are PGE₁ and PGE₂¹³⁵. Infusions of PGE₁ into the renal artery of the dog results in a marked vasodilatory response in which renovascular resistance is approximately halved¹³⁵. PGE₂ also causes a redistribution of blood away from the medulla towards the outer cortex¹³⁵ and it has been suggested by McGiff et al¹³⁶ that PGL compounds oppose the renovasoconstrictor actions of angiotensin and noradrenaline. This is supported by the work of Jickovitz¹³⁷ and McGiff. These workers showed that when synthesis of prostaglandins is prevented by the administration of indomethacin, the infusions of angiotensin result in vasoconstriction in both the medulla and the cortex. This contention that renal prostaglandins act primarily as local vasodilator hormones in the deep cortex and medulla is supported by the finding that these hormones are formed primarily in the medulla and degraded in the cortex¹³⁸. Thus when renal synthesis is increased a large fraction of blood perfuses the inner cortex and medulla; when renal synthesis is decreased, as after indomethacin

administration the fraction of inner cortical blood flow diminishes. Thus it can be seen that the prostaglandins of the E series oppose the cortical constriction caused by noradrenaline and angiotensin. In contrast to this it would appear that infusion of $\text{PGF}_{2\alpha}$ causes a marked increase in renal vascular resistance¹³⁵.

(6) Role of electrolytes in the control of renal blood flow.

It has been mentioned above that one of the mechanisms proposed for the control of renin secretion is the amount of sodium which reaches the macula densa of the juxta-glomerular apparatus. Although this theory is not universally accepted, there is good evidence that sodium concentration does affect renal blood flow due to its effect on renin secretion.

Horster and Thurau¹³⁹ found that a chronic increase in dietary sodium intake, gave rise to a redistribution of single nephron glomerular filtration rates towards the superficial nephrons. This is supported by work on intrarenal distribution of blood in man on high and low sodium diets. Here it has been shown that sodium deprivation increases renin activity and the resultant formation of angiotensin causes a redistribution of blood towards the medullary nephrons, while sodium increase has the opposite effect^{127,140,141}. In contrast to this, other workers^{142,143} have found that sodium balance has no effect on intrarenal distribution of blood. It would appear however that sodium balance certainly affects renin secretion^{127,142,143} and that it is most likely that this produces a secondary change in renal haemodynamics via the formation of angiotensin.

Potassium may also affect renin release and thereby produce secondary effects on the renal vasculature. Vander¹⁴⁴ has shown that direct intra-arterial infusions of potassium chloride in salt-depleted dogs, produced inhibition of renin release and also reduces sodium reabsorption

Para 38.

by the kidney. This has been confirmed in man^{145,146,147,148}. It has been suggested that this inhibitory effect of potassium on renin secretion is secondary to depression of sodium absorption from the proximal tubule¹⁴⁵. However a recent micro-puncture study revealed that infusions of potassium produces no increase in the delivery of sodium from the proximal tubule¹⁴⁹. Therefore the exact mechanism of renin suppression by potassium is unknown. The secondary alterations in renal haemodynamics produced by hyperkalaemia are due to the suppression of renin release and not due to primary effects of potassium, since infusion of potassium into the kidney produces no change in renal blood flow¹⁴⁹.

Summary.

The renal circulation and its innervation was described in detail. The regulation of renal blood flow was also reviewed and the role of the renal nerves and hormonal influences such as the catecholamines and the prostaglandins was described. The renin angiotensin system and its role in the regulation of renal blood flow was reviewed and in addition the influence of electrolyte alterations on renal haemodynamics was described.

METHODS OF MEASUREMENT OF RENAL BLOOD FLOW

There are many techniques of total renal blood flow. The early methods made use of radio opaque contrast material, or mercury renograms, but these are only an index of total renal function and not a specific measure of renal blood flow.

Estimation of renal blood flow by measuring the clearance of para-amino hippurate (PAH) has been used and is based on the equation $\frac{Cu}{\Delta t} = F(C_a - C_v)$

where Cu = amount of indicator substance excreted in the urine

F = rate of blood flow in mls - min

C_a & C_v = concentration of indicator substance in renal arterial and venous blood

t = change in time over which measurements were made.

This approach requires a steady state, negligible loss of indicator in renal lymph, constant renal arterial and venous blood flow rates and excretion of sufficient quantities of indicator to ensure accurate measurement. Also this method has limitations in that urine flow needs to be at a high rate and both venous and arterial renal blood need to be sampled for true accuracy. Also if alterations in blood flow distribution occur, then PAH clearance will give false readings. For example if blood is shunted away from the cortex a faulty low measurement will be obtained since fewer nephrons are being perfused.

In experimental animals the electromagnetic flow meter can be used and provides an accurate measure of total renal flow provided no kinking of the artery occurs. Also simpler methods such as measurement of renal venous drainage per unit time, and dye dilution techniques using indocyanine may be used.²⁴ Since this study was not concerned with alterations in total renal blood flow, but with intrarenal distribution of blood, the measurement of total renal blood flow will not be considered further.

MEASUREMENT OF INTRARENAL BLOOD FLOW DISTRIBUTION

1) PAH

Changes in PAH extraction have been used to measure intrarenal blood flow distribution by several workers^{150,151}. This is based on the assumption that PAH is secreted by tubular cells of the cortical nephrons and not by the cells of the juxtamedullary nephrons. A fall in renal extraction of PAH is thus interpreted as a shift away from the cortical nephrons. However Thurnau¹⁵² has shown that the concentration of dye in the vasa recta is much higher than in arterial blood, thus implying that renal cortical flow is underestimated and renal medullary flow over-estimated by this technique. This is also supported by the finding by several workers that PAH may be secreted by the juxtamedullary nephrons^{152,153}. In addition PAH may be retained in the kidney at low urine flow rates and the inaccuracy of this method are great when the extraction is low¹⁵⁴. Therefore data obtained from this method are suspect and Auckland¹⁵⁵ feels that this method is not valid under any circumstances.

Renography

This method employs the measurement of the transit time of a radioactive substance in different nephrons, as a functional basis for measuring intrarenal distribution of blood. To perform a renogram an indicator radio-isotope is injected intravenously and the radioactivity over one or both kidneys is measured by an external radiation detector. The substance first used was diodrast¹³¹ but now hippuran¹³¹ or ¹²⁵I is more commonly used^{156,157,158} because less biliary excretion of hippuran occurs than with diodrast¹⁵⁷. Radioactivity-time curves recorded are usually interpreted as normal, or abnormal depending on the rate at which radioactivity accumulates in each kidney, the maximal radioactivity measured over each kidney and the rate at which radioactivity disappears from each kidney. The rate at which radioactivity accumulates in the kidney depends on
1) the dispersion of the indicator in the arterial blood
2) the proportion of total cardiac output flowing through

each kidney.

- 3) the proportion of radioactive substance that is cleared by the kidney and remains in the organ
- 4) the rate of disappearance of the indicator from the arterial blood.

The maximal amount of radioactivity measured over each kidney depends on:

- 1) the amount of indicator injected
- 2) the renal clearance of the indicator by glomerular filtration and proximal tubular secretion
- 3) the transit time required for the radioactive substance to leave the kidney after it enters the tubule
- 4) the efficiency of the measuring apparatus.

The slope of the disappearance is determined by

- 1) the rate at which radioactivity indicator leaves arterial blood by secretion from both kidneys
- 2) the mean transit time for tubular fluids to move through the kidney which in turn depends on glomerular filtration rate, tubular volumes, reabsorption of tubular fluid.

Thus as a screening technique for detection for renal disease renography is valuable, but it does not provide quantitative measurement of renal blood flow or renal blood flow distribution, mainly because the interpretation is complicated by the passage of the marker in both glomerular filtrate and also the blood.

3) Local Methods

In this a tracer is injected into the renal artery. Fine detectors are inserted into different regions of the kidney to follow the clearance of the indicator. Auckland et al⁹¹ saturate the kidney with hydrogen gas and then follow its clearance with the volarygraphic needles that measure hydrogen concentration. Another method is to measure arrival times of red blood cells labelled with ³²P or to use thermistors to study heat dissipation.

These methods are all damaging to the kidney and measure flow in precise anatomical locations which may be composed of functionally different parts. Also any indicator used to measure distribution of blood flow from concentration-time curves, must be detectable with great accuracy and must have negligible recirculation during sampling time. Although small radiation detectors or fibre-optic catheters may provide indicator dilution curves with a suitable sensitivity, the recirculation of non diffusible indicator is difficult to quantitate by any technique⁷⁸.

Radioactive microspheres

The distribution of small particles that cannot pass through the capillaries can also be used to measure renal blood flow. If the particles are completely mixed in systemic arterial blood, the fraction of blood flowing to an organ is the same as the fraction of all the particles injected or recovered in that organ. Thus if the total number of particles are known, the blood flow to any organ or region can be determined according to the equation:

$$F_i = FT \frac{Q_i}{Q_t}$$

where F_i = rate of blood flow to the organ or region
in ml/min

FT = cardiac output or total organ flow in ml/min

Q_i = number of particles recoverable in the tissue

Q_t = number of particles injected

Similarly if blood flow to an organ is known then regional flow can be determined by the same equation. Microspheres labelled with ⁵⁸yttrbium or ⁸⁵strontium are amongst the markers used^{158,159,160}.

This method is valuable and could be anatomically precise but its main limitation is the necessity to sacrifice an organ or the entire animal for one measurement. Also it has been found that these microspheres do not pass further than the afferent arterioles and glomeruli when using microspheres 35μ in diameter, and since they do not connect

in the peri-tubular capillaries this technique cannot be used for measurement of blood flow in the medullary region.

The ^{133}Xe Xenon Technique

The technique for measurement of intrarenal blood flow distribution that has received widest application is that involving rapid intra-arterial injections of ^{85}Kr or ^{133}Xe and measurement of the rate which radioactivity appears from the kidney. By this technique measurements can be made repeatedly in the unanaesthetised and anaesthetised subject and changes in renal intrarenal distribution can be readily detected⁷⁸. Since ^{85}Kr or ^{133}Xe are highly lipid-soluble they diffuse freely across the capillary walls and their transit through the renal vascular system is thereby prolonged beyond the transit time for non diffusible indicators⁷⁸. Xenon was used in this study but the description applies equally to krypton.

The principle governing the exchange of inert gases in the tissues were outlined by Kety in 1951¹⁶¹. He stated that, at equilibrium, an inert gas will distribute itself in the blood and tissues according to its respective solubility in each. The assumption that the perfusibility of xenon is sufficiently high to maintain equilibrium between a tissue and the venous blood leaving that tissue, under all physiological condition, allows for the following equations to be formulated for the uptake and washout of an inert gas in a tissue:

$$V_i = \frac{dC_i}{dt} = F_i (C_a - C_v) = F_i \frac{(C_a - C_i)}{\lambda_i}$$

C_a = concentration of indicator in arterial blood

C_v = concentration of indicator in venous blood

C_i = concentration of the indicator in the tissue

t = time

V_i = weight of the tissues

F_i = blood flow

λ_i = partition coefficient for the indicator between venous blood and the tissues.

Page 44.

It can be assumed that following a rapid injection of the inert gas to the tissues in the desaturation period that $C_a \approx 0$ because of the very effective elimination of the gas via the lungs.

Therefore with an initial condition

$$C_i = C_{i0} \text{ at } t=0$$

$$C_i = C_{i0} e^{-k_1 t}$$

where e = exponential e

$$k = \frac{F_i}{V_i \lambda_i}$$

or for the total amount of gas in the tissue

$$Q_i = Q_{i0} e^{-k_1 t}$$

This equation is only valid for a tissue with homogenous perfusion. For an organ with different perfusion zones the total amount of indicator Q is given by:

$$Q = \sum_{i=1}^n Q_{i0} e^{-k_1 t}$$

A similar plot of Q_i/Q_{i0} against time then gives:

$$K_i = \frac{0.693}{T_{1/2}}$$

where $T_{1/2}$ = time in minutes for Q_i/Q_{i0} to be reduced to half its numerical value.

Then from k_1 the flow rate is obtained if λ_i is known from

$$\frac{F_i}{V_i} = k_1 \times \lambda_i$$

The blood flow rate in each zone can be calculated according to the above equation.

Blood flow per unit mass of renal tissue is then determined from the slope of each equation using the following formula:

$$F_i \text{ (ml/100g)} = \frac{k_i \times \lambda_i \times 100 \times 60}{E}$$

Where E is the specific gravity which can be assumed to be equal to 1

$$P_i (\text{ml./100g./min}) = \frac{0.693}{T_{1/2} (i)} \times \lambda_i \times 100 \times 60$$

In animal experiments the partition coefficient for xenon between kidney tissue and blood was found by Ladefoged to be 0.7¹⁶².

Because haemoglobin has an affinity for xenon this value for λ has in turn to be corrected using the formula:

$$\lambda = 0.7 \times \frac{1.69}{1.05 + 0.013 \text{ PCV}} \left\{ \text{From Ladefoged}^{162} \right\}$$

where PCV = haematocrit at time of measurement.

The percentage of total renal blood flow to each compartment is calculated from the zero time intercept of each component after a semi-logarithmic plot¹⁶¹. The speed of injection of ¹³³Xe does not significantly affect the blood flow/unit mass of tissue but does increase the percentage distribution to the second and third components of slow injection is used¹⁶². Therefore in all experiments a rapid injection of xenon was utilized.

It is also possible to measure the mean renal blood flow by using the half time of the initial slope of the washout curve and Ladefoged¹⁶² has obtained good correlation between a mean renal blood flow obtained using this method and simultaneous flow meter measurements. However, as shown by Ingvar and Lassen¹⁶³, the average flow of parallel compartments can only be calculated from the initial slope of the disappearance curve, measured from a whole organ, when initial concentration of indicator in the various compartments exist in identical concentrations. With differing initial concentrations a different summation curve may be obtained and the initial slope may not provide accurate measurements of mean blood flow through the whole organ. Therefore mean renal blood flow has not been calculated in this study. In recent years the formulae and applying them to the measurement of organ perfusion certain basic assumptions must be made which require consideration:

I. INSTANTANEOUS EQUILIBRIUM BETWEEN BLOOD AND TISSUE

If a calculation of tissue flow is to be valid, the gas used must be inert and freely diffusible, allowing instantaneous homogenous diffusion to occur between blood tissue in a single passage of blood through the organ. The gas will then maintain equilibrium between the tissues and venous blood leaving the tissue under all physiological conditions. The good agreement between renal blood flow measured using a radioactive inert gas technique and direct flow meter measurements supports the view that this assumption may be made¹⁶².

As mentioned there are two inert gases with the necessary diffusibility which may be used. These are ^{133}Xe and ^{85}Kr . Both may be used for measuring renal and intrarenal blood flow. ^{133}Xe is used in this study. This is because xenon is predominantly a gamma emitter¹⁶² which is more suitable for external counting than ^{85}Kr , which is mainly a beta emitter⁷⁷. The partition coefficient of xenon with respect to the kidney is 65% that of krypton, resulting in a faster "washout" of xenon from the kidney and thereby a curve which is easier to analyse²⁴. Finally xenon has a narrow energy spectrum which allows the use of a pulse height discriminator with a narrow window, thus rendering the background activity insignificant and increasing the accuracy of counting¹⁶².

II. PRESENCE OF A NUMBER OF PARALLEL ZONES EACH WITH HOMOGENEOUS PERFUSION

The equations used in the calculation of the mean tissue blood flow are based on the assumption that differently perfused zones are arranged in parallel. As described the main blood supply of the medulla is from the vasa recta which arise from the juxtamedullary zone of the cortex, thus providing a non parallel arrangement of blood supply in this area. Also while it is reasonable to assume that homogenous diffusion of blood occurs in the cortex, this

is not the case in the remainder of the kidney. The measurements of Deetjen et al¹⁶⁴ and others indicate that blood flow decreases progressively from higher values in the outer medulla to lower values in the papilla. The subdivision therefore of the medulla into distinct perfusion zones is therefore rather arbitrary. And as will be seen later when discussing the technique used in the experiments medullary flow was not considered accurate enough for comparative measurement.

III. RECIRCULATION

For Koty's principles to be valid the concentration of the gas in arterial blood must be negligible except immediately after injection. It has been shown by Chidsey¹⁶⁵ that virtually all the gas is expired during the single passage through the lungs and the little that remains is diluted in the remaining circulation. The kidney however receives a quarter of the cardiac output and therefore small amounts of gas remaining after passing in the lungs may influence the shape of the washout curve.

Ledebore¹⁶² has shown that when intra-arterial injections are used the recirculation of xenon is relatively unimportant in determining the outer cortical flow, but that it may be responsible for a small error in the juxtamedullary measurements and a considerable error in measurements of the inner medullary flow. The measurement of medullary flow in this study will be commented on later.

REMOVAL OF THE GAS BY URINE OR LYMPH

There should be negligible loss of the gas by urine or lymph. While this is almost certainly the case in the renal cortex and outer medulla due to their high rate of blood flow, in the inner medulla where blood flow is slower there may be some lymphatic drainage. It has been shown by Nashat⁸⁶ that only 4% of ¹³³Xe delivered to the inner medulla appeared in the urine. In addition the weight of blood flow through this area of the kidney is so small in comparison with the

Page 48.

rest of the kidney, that urinary loss of Xe is not significant.

Therefore from the above discussion it can be seen that the effect of recirculation and lymphatic drainage on the determination of the clearance of Xe from the inner medulla, as well as the arrangement of the blood supply to the medulla probably makes the measurement of the blood flow in the inner medulla inaccurate. Therefore, although this was measured in this study, alterations of blood flow in this area need to be treated with reserve. Other criticisms of the ^{133}Xe method will be discussed after the experimental results have been presented.

RENAL BLOOD FLOW DISTRIBUTION IN OBSTRUCTIVE JAUNDICE

It has been mentioned in the introduction to this thesis that renal failure is a common complication of decompensated cirrhosis of the liver^{2,3,4}. It has also been shown that changes in intrarenal blood flow distribution occur in cirrhosis before the onset of overt renal failure^{7,10,12,38}. These changes are a shift in blood flow distribution from the outer cortex to the juxtamedullary region.

In obstructive jaundice there is also a high incidence of renal failure particularly in the post-operative period^{15,48,49,50}. It has been suggested that the cause of this is an increased sensitivity of the kidney to periods of hypotension and ischemia^{15,17,52}. No studies have been carried out to determine whether, in common with cirrhosis, there are changes in intra-renal blood flow distribution in obstructive jaundice prior to the onset of overt renal failure. In this section therefore the changes in intra-renal blood flow in experimentally induced obstructive jaundice in the baboon monkey with the ¹³³Xe technique are presented. In addition, since periods of hypotension are associated with increased levels of circulating catecholamines¹⁶⁵, the effects of noradrenaline on renal blood flow distribution in obstructive jaundice was also studied and attempts were made to reverse these.

It was also mentioned in the introduction that in the functional renal failure of cirrhosis, there is very little change in renal histology²⁷. Braun¹⁸ has suggested that conjugated bilirubin is the factor responsible for the damage to the renal cells and in the introduction it was also mentioned that bilirubin is in fact toxic to cells. Therefore the histology of the kidney following a period of obstructive jaundice will also be presented in this

section.

Methods and Materials

27 Cane Island baboons (*Papio ursinus*) of both sexes and weighing between 10 and 20 kilograms were used in the study. They were anaesthetised with a pre-medication dose of phenylcycloidine (Sernylan - Parke Davis) followed by an anaesthetising dose of pentobarbitone (Nembutal - Abbott). The animals were then intubated and ventilated using a Harvard variable phase respirator. The left or right renal artery was exposed by a para-costal incision into the retroperitoneal space. No renal capsular stripping was done and the kidney was handled as little as possible. A twenty-five gauge, 1 cm long needle was inserted into the lumen of the renal artery in the direction of flow. The needle was attached to polythene tubing at the other end of which was fitted a three way tap to allow a constant infusion of saline, or test solution, by a Harvard infusion pump. Bolus injections of ^{133}Xe (250 μCi in 50 mls) into the renal artery were also made via this system.

Phasic and mean arterial blood pressure was monitored through a femoral artery catheter by a Statham pressure transducer connected to a Beckman 411 dynograph. The electrocardiogram and heart rate were continually recorded and in each experiment periodic measurements of PaCO_2 and PaO_2 using an Astron (Radiometer) were also done. Packed cell volume (PCV) was determined every hour and this value was used to control the rate of intravenous saline infusion to replace fluid lost during the experiments. A diagrammatic representation of the experimental apparatus used, can be seen in Figure 3.

Three measurements of intrarenal blood flow were performed on each animal and then the common bile duct was ligated via an abdominal approach. All wounds were then sutured and the animals given 2 million units of crystalline penicillin. In 5 animals repeat measurements of flow were

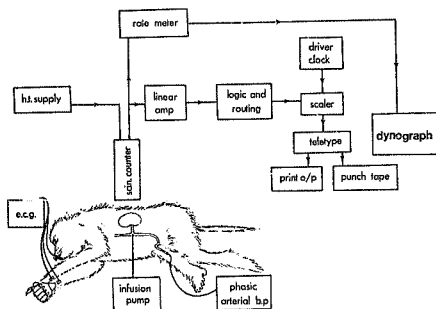


Figure 3. SCHEMATIC REPRESENTATION OF THE EXPERIMENTAL APPARATUS.

made one week after surgery and in 13 animals two weeks after ligating the common bile duct.

In both groups noradrenaline in normal saline was infused directly into the renal artery in doses ranging from 0.005 to 0.5 μ g/min. The dose response curves were compared with those previously reported in normal anaesthetised baboons¹⁶⁷. It has also been shown that an alpha adrenergic blocking agent can attenuate noradrenaline induced vaso-constriction¹⁶⁷. In 5 experiments the alpha adreno-receptor blocker phenoxybenzamine (PBZ) was infused (20 μ g/min for 30 mins.) simultaneously with a constrictor dose of noradrenaline (0.5 μ g/min for 30 mins) into the renal artery. The effect of the beta adreno-receptor blocker propranolol (10 μ g/min for 30 mins) was assessed in the same way. In a further four animals phenoxybenzamine was infused alone i.e. without a constrictor dose of noradrenaline.

Five animals were subjected to sham operations in which the common bile duct was manipulated but not ligated, but all other procedures were identical. In these animals repeat intra-renal blood flow measurements were done two weeks after the control flow.

In each experiment blood was taken for the measurement of bilirubin (direct and total) transaminases, alkaline phosphatase, urea, sodium, potassium, and plasma renin. Blood samples were collected one hour after the anaesthetic and before any surgery in each case. All statistical analyses were done on absolute values by a paired Students "t" test or by a Wilcoxin Rank test for the percentage changes, since these are not normally distributed.

Application of the Renon technique

For the measurement of tissue blood flow by the radioactive washout method the assumption is made that a highly diffusible substance will equilibrate between blood and tissue in a single passage of the indicator through the capillary bed¹⁶¹. ¹³³Xe injected into the renal artery

Page 53.

comes into diffusion equilibrium with the renal tissues as it passes through the capillary. The activity in the tissues then declines because subsequent blood flowing through the kidney contains very little ^{133}Xe . The gas then diffuses back from the renal parenchyma and is removed by the venous drainage. The speed at which the activity declines is proportional to the blood flow¹⁶¹. The washout of the gas is then a measure of the nutrient blood flow and can be calculated from a recording of the disappearance rate of the isotope measured with an external scintillation counter. In these experiments the washout of the isotope from the kidney was recorded by means of a 5 cm diameter collimated scintillation detector placed over the kidney. The probe was connected to a rate meter and pulse height analyzer assembly and the output recorded on a teletype printout and on punch tape for subsequent computer and manual analysis. The discriminator window was set at E 4 and $\Delta E = 5$. Peak radio-activity counts recorded were at least four times the background count and a time constant of one second used throughout.

The xenon washout curve recorded from the kidney is multi-exponential and can be resolved into a series of components, each associated with different blood flow rates through localized regions of the kidney¹⁵². The decrease in radio-activity as a function of time is plotted on a semi-log plot¹⁶². A straight line is fitted to the terminal linear part of the curve and extrapolated to zero time. This represents the slow component of the tracer. The counts per second at each point on the extrapolated line are then subtracted graphically from the corresponding point on the original curve, producing a new curve. A straight line drawn through the terminal linear part of the curve and extrapolated to zero time represents the next component. This procedure is repeated on the new curve until the resulting component is a straight line¹⁶³. The quantity of inert gas received by each component is propor-

tional to the fraction of the total renal blood flow constituting that component¹⁷⁰. Random error may be introduced in the translocation of the washout curve from the recorder writer to the semi-log plot, and in the visual estimation of the slope and hence the half time of the curve¹⁷¹. Therefore more accurate curve readings can be obtained using an analog or digital computer. In these experiments a digital computer (IBM 370) was used and the curve analysed by the method of least squares. Hand analysis was only used when abnormal curves such as fusion curves (see below) were obtained.

It has also been suggested by Zierler¹⁷² that Kety's equations which have been derived above, do not give an accurate representation of blood flow through the tissues since these equations assume that blood is flowing through separate compartments. He believes that by analysing the area under the curve one is able to obtain a more accurate estimation of the blood flow through a tissue bed. However using this method, it is only possible to obtain total blood flow rates through the particular tissue being investigated. As this study was concerned with blood flow distribution changes within the tissue it was felt that Zierler's method did not apply and exponential analysis of the curve using the Kety's equations was used.

In baboon kidney the multi-exponential curve for xenon can be resolved into three components. Thorburn¹⁷³ using ⁸⁵Kr in the dog kidney showed that the early content of radio-activity was located in the cortex. This had been washed out of the kidney after two minutes and therefore component I, the fastest component, represents flow through the outer cortex. Thorburn suggests that the second component C II represents flow through the inner cortex and outer medullary area and C III the slowest clearance, flow through the inner medulla. A fourth component has also been described in dog kidney which probably represents flow through the hilar and peri-renal fat, renal capsule, ureter and parts of the adrenal¹⁷¹. Only three components could be recognised in the baboon kidney in our experiment.

and an example of a typical washout curve resolved into these components is shown in Figure 4.

The anatomical validity of this compartmental analysis was confirmed in dogs by Rosen¹⁷⁴ using auto-radiography and in man by Ter Pofossion et al¹⁷⁵ with rapid cinecinematography. Further confirmation of this compartmental analysis is provided by Logan using radio-active microspheres¹⁶⁰, Also the clearance values obtained using the washout technique compare well with the figures for outer medullary flow by Deetjen¹⁶⁴ and renal arteriography has demonstrated a close relationship between the second component and juxtamedullary and outer medullary vascular density¹⁷⁵.

For the reasons mentioned above namely the effect of re-circulation and lymphatic drainage on the tail of the washout curve, the third component is of questionable value and although measured in these experiments no significance was attached to alterations of this component. The effect that inaccuracies of this in measurements of this component has on the remaining analysis will be discussed further below.

The washout tracings were measured for 17 minutes and Ladefor¹⁶² has shown that the amount of indicator in the kidney after 20 minutes is only 1-2% of the initial value, and that omission of the tail of the curve caused insignificant changes in the parameter of the first two components. Also it has been shown by Bomzon¹⁶⁷ that continuing the curve beyond this time did not significantly improve the resolution of the compartmental analyses.

For each component, flow was determined by the formula

$$F = \frac{\lambda_{10r} \cdot 10^3}{T_{1/2}} \times 100 \times 60$$

where F = Flow ml/min/100g tissue

λ = the tissue partition coefficient and equal to 0.7 (16.7)

$T_{1/2}$ = The half decay time.

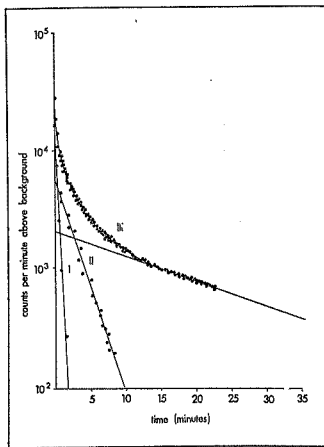


Figure 4. WASHOUT CURVES OF ^{133}Xe FROM THE RAT KIDNEY

C I = Outer cortical component.

C II = Inner cortical - outer medullary component.

C III = Inner medullary component.

Page 57.

It has been mentioned that because xenon has a high affinity for hemoglobin, therefore the partition coefficient should be corrected. This was done using the formula

$$\lambda = \frac{1.69}{1.05 + 0.013 \text{ PCV}} \quad (\text{Ladoford}^{162})$$

where P.C.V. = packed cell volume.

In addition the relative distribution of the blood within the kidney was calculated by determining the intercept of each component with the ordinate (t_0). This has been discussed above. The limitations and inaccuracies of this technique will be discussed when considering the results obtained using this method.

Histological examination

Three kidneys were examined. These were removed from the animal after two weeks of jaundice. In each kidney, 5 wedge-shaped areas of the organ, containing areas from both the medulla and cortex, were cut. These were then sectioned serially from medulla to cortex and stained with haematoxylin and eosin. Two normal kidneys from baboons in whom sham operations were done were also prepared in the same way and examined for comparison.

Results

Intrarenal blood flow and percentage volume of blood distributed to each component after one week and two weeks of jaundice are shown in Figures 5 and 6 respectively. Each result is expressed as a percentage of the mean control flow obtained before ligation of the common bile duct. These percentages are plotted on a log scale so that a doubling or a halving of the flow are shown to be equidistant from the control measurement. As mentioned G III values are not presented because of their questionable value. A computer derived curve before and after two weeks of jaundice is shown in Figure 7.

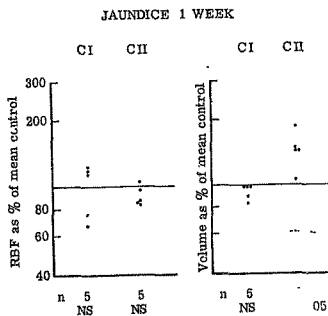


Figure 5. Changes in blood flow and percentage distribution after one week of jaundice are shown as alterations from the mean control values. There is a significant increase in Component II distribution of blood. There is no significant alteration of blood flow rates.

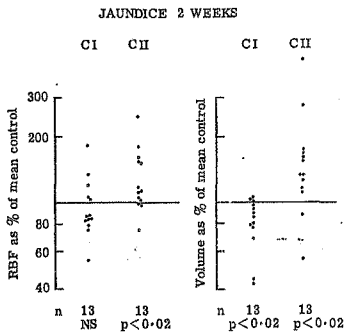


Figure 6. Changes in blood flow rates and percentage distribution after 2 weeks of jaundice are shown as alterations from the mean control values. There is a significant increase in Component II flow rates and percentage distribution. There is a significant decrease in Component I distribution of blood volume.

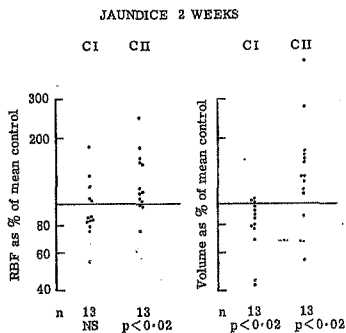


Figure 6. Changes in blood flow rates and percentage distribution after 2 weeks of jaundice are shown as alterations from the mean control values. There is a significant increase in Component II flow rates and percentage distribution. There is a significant decrease in Component I distribution of blood volume.

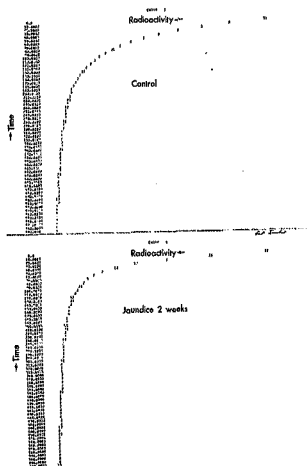


Figure 7. Shows computer derived analysis of a ^{133}Xe washout curve in the control period and after two weeks of jaundice.

TABLE 3

Jaundice 1 week

Table showing the mean values of Component I & II flow rates and percentage of flow in each animal after one week of jaundice. The changes (Δ) of the values are also shown.

No.	Control					Jaundiced									
	Flow ml/min/100gr					Volume %					Flow ml/min/100gr				
	C I	C II	C I	C II	C I	C I	C II	C I	C II	C I	C I Δ	C I	C II	C I Δ	C II Δ
1.	303.1	101.7	75.0	22.0	350.4	+ 47.3	89.3	- 12.4	72.6	- 2.4	23.3	+ 1.3			
2.	416.5	84.5	83.9	8.9	501.8	+ 85.3	88.9	+ 4.4	69.2	- 14.7	16.4	+ 7.5			
3.	526.2	71.1	88.2	10.3	353.6	- 172.6	62.5	- 8.6	78.7	- 9.5	15.4	+ 5.1			
4.	266.5	90.6	76.1	11.7	199.0	- 67.5	78.4	- 12.2	75.2	- 0.9	16.7	+ 5.0			
5.	311.3	67.8	90.6	4.7	354.1	+ 42.8	66.1	- 1.7	88.7	- 1.9	6.7	+ 2.0			

It can be seen that there is no significant change in blood flow rates in the animals who had been jaundiced for one week. However, the percentage distribution of blood to each vascular bed is altered. There is a significant decrease ($p < 0.05$) in C I distribution with concomitant increase in C II distribution ($p < 0.05$). The mean flow rates and percentage distributional changes in each experimental animal can be seen in Table 3. The mean changes in C I distribution and C II distribution were -5.8 ± 2.6 and 4.1 ± 1.1 percent respectively. (Table 4)

After two weeks of jaundice there was a mean decrease in C I flow rate of 28.5 ± 32.9 mls/min/100g although this change was not significant. (Table 4). There was however significant ($p < 0.1$) increase in C II flow rate. This was 21.4 ± 7.5 ml/min/100g (Table 4). The percentage of blood distributed to each component was significantly altered ($p < 0.05$). There was a decrease in C I distribution of $-12.7 \pm 4.3\%$ with an increase of C II distribution of $12.2 \pm 4.4\%$ (Table 4). The mean flow rates and percentage distribution changes in each animal are shown in Table 5.

In the sham operated animals there was no significant change in percentage distribution or flow rate (Table 6).

The results obtained when noradrenaline was infused directly into the renal artery in doses from 0.005 to 0.5 μ g/min are shown in Figure 8. These are compared with results in normal baboons using the same doses of noradrenaline obtained by Bomzon et al¹⁶⁷ which were performed in the same laboratory with the same technique and are presented with his permission. At one week after common bile duct ligation there was a significant reduction ($p < 0.05$) in outer cortical (C I) flow in response to a dose of 0.5 μ g/min. At two weeks there was a significant reduction in C I flows at doses of 0.05 and 0.5 μ g/min ($p < 0.01$ and $p < 0.001$). No changes occurred in normal animals at these doses where constriction was only obtained at doses greater than 5 μ g/min and this was accompanied by

TABLE 4

Shows the total mean changes in CI and CII flow rate and percentage distribution after one week and 2 weeks of jaundice, Standard deviation (SD), Standard error (SE) and significance are also shown.

Measurement	Jaundice 1 week					Jaundice 2 weeks				
	Mean	SD	SE	Significance		Mean	SD	SE	Significance	
C I (Flow)	-12.9	105.9	± 47.3	N.S.		-28.5	118.9	± 32.9	N.S.	
C II (Flow)	- 6.1	7.2	± 3.2	N.S.		+21.4	27.2	± 7.5	$p < 0.01$	
C I %	- 5.8	5.9	± 2.6	N.S.		-12.7	15.6	± 4.3	$p < 0.05$	
C II %	+ 4.1	2.5	± 1.1	$p < .05$		+12.2	15.9	± 4.4	$p < 0.05$	

Table 5
Jaundice 2 weeks

Table showing mean values of Component I and II flow rates and percentage distribution of flow in each animal. The changes (Δ) of these values are also shown.

Control

Jaundiced

No.	Flow mls/min/100g			Volume %			Flow mls/min/100g						Volume %					
	C I	C II		C I	C II		C I	Δ C I	C II	Δ C II		C I	Δ C I	C II	Δ C II			
1	193	49.9	71.4	23.1	350.1	+157			122.4	+72.5		31.6	-39.8	62.7	+39.6			
2	405.0	108.0	59.2	29.9	339.3	-65.7			83.0	-25		62.1	+2.9	34.8	+4.9			
3	348.0	71.1	58.4	33.0	460.8	+112.8			76.8	+5.7		62.7	+4.3	29.0	-4			
4	309.2	85.8	66.8	26.4	371.1	+61.9			138.4	+52.6		53.0	-13.8	40.7	+14.3			
5	643.7	81.7	80.7	16.8	346.2	-297.5			81.4	-0.3		73.5	-6.5	21.6	+4.8			
6	434.4	84.3	70.6	23.7	378.0	-56.4			130.1	+45.8		56.1	-14.5	38.1	+14.4			
7	333.6	79.7	70.0	23.7	355.2	+21.6			85.4	+5.7		63.6	-7.0	31.3	+7.6			
8	325.2	79.7	75.4	20.1	277.0	-48.2			79.9	+0		59.3	-16.1	35.3	+15.2			
9	312.2	53.1	81.1	12.6	322.2	+10			97.8	+44.7		34.4	-46.7	57.0	+44.4			
10	401.4	59.5	67.9	28.2	348.8	-52.6			68.3	+8.8		72.2	+4.3	15.0	-13.2			
11	326.0	72	65.8	29.5	375.5	+49.5			106.4	+39.2		55.4	-10.4	38.8	+9.3			
12	460.1	107.3	59.1	31.1	337.4	-122.7			128	+20.7		40.7	-18.4	52.2	+21.1			
13	545.3	74.1	83.5	15.4	405	-140.3			83.5	+9.1		80.5	-3.5	54.5	+1.1			

TABLE 6

Table showing the mean values of C I and C II flow rates and percentage distributions after sham operations. The changes (Δ) of these values are also shown.

Page 10

No.	Control				After Operation							
	Flow ml/min/100gr				Volume %				Flow ml/min/100gr			
	C I	C II	C I	C II	C I	C II	Δ C I	Δ C II	C I	C II	Δ C I	Δ C II
1	335.4	102.7	70.2	23.9	277.0	74.7	-58.4	-28.0	81.9	12.5	+11.7	-11.4
2	420.1	75.8	69.9	24.1	419.0	77.6	-1.1	+1.8	79.9	15.4	+10.0	-8.7
3	531.0	77.3	59.8	22.4	377.9	77.3	-154.0	0.0	50.5	30.1	-9.3	+7.7
4	432.2	113.3	61.3	30.7	463.6	33.0	+31.4	-80.0	63.9	12.7	+2.6	-18.0
5	416.8	78.2	72.4	26.8	521.0	77.4	+104.2	+0.8	73.1	27.4	+0.7	+0.6

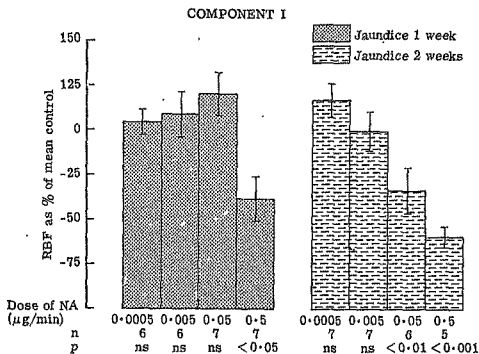


Figure 8. Changes in Component I flow rates are shown after infusion of 0.0005 - 0.5 µg/min of noradrenaline. After one week of jaundice significant constriction occurred during infusion of 0.5 µg/min of noradrenaline and after two weeks with infusion of 0.05 and 0.5 µg/min of the hormone.

alterations in blood pressure. There was no change in blood pressure or pulse rate in the jaundiced animals compared to levels found before surgery and also infusions of noradrenaline produced no change in these values. The mean changes in C I flow rates are shown in Table 7. Although C II rates are shown these were not considered for statistical analysis because the increased vascular resistance produced extremely slow flow rates and recirculation may have had an effect. In addition, these curves were often fusion curves i.e. when outer cortical flow rate has slowed to equal inner cortical - outer medullary flow, and only two components can be analysed. This means that C I represents flow through the entire cortex and C II represent medullary flow. The inaccuracies in measuring medullary flow have already been discussed. An example of such a two component fusion curve is shown in Figure 9.

The results obtained after infusion with 20 μ g of phenoxybenzamine and 10 μ g of Propranolol are shown in Table 8. PBZ attenuated the vasoconstrictor effects of 0.5 μ g/min noradrenaline in five jaundiced animals while propranolol had no effect. Furthermore an infusion of PBZ alone increased component I flow rate in a further 4 animals with jaundice (Table 9). There was no change in blood pressure or pulse rate when PBZ or propranolol infused.

No correlation could be found between the changes in perfusion or sensitivity to noradrenaline, and the plasma bilirubin, urea, alkaline phosphatase, transaminase and plasma renin activity. These values may be seen in Table 10. Plasma renin measurements were only performed in the two week group.

Histological findings

Serial sections of the three kidneys revealed no gross damage although there was considerable bile staining of

TABLE 7

Shows mean % change in Component I and II flow rates after infusion of noradrenaline.

	Mean % change in C I flow					Mean % change in C II flow				
	5×10^{-10}	5×10^{-9}	5×10^{-8}	5×10^{-7}		5×10^{-10}	5×10^{-9}	5×10^{-8}	5×10^{-7}	
Dose NA (units) grams										
Jaundice one week	+ 3.4±6.5	+8.5±10.8	+20.3±8.4	-38.9±9.7		+19.0±38.0	+18.0±24.0	+32.0±24.0	- 4±18	
Jaundice two weeks	+13.4±6.0	-0.9±11.0	-30.2±11.1	-65.5±46.0		+ 4.0± 7.0	-12.0± 5.0	- 5.0±16.8	+13±16	

TABLE 7

Shows mean % change in Component I and II flow rates after infusion of noradrenaline.

	Mean % change in C I flow					Mean % change in C II flow				
	5×10^{-10}	5×10^{-9}	5×10^{-8}	5×10^{-7}		5×10^{-10}	5×10^{-9}	5×10^{-8}	5×10^{-7}	
Dose NA (units) grams										
Jaundice one week	$+3.4 \pm 6.5$	$+8.5 \pm 10.8$	$+20.3 \pm 8.4$	-38.9 ± 9.7		$+19.0 \pm 38.0$	$+18.0 \pm 24.0$	$+32.0 \pm 24.0$	-4 ± 18	
Jaundice two weeks	$+13.4 \pm 6.0$	-0.9 ± 11.0	-30.2 ± 11.1	-65.5 ± 46.0		$+4.0 \pm 7.0$	-12.0 ± 5.0	-5.0 ± 16.8	$+13 \pm 16$	

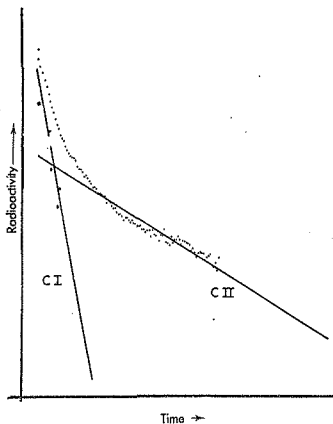


Figure 9. A fusion curve in which there are only two components is shown. C I represents flow in the whole cortex, C II represents flow in the nodules.

TABLE 8

The Effects of Sympathetic Blocking Agents - The table shows the effect on Component I flow rates of 5×10^{-6} E/min of noradrenaline alone and with $20 \mu\text{g/min}$ phenoxybenzamine (PBZ) and $10 \mu\text{g/min}$ propranolol.

Baboon No.	Flow mls/min/100g (jaundiced)	Noradrenaline 5×10^{-6} gr/min		Noradrenaline 5×10^{-6} gr/min $20 \mu\text{g/min}$ PBZ		Noradrenaline 5×10^{-6} gr/min $10 \mu\text{g/min}$ Propranolol	
1.	417 ± 32 n = 3	192.0 n = 1		437 n = 1		197 n = 1	
2.	522 ± 36 n = 3	197 n = 1		341 ± 40 n = 2		236 ± 17.5 n = 2	
3.	369 ± 10 n = 3	240 ± 14 n = 2		319 ± 32 n = 2		239 ± 5.5 n = 2	
4.	375 ± 11 n = 4	185 ± 14 n = 2		400 ± 10 n = 4		190 ± 12 n = 4	
5.	412 ± 20 n = 3	172 ± 15 n = 2		425 ± 8 n = 4		184 ± 8 n = 4	

TABLE 9

Infusion of phenoxybenzamine (PBZ) - Shows mean effect on component I flow and percentage distribution after infusion of 10 μ g/min PBZ in animals two weeks after common bile duct ligation.

Page 71.

Control				Jaundiced				PBZ			
CI ml/min/100g	CI %	CI ml/min/100g	CI %	CI ml/min/100g	CI %	CI ml/min/100g	CI %	CI ml/min/100g	CI %	CI ml/min/100g	CI %
389.8 n = 4	74.8 n = 4	322.0 n = 3	83.3 n = 3	552.5 n = 3	80.9 n = 3	230.5 n = 3	- 2.4 n = 3				
354.6 n = 4	63.3 n = 4	420.4 n = 3	82.5 n = 3	521.3 n = 3	78.7 n = 3	100.9 n = 3	- 3.8 n = 3				
232.3 n = 2	81.5 n = 2	401.0 n = 3	51.5 n = 3	606.3 n = 3	62.2 n = 3	205.0 n = 3	+10.7 n = 3				
398.6 n = 5	77.7 n = 5	240.3 n = 3	76.6 n = 3	272.7 n = 3	79.4 n = 3	32.4 n = 3	+ 2.8 n = 3				

TABLE 10

The Biochemical Data - The table shows the mean biochemical data for all experiments.

SGOT = Serum glutamic oxaloacetic transaminase.

SGPT = Serum glutamic pyruvic transaminase.

	Bilirubin Total (Conjugated) mg %	S.G.O.T. units	S.G.P.T. units	Na ⁺ meq/l	K ⁺ meq/l	Urea mg %	Alk. Phos. K.A. units	Plasma Renin activity pg/ml/hr.
Control	0.5	60.8 ± 8.3	44.11	145.5	3.7	45.45	16.58	352
	0.2		± 8.1	± 1.6	± 0.2	± 3.4	± 2.6	± 81.3
Jaundice	8.2 ± 1.5	105.2	356	138.7	4.1	39.0	322.2	-
1 Week	(5.2 ± 1.0) ±	26.2	± 67.2	± 2.1	± 0.4	± 1.4	± 85.7	-
Jaundice	10.3 ± 3.6	100.2	201.8	142.5	3.9	52.4	370.2	385
2 Weeks	(7.8 ± 2.8) ±	12.4	± 53.7	± 1.7	± 3.3	± 3.6	± 70.9	± 66.4

the sections. The glomeruli were normal and there was no apparent damage to the vessels. The proximal tubules showed some degenerative changes but the distal tubules appeared normal. Two representative sections are shown in Figures 10 and 11.

Discussion

1) Criticism of Xenon technique

The following are the main criticisms of the method:

- i) The rate of uptake of the gas and its equilibration within its volume of distribution is a limiting process. The considerable solubility of Xe in lipids, (the fat - H_2O partition coefficient for Xe is 20:1⁷⁶) ensures the rapid uptake by cellular structures within the kidney of the gas. Furthermore Lodeford¹⁶² showed that the Xe clearance curve is the same whether obtained after a single injection of ^{133}Xe , assuming constant equilibration, as that following the termination of a continuous infusion, permitting a much longer time for equilibration. Thus rate-limited uptake does not appear to be a practical problem within the conditions of these experiments.
- ii) In principle the increasing background due to recirculation of the gas diffusion from one region to another results in an unreliable terminal slope from which all subsequent determinations are made. Lodeford¹⁶² however, has shown that the parameters of the first and second components did not alter significantly when the curves were corrected for recirculation. Also in these experiments this problem is eliminated by the use of a curve fitting programme in which the "needing-off" technique was not used but rather the computer calculated each variable of the equation

$$C_i = C_{i_0} e^{-kt}$$

for each component and therefore calculations of C_i

Page 74.

A

B

C

Figure 10. SECTION OF KIDNEY AFTER STAINING WITH PAS AND HEIDENHAIN IRON-VERMILION.

- A = Glomerulus
- B = Proximal tubule
- C = Distal tubule

Page 75.



Figure 11. SECTION OF KIDNEY AFTER 2 WEEKS OF SAUNDICE x 400.

- A = Glomerulus
- B = Proximal tubule
- C = Distal tubule

Para 6.

and C II could not be influenced by CII values. C III values are acknowledged to be unreliable and as mentioned, are not presented in this study. The "peeling off" method was however used when the curves became fused, or a two component system, and it is possible that here, there is some source of error. However the findings by Ladeford quoted above would indicate that these errors are most likely to be insignificant. Nevertheless as mentioned cognisance has only been taken of component I values which represents flow through the entire cortex, when a two component curve was encountered.

- iii) It may also be argued that the equations from which the calculations are made, do not describe biological relationships since they are derived from mathematical models. Also the addition of experimentally derived values for the partition coefficient and the values for specific gravity introduce artefacts resulting from the determination of these quantities. However, there is good agreement found between isotope and flow meter methods for the measurement of renal blood flow^{162,171} and it would seem that these factors do not cause a significant error in the determination of flow. Also since each animal acts as its own control the results after operation are influenced by the same error as the controls and therefore comparisons are not likely to be influenced by any errors in measurement.

Thus it can be seen that the main objection to ¹³³Xe method is that the third component measurement is inaccurate and this influences the other measurements. In these experiments we have used computer analyses for most measurements and also the third component was ignored. In the cases where hand analysis was done using the "peeling off" technique some error may be present. It is felt, however, that this is likely to be small.

Britton et al¹⁷⁶ also feel that the kidney does not represent the compartmental model which is required for analysis. However it is argued by Kew²⁷ that the xenon curve can be clearly resolved into three exponential components, each differing by at least a factor of three, which favours a homogenous distribution of indicator through each compartment and insignificant diffusion between them.

It is felt therefore that the ¹³³Xe method provides a valid measurement of intrarenal blood flow distribution, but that errors do occur, particularly when using the stripping off method of analysis. These errors are largely avoided in this study by the use of computer analysis.

2) Discussion of the Results

These results show that in obstructive jaundice there are changes in intrarenal blood flow distribution of blood in the baboon kidney. There is an alteration of blood flow distribution from the outer cortical to the inner cortical and outer medullary areas. The finding of an insignificant alteration in C I flow rate together with a decreased volume distribution is similar to the findings of Carriere¹⁰³ when the renal nerves are stimulated. He feels that the closure of resistance vessels in the kidney is patchy and therefore the flow rate may be the same but the volume of the vascular bed decreases. This finding is also similar to the findings by other workers in cirrhosis of the liver^{4,10,11,12,38} and other liver diseases^{13,14}. Also in common with the findings in functional renal failure of cirrhosis there appears to be no significant alteration of renal structure and the changes seem to precede the onset of frank renal failure⁶. It would seem therefore that the changes in obstructive jaundice are functional in nature.

It has been suggested by Kew⁶ that the changes in renal blood flow distribution in cirrhosis may be due to an increase in sympathetic activity. This contention is supported by the findings of Hori²⁶ that the sympathetic

nervous system of liver and kidney are inter-related. Further evidence that the changes produced in the kidney in liver diseases may be due to sympathetic activity is the similarity of the distributional changes to those produced by infusion of noradrenaline or stimulation of the renal nerves^{102,103}. Epstein, however, showed that the renal resistance was not decreased by intravenous infusion of an alpha blocking agent and felt that the sympathetic activity was not involved. In his studies however infusion of PBZ produced a fall in blood pressure and when this is prevented Baldus⁴ was able to improve renal blood flow with dibenzylene.

In this study similar results have been obtained which indicate that the sympathetic nervous system is involved. It was found that infusions of PBZ reversed the vasoconstrictor effect of noradrenaline and also improved blood flow through the cortex. In addition the kidneys were unduly sensitive to the effects of noradrenaline. Thus it would seem that the primary disturbance of renal haemodynamics in obstructive jaundice may be explained by an increased sensitivity of the kidney to circulating catecholamines.

This hyper-sensitivity to noradrenaline may also explain some of the clinical features accompanying obstructive jaundice. As mentioned in the introductory section there is a high incidence of acute renal failure after surgical operations on patients with obstructive jaundice^{5,15,48,50}. There is also evidence that in cholestasis the kidney is more sensitive to ischaemia^{15,17,50}. The results found here suggest that there are changes in intrarenal blood flow distribution which precede the onset of overt renal failure. These changes are similar to those produced by renal nerve stimulation or infusion with noradrenaline into the kidney^{102,103}, and therefore imply that sympathetic activity may be responsible for changes seen in this disease. The finding of an increased sensitivity to noradrenaline provides an explanation for the

renal distribution changes which may be in fact occurring in the presence of normal sympathetic activity. In addition this potentiated response to the effects of noradrenaline may explain the subsequent hyper-sensitivity of the kidney to periods of ischaemia, or hypotension when excessive catecholamines could be expected to be present. Thus this would also account for the increased incidence of renal failure found during operations on patients with obstructive jaundice. This could then be explained on the basis of sympathetic hyper-activity on a kidney with a heightened response to the effects of noradrenaline.

The investigations to find a factor or factors causing this hyper-sensitivity will be described in section 4 of this thesis.

Summary

In this section the renal circulation, its control and methods of measurement is described. The findings in obstructive jaundice using the ^{133}Xe technique are presented and are summarised in Figure 12. These are a shift in blood flow distribution from the outer cortex to the inner cortical and outer medullary areas. Also there is a hyper-sensitivity of the kidney to noradrenaline which may explain some of the clinical findings in obstructive jaundice, such as post-operative renal failure.

If the factor or factors causing the increased sensitivity of the renal vasculature to noradrenaline, are blood borne, then it should be possible to produce similar effects on other vascular beds. The next section will describe the effect of obstruction jaundice on cerebral blood flow.

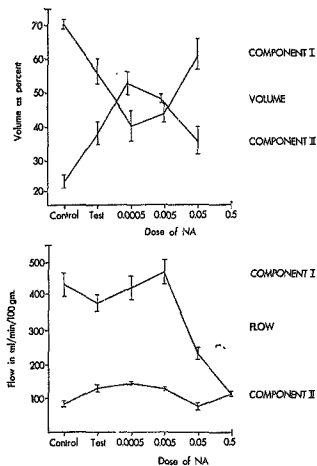


Figure 12. Changes in flow rate and percentage distribution during jaundice and during perfusion with norepinephrine in doses from 0.0005 to 0.5 μ r/min. It can also be seen that infusion of .0005 μ r/min of norepinephrine reduces the percentage distribution but not the flow rate. This is similar to the findings following 2 weeks of bile duct obstruction.

Page 81.

SECTION III.

THE EFFECT OF OBSTRUCTIVE JAUNDICE ON CEREBRAL BLOOD FLOW.

CONTROL OF CEREBRAL BLOOD FLOW

1. Autoregulation

In the normal brain cerebral blood flow is maintained at a relatively constant level despite variations in cerebral perfusion pressure. In baboons, flow remains constant despite variations in pressure from 80-150 mmHg¹⁷⁷. It is felt that autoregulation results from the myogenic response of the smooth muscle cells of the arteriolar wall to stretch-induced variations in transmural pressure¹⁷⁸. The autonomic nervous system may however have some role in modifying the autoregulatory responses. Yoshida¹⁷⁹ demonstrated that the rise in cerebrovascular resistance which occurs following an abrupt rise in intravascular pressure could be prevented by prior section of the cervical sympathetics. James¹⁸⁰ demonstrated that the normal relationship between perfusion pressure and cerebral blood flow could be abolished following section of the cervical sympathetic nerves. He also¹⁸¹ has shown in experiments in which the carotid sinus was vascularly isolated, that changes in perfusion pressure to the isolated sinus, induced changes in cerebrovascular resistance, thereby demonstrating the importance of neurogenic mechanisms in mediating the autoregulatory response.

It has also been postulated that autoregulation is due to alterations in pH around the cerebral vessels. Zwetnow¹⁸² demonstrated an increase in jugular bulb lactate and pyruvate concentrations in dogs following a reduction in perfusion pressure. It is suggested that decrease in perfusion pressure results in a decreased cerebral blood flow, resulting in lactic acidosis which produces a vasodilatation and a decrease in cerebrovascular resistance. This is supported by the finding of abnormal autoregulation in hypercapnia and hypoxia^{183,184}. An increase in resistance which would be expected to occur in alkalosis has not,

however, been demonstrated. Thus it is likely that the induced vasodilatation seen in acidosis merely over-rides the myogenic mechanism.

Chemical Control

Variations in arterial P_{CO_2} exert a profound effect on cerebral blood flow. Hypercapnia causes cerebral vasodilatation and hypocapnia causes a marked vasoconstriction. Lassen¹⁷⁸ states that cerebral blood flow changes 4% per mmHg change in arterial P_{CO_2} .

The CO_2 reactivity is mediated by variations in pH of the cerebral blood flow around the arterioles^{185,186}. The pH at this site also depends on local cerebro-spinal fluid bicarbonate concentration¹⁷⁸. CO_2 -induced changes in cerebro-spinal fluid also alter pulmonary ventilation so that cerebral blood flow and ventilation combine to keep brain extracellular pH constant⁸².

Moderate changes in oxygen tension in arterial blood do not influence cerebral blood flow measurably¹⁷⁸. Thus in moderate hypoxic or arterial hyperoxic cerebral blood flow remains unchanged. With marked hypoxia, however, flow increases. This is not seen until arterial P_{O_2} falls below 50 mmHg¹⁷⁸ which is the same level at which brain tissue lactic acidosis becomes apparent¹⁷⁸. This finding suggests that oxygen has no direct effect on cerebral blood vessels but the changes seen in hypoxia may be due to local changes in pH.

Neurogenic Control

The pial arteries on the surface of the brain have a rich supply of sympathetic and parasympathetic nerve fibres. Although early workers¹⁸⁷ doubted whether intra-cerebral vessels were similarly innervated, it is clear from recent studies using silver stains, electron microscopy and fluorescent histological methods, that a rich supply of nerves innervate these vessels.

Penfield¹⁸⁸ used a silver staining technique, showed that the nerve fibres accompanied inter-cerebral arteries and this has been confirmed by other workers using light microscopy^{189,190}. Electron microscopy has substantiated findings made by the light microscope and showed nerve fibres on pial vessels as small as 15-20 microns in diameter¹⁹⁰. Also, Falk using a histo-chemical fluorescent stains has identified adrenergic nerves which are most profuse on the larger vessels leaving the Circle of Willis, but are also present on the smaller internamchymal vessels¹⁹¹. Degeneration studies¹⁸⁸ have suggested that a large proportion of these fibres originate in the cervical sympathetic chain.

It has also been shown by electron microscopy that there are two types of nerve terminal on the vessels. The one contains electron-dense vesicles which are probably adrenergic, and the other translucent non-granular vesicles which are probably cholinergic since these do not show degenerative changes following sympathectomy¹⁹². Also further evidence that cholinergic fibres are present, is provided by the findings of Lavrentev et al¹⁹³ of the presence of acetyl-choline in the nerves surrounding the pial vessels.

The functional importance of the cerebrovascular nerves is however controversial. Constriction of the pial vessels occurs following sympathetic stimulation and dilatation occurs following a nerve-sympathetic stimulation^{194,195}. The influence of the nervous system on the intra-cerebral vessels is however much less evident. James¹⁸⁰ has demonstrated a decrease in cerebral blood flow following a stimulation of the sympathetic nerves and an enhancement of the vascular response to CO_2 following sympathectomy. Purves¹⁹⁶ also feels that the sympathetic nervous system has a strong influence on cerebral blood flow. In contrast to this, removal of the superior cervical ganglion appears to have very little effect on total cerebral blood flow,

or on the ability of the cerebral vasculature to autoregulate or respond to CO_2 ^{197,198,199}. Lassen¹⁷⁸ feels that the experiments in which sympathetic nerves were stimulated in the neck, interfered with the arterial blood supply to the brain and points out that even mild handling of the carotid artery causes a spasm which reduces the maximal inflow to the brain.

It could be expected that the infusion of vasoactive adrenergic drugs would elucidate the role of the sympathetic nervous system in the control of cerebral blood flow. Corpi²⁰⁰ in a review of 40 papers in which the effect of adrenaline or noradrenaline on cerebral blood flow is studied, found no consistent pattern to emerge. The wide variation in the results may be due to differences in the anesthetic used, local-arterial effects, to autoregulatory responses to the systemic blood pressure effects of these drugs, particularly when given intravenously or due to non-uniform effects i.e. a redistribution of blood flow from some areas to others.

It has however been shown by Rosenzweig and Granston¹⁹⁹ that if noradrenaline is applied to the adventitial surface of the intra-cerebral vessels that vaso-constriction occurs. Rosenblum²⁰¹ has suggested that the effectiveness of noradrenaline on cerebral vessels is reduced by avid neuronal uptake mechanisms and this contention is supported by the work of Rosenzweig, where these mechanisms would be saturated by the high concentrations used by these workers.

These findings do not explain the lack of response to blood borne noradrenaline which would be expected to reach the receptor before the neuronal uptake could be achieved. It is possible that there may be an avid uptake of infused noradrenaline into the vascular smooth muscle - uptake 2 (Iverson²⁰²) which prevents circulating noradrenaline from reaching the receptors. This will be discussed further in this thesis when considering the alterations in cerebro-vascular perfusion obtained by infusing noradrenaline in

baboons with bile duct ligation.

It may also be possible to elucidate the role of the autonomic nervous system by using blocking agents. It was reported by Lende²⁰³ that the spasm of the cerebral vessels induced by mechanical stimulation, could be reversed by local application of beta adrenergic blocking agents. Falke²⁰⁴ has also shown that induced vasoconstriction from hyperventilation could be reversed with a beta blocking agent. Although barium/chloride induced vasoconstriction can be reversed by beta blocking drugs and not by alpha blocking agents²⁰⁴, evidence is accumulating that alpha receptors are also present on the cerebral vessels. Fraser²⁰⁵ showed that phenoxymethamine (PEM) applied locally to cerebral vessels reversed the vaso-spasm induced the local application of blood. Recently Kawamura²⁰⁶ has shown that PEM reduces the vasoconstrictor tone of cerebral vessels during hypocapnia and raised cerebral perfusion pressure. It also enhances the vasodilator response to CO₂ inhalation.

It can be seen from this brief review that there is a rich nerve supply to the intra-cerebral vessels and most evidence would suggest that the sympathetic nervous system is involved in the vascular response to raised PCO₂ and raised cerebral perfusion pressure. That infusions of nor-adrenaline produce conflicting results may be explained by the fact that this hormone may not be reaching receptor sites when infused intra-arterially.

MEASUREMENT OF CEREBRAL BLOOD FLOW

1. Direct observation of vital vessels

Measuring vital artery diameter is a classical method of assessing variations in cerebral blood flow and was first described by Donders in 1859²⁰⁷. However acid-base changes, trauma and hypothermia or hypoxia of the exposed cortical surface tends to abolish the normal vasomotor reactions.

Although currently in use to study the response of pial vessels to local changes in cerebro-spinal fluid²⁰⁸ its main drawback, in addition to those mentioned above, is the uncertain relationship between pial vessel diameter and flow within the vessel. It is however a useful technique to study the pharmacology of brain arteries in vivo in response to locally applied drugs, without the complications of systemic drug effects.

2. Flow in neck vessels

a) Arterial blood velocity

Electro-magnetic flow meters²⁰⁹ have been applied to neck vessels and are capable of measuring the velocity of blood moving in the carotid vessels. However, the results will be inaccurate since only the blood flow in the carotids are measured, and blood flowing in other arteries such as the vertebral is ignored. Also the volume of blood flowing in the blood vessels depend on the vessel diameter as well as the velocity. Therefore since the flow meter does constrict the vessel to a certain extent, and mechanical stimulation also constrict the vessel¹⁷⁸, measurements of total cerebral flow using this method are inaccurate.

b) Venous outflow

This may also be measured by electro-magnetic flow meters²⁰⁹ as well as by thermodilution and by dye dilution techniques²¹⁰. Jugular vein cannulation is required and the methods are only semi-quantitative, unless calibrated against a second and simultaneous method which provides absolute flow figures²¹¹. Also inaccuracies arise from turbulence¹⁹⁵ unless this and the flow profile of the vessel are known, the accuracy of the derived flow is very difficult to determine.

c) Mean transit time

The cerebral circulation time for non-diffusible radioactive indicators injected intravenously to a peripheral vein, has been used to provide an index of cerebral blood flow^{212,213}. With this method, uptake and clearance of the isotope is followed by a detector placed over the cranium. The activity

curve is differentiated and the mean circulation time is measured. The technique is simple but there is no simple relationship between the transit time and cerebral perfusion, although a linear relationship between flow and transit time at low cerebral blood flow has been demonstrated²¹⁵. It would seem, that mean transit time, produce meaningful estimations of cerebral blood flow changes only when cerebral blood volume changes are simultaneously determined and this requires continuous monitoring of cerebro-spinal fluid pressure²¹⁵.

4. Inert diffusible gas methods

a) Arterio-venous difference of an inert gas

Kety and Schmidt²¹⁶ were the first to apply Fick principle to the measurement of cerebral blood flow. In this method the patient inhales a mixture of air and nitrous oxide for 10 minutes, and blood samples were drawn throughout the period from the external carotid artery and the internal jugular vein. The mean cerebral blood flow can be measured by applying the equation

$$F = \frac{C_a t}{C_a - C_v}$$

F = Flow in unit time, C t = Quantity of substance taken up in time t.

C_a = Concentration of substance in arterial blood.

C_v = Concentration of substance in venous blood.

which yields a value for the average cerebral blood flow without measurement of total brain flow and total brain weight. ⁸⁵Kr, ¹³³Xe may also be used. A further variation has been the substitution of hydrogen as the indicator²¹⁷. With this method blood is withdrawn through cuvettes in which are incorporated H₂ sensitive electrodes and subsequently re-circulated to the patient. The method allows semi-continuous measurements of cerebral blood flow.

This is a valuable method and allows measurements of high flow rates obtained during marked hyperemia. It does however assume that the cerebral blood flow remains

constant over the 10-15 minutes during which the measurements are made and Lassen has found that this assumption may be made, provided there are no changes in arterial gas tensions or perfusion pressures²¹⁸. In addition in slowly perfused areas of the brain the inert gas may not have reached equilibrium with the arterial and mixed venous bloods at the time the measurements are made, so that there will be errors in the calculated flow. These difficulties can be surmounted by prolonging the saturation time and extrapolation of the saturation curves to infinity²¹⁸. The main drawback of the method is in the technical difficulty of drawing multiple samples at frequent intervals. This usually requires numerous assistants and each study also uses 50-80 ml of blood which, in small animals, can introduce a considerable blood loss. Also results are not available until analysis of the indicator is completed and artefacts cannot be recognised until calculations have been completed.

b) Intra-arterial ^{133}Xe injection method

This method is based on measurement of the mean clearance of the tracer gas with externally placed scintillation detectors. The flow rate is then calculated by the same method as described for the ^{133}Xe technique in the kidney.

The large number of anastomoses between intra and extra-cranial circulation makes selective labelling of brain tissues difficult. In the experimental animal however, this can largely be avoided by surgical removal of the soft tissue, ligation of the external carotid artery and careful collimation of the external scintillation counter. However spasm of the artery to the brain following surgery may induce error in animal experiments and must be taken into account.

The clearance curve is normally analysed into two exponential components to provide a derivation of flow in grey and white matter¹⁷⁸.

Although errors may arise from mechanical trauma,

Para 90.

anaesthesia, and rates of injection, it is felt that of all the methods available, the ^{133}Xe injection technique is the method which is least open to theoretical objection provided the limitations of the technique are taken into account¹⁹⁵. Some of the difficulties which are inherent in methods which depend on cannulation of the internal carotid artery are avoided by a method in which ^{133}Xe is inhaled and the clearance rate over the cranium calculated in a similar manner. Here, however, re-circulation of isotope and contamination by radio-activity arising outside the brain also introduce errors. In man the objections to cannulating an artery over-ride this source of error and it is probably here that the inhalation technique has most application. In experimental animals the direct infusion of ^{133}Xe into the carotid artery is at present the method of choice.

Other methods

Several attempts have been made to develop methods for study of cerebral blood flow. There are many such methods such as the use of radio-active microspheres²¹⁹ arterio-venous oxygen differences²²⁰ and determination of heat clearance from the cerebral cortex or internal jugular vein²²¹. These are of specific value in specific contexts but in this study it was decided that although ^{133}Xe xenon technique does have limitations it was most useful for the study of cerebral blood flow in experimental animals and therefore was used throughout in this study.

THE EFFECT OF OBSTRUCTIVE JAUNDICE ON CEREBRAL BLOOD
FLOW IN MONKEYS

Introduction

The experiments in the previous sections showed that in obstructive jaundice the alterations in renal blood flow are similar to those found in other liver diseases. It was also demonstrated that in addition to these changes there is an increased reovascular sensitivity of some organs. It was suggested that this hyper-sensitivity may explain the distributional changes found in the kidney, as well as the high incidence of renal failure seen post-operatively in obstructive jaundice. It was also felt that if this hyper-sensitivity to noradrenaline is due to some factors produced during obstructive jaundice other vascular beds should also be hyper-sensitive to the hormone.

As mentioned the ¹³³Xenon technique is particularly well-suited for the study of cerebral circulation. Also as has been mentioned the cerebral circulation is relatively insensitive to the effects of noradrenaline and the role of the hormone in the control of cerebral blood flow is uncertain. It was therefore anticipated that the obstructive jaundice model might be useful in delineating the role, if any, of noradrenaline in normal cerebral vascular physiology. This section then, will deal with studies made on cerebral blood flow during obstructive jaundice.

Materials and Methods

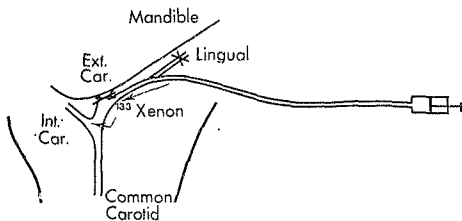
Studies were carried out on forty-nine adult baboons (*Papio ursinus*) weighing 9-16 kilograms. In 7 of the animals obstructive jaundice was produced by surgical ligation of the common bile duct two weeks prior to the experiments on cerebral blood flow. The rest remaining 9 animals were all normal baboons. All the animals were sedated with 0.5 mg/kg Ketamine hydrochloride (Ketalar by Parke-Davis) administered by intramuscular injection, and full anaesthesia was induced with intravenous injection of 20-30 mg/kg pentobarbitone

sodium (Nembutal, Abbott). The animals were then intubated and anesthesia was maintained with a 3:2 mixture of oxygen and nitrous oxide with similar quantities of barbiturate given to each group as required. A catheter was introduced into the carotid artery to measure intra-arterial blood pressure via an electro-manometer (Statham P23AA) and to enable blood sampling for determinations of PaCO_2 , PaO_2 and pH on an Instrumentation Laboratories blood gas analyser (IL313). The right carotid bifurcation was exposed and a fine catheter was inserted into the lingual artery until its tip just entered the external carotid artery. The external carotid artery and all its remaining branches were then ligated (Figure 13). Cerebral blood flows (CBF) were measured by an intra-carotid ^{133}Xe washout technique. A bolus of 30 μCi of ^{133}Xe in 0.2 ml of saline was injected into the lingual catheter and was washed retrograde down the external carotid artery into the internal carotid artery. The cerebral uptake clearance of ^{133}Xe was monitored using a 5 cm. diameter sodium iodide detector placed posteriorly over the parietal region. A high degree of collimation was used to exclude possible radiation arising from non-cerebral orbital tissues and in addition the soft tissue over the skull was removed. That all possible radiation was excluded was confirmed by the absence of a third flow component (extra-cranial perfusion) in the clearance curves. After the initial peak of activity the clearance data was recorded for at least 15 minutes in digital form with a logging system similar to that described in the kidney experiments. When exceptionally slow flows were found this record was extended to 20 minutes. The washout curves were analysed manually and by computer (using a least squares fitting programme) into two exponential components representing flow through cerebral grey and white matter respectively.

When surgery was completed the bladder was catheterized and allowed to drain freely. The animals were nursed with 10 mg/kg of intravenous Seoline (Glaxo-Allenburys) and

sodium (Nembutal, Abbott). The animals were then intubated and anaesthesia was maintained with a 3:2 mixture of oxygen and nitrous oxide with similar quantities of barbiturate given to each group as required. A catheter was introduced into the carotid artery to measure intra-arterial blood pressure via an electro-manometer (Statham P23AA) and to enable blood sampling for determinations of PaCO_2 , PaO_2 and pH on an Instrumentation Laboratories blood gas analyser (IL313). The right carotid bifurcation was exposed and a fine catheter was inserted into the lingual artery until its tip just entered the external carotid artery. The external carotid artery and all its remaining branches were then ligated (Figure 13). Cerebral blood flows (CBF) were measured by an intra-carotid ^{133}Xe washout technique. A bolus of 30 μCi of ^{133}Xe in 0.2 ml of saline was injected into the lingual catheter and was washed retrograde down the external carotid artery into the internal carotid artery. The cerebral uptake clearance of ^{133}Xe was monitored using a 5 cm. diameter sodium iodide detector placed posteriorly over the parietal region. A high degree of collimation was used to exclude possible radiation arising from non-cerebral orbital tissues and in addition the soft tissue over the skull was removed. That all possible radiation was excluded was confirmed by the absence of a third flow component (extra-cranial perfusion) in the clearance curves. After the initial peak of activity the clearance data was recorded for at least 15 minutes in digital form with a logging system similar to that described in the kidney experiments. When exceptionally slow flows were found this record was extended to 20 minutes. The washout curves were analysed manually and by computer (using a least squares fitting programme) into two exponential components representing flow through cerebral grey and white matter respectively.

When surgery was completed the bladder was catheterised and allowed to drain freely. The animals were paralysed with 10 mg/Kg of intravenous Scoline (Glaxo-Allenburys) and



CANNULATION TECHNIQUE

Figure 13. Shows a diagrammatic representation of the technique for infusing ¹³³Xe via a catheter in the lingual artery.

were ventilated using a Harvard variable volume respirator. End tidal CO_2 concentration together with electroencephalograph, pulseatile and mean arterial blood pressure, central venous blood pressure and the analogue xenon washout curve were recorded on a Beckman 411 dynograph recorder. The electroencephalograph was used to standardize the depth of anaesthesia. Measurements of cerebral blood flow were only made when the electroencephalograph was stable and showed a frequency of 9-12 Hz. Throughout the experiment the arterial PaCO_2 , PbO_2 were kept approximately at 35 and 120 mmHg respectively and body temperature was kept at 37°C by radiant heating. After a stabilization period of approximately one hour, two control cerebral blood flow measurements were made. Noradrenaline (NA) was then infused via the lingual artery at $8 \mu\text{g}/\text{min}$ and after 10 minutes the flow again determined. The NA was then stopped and after a further 10 minutes another control measurement was made. Using this protocol the cerebrovascular response to $8 \mu\text{g}/\text{min}$, $16 \mu\text{g}/\text{min}$ and $32 \mu\text{g}/\text{min}$ of NA was determined.

In addition arterial blood samples were taken for serum urea, sodium, potassium, glucose, bilirubin (direct and indirect), alkaline phosphatase and renin measurements. An attempt was made to determine the degree of cerebrovascular constriction in response to noradrenaline with the alteration in these serum constituents after bile duct ligation.

Because it has been suggested that the cerebrovascular response to increased levels of PaCO_2 is mediated via a neural mechanism in a further 15 baboons with obstructive jaundice the cerebrovascular response to graded 10 mmHg alterations of PaCO_2 (range 35-70 mmHg) was compared with the responses of 16 control baboons to similar increments of PaCO_2 . The animals were also hyperventilated to reduce the PaCO_2 to between 20-24 mmHg. In 6 of the experimental animals this was repeated after alpha adrenergic blockade with Phentolamine (5 mg I.V. Siba).

TABLE 11

Mean grey matter flows (CBF) in nine normal animals and seven jaundiced animals following infusions of noradrenaline (NA).

	CBF (ml/min/100g)			
	0 μ g NA	8 μ g NA	16 μ g NA	32 μ g NA
<u>Control</u>				
1	72.8	79.1	-	-
2	47.5	53.6	51.9	-
3	52.5	87.5	97.8	-
4	51.9	42.6	55.4	47.5
5	63.9	83.1	85.2	-
6	62.7	75.5	66.5	75.5
7	49.8	55.4	46.2	33.9
8	56.7	53.6	48.9	50.4
9	58.3	61.6	60.4	63.9
<u>Jaundiced</u>				
1	60.2	50.1	48.7	55.2
2	43.0	37.4	-	37.8
3	50.0	35.6	22.6	46.4
4	57.4	57.4	47.5	51.9
5	64.1	59.4	66.5	68.8
6	86.7	63.9	73.8	75.5
7	52.2	45.4	43.8	49.0

Results

1) NA infusion

The mean grey matter blood flow (CBF) was the parameter used in this study. White matter flows although measured are not included since the flow here is slow and less sensitive to physiological influences and Lassen feels that measurements of white matter flow are not as accurate because of the extremely slow flows and the possibility of recirculation inaccuracies¹⁷⁸. The mean grey matter flow (CBF) in the control animals was 57.3 ± 2.7 ml/min/100gr tissue. Infusion of $8 \mu\text{g}$ of noradrenaline per minute produced a mean increase in flow to 65.8 ± 5.2 ($+8.4 \pm 4.3$) ml/min/100gr tissue. $16 \mu\text{g}$ increased flow to 64.0 ± 6.5 ($+8.6 \pm 6$) ml/min/100gr tissue and $32 \mu\text{g}$ of noradrenaline/minute reduced flow to 54.2 ± 12.2 (-1.67 ± 4.9) ml/min/100gr tissue.

In the jaundiced animal the mean baseline grey matter flow was 59.1 ± 5.3 ml/min/100gr tissue. Infusion of $8 \mu\text{g}$ of noradrenaline/minute reduced flow to 49.9 ± 4.1 (-9.4 ± 2.8) ml/min/100gr tissue. $16 \mu\text{g}$ of noradrenaline/minute reduced flow to 50.5 ± 7.3 (-11.3 ± 3.9) ml/min/100gr tissue. $32 \mu\text{g}$ of noradrenaline/minute reduced the flow to 54.8 ± 4.9 (-4.1 ± 1.8) ml/min/100gr tissue.

The changes in flow in the jaundiced animals were significantly different from the control values after infusion of $8 \mu\text{g}$ and $16 \mu\text{g}$ of noradrenaline/minute ($p < 0.025$). The decrease in flow seen with $32 \mu\text{g}$ of noradrenaline/minute was not significantly different from the control value.

The mean grey matter flow (CBF) in each animal can be seen in Table 11.

The total mean values of all experiments are shown in Table 12. This table also shows the mean change in CBF (Δ CBF).

TABLE 12

Mean grey matter cerebral blood flow (CBF); mean change in cerebral blood flow (Δ CBF); and mean arterial blood pressure (M.B.P.) in nine control animals and seven jaundiced animals following infusions of noradrenaline (NA). All values are shown $\pm$ one standard error of the mean.

(μ g/min) NA	(mls/min/100g) CBF	(mls/min/100g) Δ CBF	(mm Hg) M.B.P.	(mm Hg) P _{CO2}
<u>Control</u>				
0	57.3 $\pm$ 2.7	-	108.3 $\pm$ 7.7	36.7 $\pm$ 1.0
8	65.8 $\pm$ 5.2	8.4 $\pm$ 4.3	112.3 $\pm$ 7.2	34.6 $\pm$ 0.6
16	64.0 $\pm$ 6.5	8.0 $\pm$ 6.0	117.8 $\pm$ 8.8	34.7 $\pm$ 0.8
32	54.2 $\pm$ 12.2	-1.6 $\pm$ 4.9	126.7 $\pm$ 9.5	35.0 $\pm$ 0.3
<u>Jaundiced</u>				
0	59.1 $\pm$ 5.3	-	110.1 $\pm$ 6.2	34.7 $\pm$ 0.9
8	49.9 $\pm$ 4.1	-9.4 $\pm$ 2.6	120.7 $\pm$ 6.7	35.2 $\pm$ 0.8
16	50.5 $\pm$ 7.3	-11.3 $\pm$ 3.9	116.8 $\pm$ 4.6	35.5 $\pm$ 0.8
32	54.9 $\pm$ 4.9	-4.1 $\pm$ 1.8	127.1 $\pm$ 3.6	34.9 $\pm$ 0.4

The mean percentage changes in CBF in each animal are shown in Table 13.

The change in grey matter flow is shown in graphical form in Figure 14 and the mean percentage change in CBF in Figure 15.

The infusion of noradrenaline was associated with an increased systemic blood pressure and in order to take this into account the cerebral vascular resistance (pressure/flow in mmHg) was calculated (CVR).

In the control animals 8 μ g of noradrenaline/minute produced a decrease in CVR of 0.12 ± 0.1 mmHg while 16 μ g and 32 μ g of noradrenaline produced an increase of 0.01 ± 0.1 and 0.44 ± 0.2 mmHg respectively.

In the jaundiced animals 8 μ g of noradrenaline/minute increased CVR by 0.66 ± 0.2 mmHg while 16 μ g and 32 μ g of noradrenaline/minute increased CVR by 0.92 ± 4.7 and 0.71 ± 0.3 mmHg respectively. The increase was significantly different from the control values only after infusion of 8 μ g of noradrenaline/minute.

The values of CVR in each animal can be seen in Table 14 and the percentage changes in Table 15. The mean changes are shown graphically in Figures 16 and 17.

The arterial blood pH P_{CO_2} and P_{O_2} were not significantly changed throughout the experiments. The range of pH was from 7.41 - 7.43. Increments in systemic arterial blood pressure were recorded during perfusion of noradrenaline. The increase was, however, similar in both groups of animals and the mean blood pressure ranged from 100-126 mmHg which is well within the cerebral autoregulatory limits for the baboon¹⁷⁷. The changes in pH P_{CO_2} and P_{O_2} were minor, and would not be expected to significantly alter the cerebral blood flow. The measured values for bilirubin and other serum variables in each animal are shown in Table 16 and the mean values in Table 17. There was no good correlation between the size of the cerebrovascular response to noradrenaline and the levels of bilirubin, or the hepatic

TABLE 13

Shows percentage change in cerebral blood flow (CBF) in nine normal animals and seven animals with obstructive jaundice following infusions of noradrenaline (NA).

Mean Percentage Change				
CBF				
<u>Control</u>	O	8 μ g NA	16 μ g NA	32 μ g NA
1	-	+ 8.6	-	-
2	-	+12.8	+ 9.2	-
3	-	+66.5	+86.1	-
4	-	-17.9	+ 6.7	- 8.5
5	-	+29.9	+33.2	-
6	-	-20.3	+ 5.9	+20.3
7	-	+11.2	- 7.2	-31.9
8	-	- 5.5	-13.8	-11.1
9	-	- 5.5	+ 3.5	+ 9.5
Mean change		+ 8.8	+15.4	- 4.3
<u>Jaundiced</u>				
1	-	-16.7	- 1.9	-41.5
2	-	-13.0	-	-12.0
3	-	-28.8	-54.8	- 7.2
4	-	1.7	-20.0	-12.6
5	-	- 4.1	+ 5.2	+ 8.8
6	-	-26.2	-19.8	-12.9
7	-	-12.9	-16.1	- 6.2
Mean change		-14.8	-17.9	-11.9

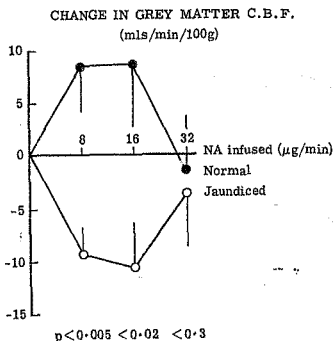


Figure 14. CHANGE IN GREY MATTER CEREBRAL BLOOD FLOW WITH
NORADRENALINE

The mean change in grey matter cerebral blood flow (ml/min/100g tissue) during intra-carotid infusion of noradrenaline (at 8 µg/min, 16 µg/min and 32 µg/min) in control animals (closed circles) and in animals with obstructive jaundice (open circles). The vertical lines represent plus or minus one standard error of the mean and the p value is calculated between the control and the jaundiced groups at the same dose of noradrenaline.

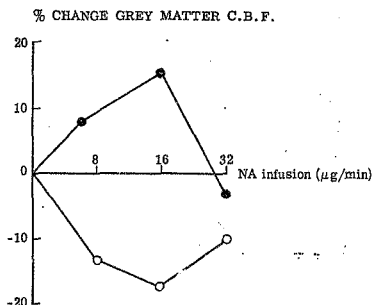


Figure 15. MEAN PERCENTAGE CHANGE (CBF)

Shows mean percentage change in grey matter flow (CBF) during infusion of 8 μg , 16 μg and 32 μg of noradrenaline (NA) in control animals (closed circles) and in animals with obstructive jaundice (open circles).

TABLE 14

Shows mean cerebral vascular resistance (CVR) in nine control animals and seven animals with obstructive jaundice. The mean change (Δ) in CVR is also shown.

CVR (mm Hg/ml/min)				
<u>Control</u>	0 g	8 μ g NA	16 μ g NA	32 μ g NA
1	1.5	1.5	-	-
2	2.2	1.8	2.0	-
3	1.2	0.8	0.8	-
4	1.7	2.0	1.6	2.0
5	1.9	1.5	1.7	-
6	1.4	1.5	1.7	1.7
7	2.5	2.1	2.8	3.6
8	2.2	2.3	2.7	2.6
9	2.3	2.3	2.2	2.4
Mean change	-	-0.12 $\pm$ 0.1	+0.01 $\pm$.1	+0.44 $\pm$ 0.2
<u>Jaundiced</u>				
1	1.6	2.2	2.1	3.7
2	3.1	3.9	-	3.8
3	1.8	3.7	5.0	2.5
4	2.2	2.3	2.9	2.6
5	1.7	1.8	1.8	1.7
6	1.1	1.4	1.4	1.6
7	1.9	2.7	2.6	2.5
Mean change	-	+0.66 $\pm$ 0.2	+0.92 $\pm$ 4.7	+0.71 $\pm$ 0.3

TABLE 15

Shows percentage change in cerebrovascular resistance (CVR) in nine control animals and seven animals with obstructive jaundice following the infusion of noradrenaline (NA).

Mean Percentage Change				
CVR				
	0	8 μ g NA	16 μ g NA	32 μ g NA
<u>Control</u>				
1	-	- 1.8	-	-
2	-	-17.1	- 8.5	-
3	-	-33.5	-34.4	-
4	-	+20.2	- 6.3	+17.8
5	-	-23.1	- 9.9	-
6	-	+ 4.1	+18.3	+20.3
7	-	-15.2	+11.4	+42.9
8	-	- 4.0	+20.0	+16.5
9	-	- 0.3	- 0.5	+ 5.3
Mean change	-	- 7.8	- 1.2	+20.6
<u>Jaundiced</u>				
1	-	42.1	+32.0	+129.0
2	-	+32.0	-	1.2
3	-	+85.0	+163.0	+ 32.0
4	-	+ 4.4	+ 29.0	+ 18.0
5	-	+12.0	+ 9.6	+ 6.0
6	-	+ 8.6	+ 8.6	+ 25.8
7	-	+24.0	+ 40.1	+ 34.9
Mean change	-	+29.7	+ 47.1	+ 35.3

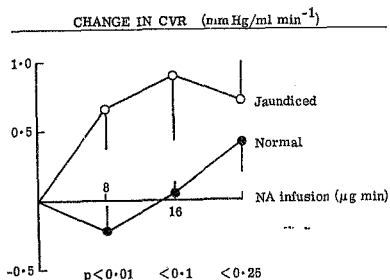


Figure 16. CHANGES IN GREY MATTER CEREBROVASCULAR RESISTANCE WITH NORADRENALINE

The mean changes in cerebrovascular resistance (mm Hg/ml/min $^{-1}$) during intra-carotid infusion of NA (8 $\mu\text{g/min}$, 16 $\mu\text{g/min}$ and 32 $\mu\text{g/min}$) in control animals (closed circles) and jaundiced animals (open circles). The vertical lines represent plus or minus one standard error of the mean and the p values are calculated for the differences between control and the jaundiced groups at the same dose of noradrenaline.

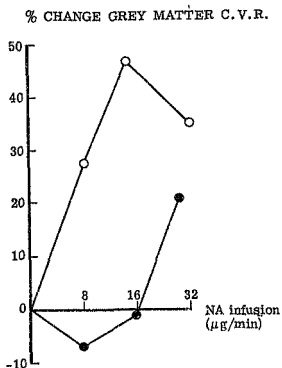


Figure 17. MEAN PERCENTAGE CHANGE (CVR)

Shows the mean percentage change in cerebrovascular resistance (CVR) during infusion with 8 μg , 16 μg and 32 μg of noradrenaline (NA) in control (closed circles) and jaundiced animals (open circles).

TABLE 16

Page 106.

The biochemical data from each of the jaundiced animals before and after indication of jaundice.

Number	Bilirubin		SGOT units	SGPT units	Alkaline Phosphatase mEq/litre	Na ⁺ mEq/litre	K ⁺ mEq/litre	Urea mg %	Plasma renin activity pg/ml/hr
	Total & (unconjugated) mg%	Free mg%							
1	Before	0.5 (0.2)	36	41	33.4	141.0	4.2	28	525
	After	11.5 (8.2)	78	76	624.0	145.0	3.8	43	470
2	Before	0.5 (0.2)	40	52	17.0	143.0	4.3	48	465
	After	9.0 (5.9)	62	195	145.0	145.0	4.0	47	720
3	Before	0.5 (0.2)	38	43	24.6	138.0	3.8	33	720
	After	22.1 (13.3)	50	50.	433.0	138.0	4.0	48	600
4	Before	0.5 (0.2)	47	45	9.5	142.0	4.0	52	427
	After	16.5 (9.5)	78	78	532.0	146.0	4.2	30	300
5	Before	0.5 (0.2)	53	47	10.8	140.0	3.0	35	780
	After	10.3 (6.6)	99	99	237.0	138.0	3.8	65	990
6	Before	0.5 (0.2)	39	27	15.8	142.0	4.0	54	450
	After	7.5 (4.1)	47	37	550.0	143.0	3.9	52	420

7	Before	0.5 (0.2)	27	51	10.8	145.0	3.8	51	300	Page 107
	After	18.5 (11.5)	54	78	3.32	140.0	4.2	49	336	

TABLE 17

The mean biochemical data from all of the jaundiced animals before and after bile duct ligation. The mean values are shown plus one standard error of the mean.

Bilirubin		SGOT	SGPT	Alkaline Phos	Na+	K+	Urea	Plasma Renin
Direct	Indirect	Units	Units	Units	meq/l	meq/l	mg %	pg/ml/hr
BEFORE	0.5	0.2	40 ± 3	44 ± 3	141.0 ± 3.3	3.9 ± 0.2	43 ± 4	524 ± 92
AFTER	13.6 ± 2.0	8.4 ± 1.2	67 ± 7	88 ± 19	142.0 ± 88.4	4.0 ± 0.1	48 ± 4	548 ± 92

TABLE 17

The mean biochemical data from all of the jaundiced animals before and after bile duct ligation. The mean values are shown plus one standard error of the mean.

	Bilirubin		SGOT		SGPT		Alkaline Phos		Na+		K+		Urea		Plasma Renin	
	Direct	Indirect	Units		Units		Units		meq/l		meq/l		mg %		I./ml/hr	
BEFORE	0.5	0.2	40 ± 3		44 ± 3		17.5 ± 3.3		141.0 ± 0.8		3.9 ± 0.2		43 ± 4		524 ± 92	
AFTER	13.6 ± 2.0	8.4 ± 1.2	67 ± 7		88 ± 19		360 ± 88.4		142.0 ± 1.3		4.0 ± 0.1		48 ± 4		548 ± 92	

dysfunction as determined by any of the biochemical tests carried out. The plasma renin levels also not significantly altered in the jaundiced animals. Thus none of these variables appear to be responsible for the alteration of cerebrovascular response in the jaundiced animals as compared to that found in the control animals.

CO₂ experiments

The mean resting grey matter flow at various levels of P_{aCO_2} in the control and jaundiced animals are shown in Table 18 plus or minus one standard error of the mean. In the control animals the mean P_{aCO_2} was 35.4 mmHg and as CO_2 was added the mean increase in tension rose to 43.1; 54.2 and 63.6 mmHg respectively. The corresponding values for cerebral blood flow were 60.9 ml/min/100gr tissue at rest and 80.1; 119.6 and 135.3 ml/min/100gr tissue. During hyperventilation the P_{aCO_2} fell to a mean level of 26.5 mmHg with corresponding decrease in cerebral blood flow to 55.1 ml/min/100gr tissue. In the jaundiced animals the resting P_{aCO_2} was 35.5 mmHg and the mean increase in tension rose to 45.8; 53.4 and 64.2 mmHg respectively. The corresponding values of CBF were 50.0 ml/min/100 gr tissue at rest and 74.6; 77.9 and 92.3 ml/min/100gr tissue respectively. During hyperventilation the CBF fell to 34.8 ml/min/100gr tissue at a mean P_{aCO_2} of 24.8 mmHg. After alpha blockade the mean values for CBF were 52.6 at rest and increased to 75.5 and 85.1 ml/min/100gr tissue, while the fall of CBF with hyperventilation was to 40.7 mmHg. These values are also expressed graphically in Figure 10. The response to raised pCO_2 was significantly attenuated in the jaundiced animals ($n < 0.0005$) at 40-49 mmHg and $p < 0.05$ at 50-59 mmHg). Hyperventilation produced a significant decrease compared to controls ($n < 0.05$). The CBF response following alpha adrenergic blockade was lower than the jaundiced animals ($n < 0.005$ at 40-49 mmHg).

TABLE 18

RESPONSE TO PaCO_2

Shows the mean values of grey matter flow (CBF in ml/min/100gr) in response to raised PaCO_2 in control animals and jaundiced baboons. The response after 5 $\mu\text{g}/\text{min}$ of phentolamine is also shown.

	PaCO_2 mmHg	20 - 29	30 - 39	40 - 49	50 - 59	60 - 69
Control	Mean PaCO_2	26.5 ± 1.1	35.4 ± 2.6	48.1 ± 0.7	54.2 ± 1.6	63.6 ± 2.8
	CBF	55.1 ± 5.1	60.9 ± 6.1	88.1 ± 14.4	119.6 ± 13.2	125.2 ± 1.7
Jaundiced	Mean PaCO_2	24.8 ± 1.6	35.5 ± 1.7	45.8 ± 1.8	53.4 ± 2.1	64.2 ± 2.6
	CBF	34.8 ± 3.1	52.0 ± 3.4	54.6 ± 3.3	77.1 ± 6.3	92.2 ± 2.1
Jaundice and Phentolamine	Mean PaCO_2	25.1 ± 1.7	35.2 ± 1.7	46.1 ± 1.2	52.1 ± 2.1	-----
	CBF	42.7 ± 6.8	52.6 ± 6.2	75.5 ± 3.7	85.1 ± 6.8	-----

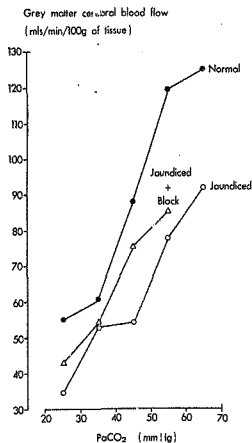


Figure 18. CO₂ RESPONSE CURVES

The response of grey matter flow (GMF) during 10 mm Hg increments in P_{rCO_2} in control animals (closed circles) jaundiced animals (open circles) and after alpha blockade with phentolamine (triangles). (5 mg/min I.V.) Responses to P_{CO_2} along x axis shown in decades.

Page 112.

Discussion

These findings indicate that in baboons after ligation of the bile duct, there is an altered responsiveness of the cerebral vasculature to NA and CO_2 . When increments of CO_2 were administered the response of the blood vessels was diminished compared to the control animals. There was less vasodilatation and also hyperventilation produced a much greater reduction in CBF than the normal animals. This returned towards normal after alpha blockade. These results could be explained by a tonic constriction of the cerebral blood vessels which prevents the normal response of these vessels to raised CO_2 . This could be produced by an increased sympathetic tone, since a rich sympathetic nerve supply to the brain has been clearly demonstrated²². Alternatively there could be an increased sensitivity to the sympathetic hormones, similar to that demonstrated in the kidney (Section II).

It would appear from these studies that the latter explanation is more likely and that the cerebral vasculature is common with that of the kidney is hypersensitive to the effects of noreadrenaline. This confirms the findings in the kidney and indicates that the factor or factors causing the changes found in that organ are general since similar effects can be found in both organ beds. In addition these findings may help elucidate the role of noreadrenaline in cerebrovascular control.

The effects of infused NA in the normal cerebral circulation have been intensively studied but no consistent pattern of response has emerged. Reported results of infusion include cerebral-vaso constriction²³ vasodilatation²⁴ or no effect²⁵. Although much of these discrepancies may relate to the routes of administration and the anaesthesia used other mechanisms may be operative. It has been discussed in the introduction that Rosenblum²⁰ has suggested that the effectiveness of noreadrenaline on cerebral vessels may be reduced by an avid neuronal uptake mechanism, which prevents noreadrenaline from reaching

receptor sites in sufficient concentration to exert an effect. The work of Rosendorff and Granston¹⁹⁹ support this finding in that they demonstrate cerebro-vaso constriction when noradrenaline was applied locally in high concentration to the vessel surface. This latter result suggests a possible saturation of neuronal uptake mechanisms allowing sufficient noradrenaline to reach the smooth muscle receptors.

These findings do not explain the lack of response to blood-borne NA which would be expected to reach vascular muscle receptors before neuronal uptake (uptake I) could be achieved. It is possible, however, that blood-borne noradrenaline is avidly taken up by extra neuronal processes (uptake II)²⁰². The noradrenaline is taken up into the smooth muscle cell and is there metabolised mainly by the enzyme catechol-o-methyl-transferase (COMT)²²⁶. Normally the amount of noradrenaline taken up by the smooth muscles does not exceed the amount diffusing into the vascular wall from the blood. It has been shown that inhibition of COMT slows down the uptake II process and increases the availability of NA to the receptor and potentiates the effect of NA²²⁷. Conversely, if Uptake II is very avid the effects of noradrenaline would be reduced. McCalden and Eidelman have demonstrated that inhibition of COMT results in an enhanced cerebrovascular response to infused noradrenaline²²⁸, which is analogous to the findings reported here. This indicates that avid metabolism of NA within the vascular smooth muscle leading to an increase in uptake II may account for the normal relative lack of response of the cerebral vessels to NA.

The present findings of cerebral vascular constriction at low infusion rates of NA in jaundiced animals, as opposed to dilatation in the normal animals, is as mentioned similar to that found by McCalden and Eidelman in normal animals after COMT blockade. The dilatation observed in the normal probably relates to NA reaching the receptors in low concentrations and exerting a primary beta-adrenergic effect²²⁹. In the jaundiced animals the NA concentration

at the receptor sites is probably higher and this produces an alpha-adrenergic stimulation resulting in vasoconstriction²²⁹. This suggests that some factor or factors in the serum of animals with obstructive jaundice may be acting on a vascular smooth muscle and inhibiting catecholamine uptake mechanisms. It is possible, however, that there are other explanations for the increased sensitivity of the vasculature to the effects of norepinephrine. This will be discussed in the final section of this thesis.

The specific underlying factor or factors responsible for the enhanced responses to norepinephrine is speculative and has been extensively covered when dealing with the kidney. There was no correlation between the serum bilirubin levels (direct or indirect), transaminase concentration, enzyme concentration and plasma renin activity and the magnitude of the cerebral vasoconstrictor response following infusions of NA. As shown in Section II of this study the sensitivity of the kidney vasculature to norepinephrine also could not be correlated with these variables.

It may be argued that cerebral pressor response to norepinephrine found in the present study is related to the prior bile duct surgery. Numerous sham operations were conducted with animals which had previous bile duct manipulation but not ligation and it was found that both the resting cerebral blood flow and the response of the cerebral vasculature to infused norepinephrine was identical to the control animals used here. Also the fact that resting cerebral blood flow, blood pressure and heart rate were little changed in the jaundiced animals would indicate that they had recovered from the trauma of surgery.

In this method the NA was infused through the carotid bifurcation before entering the internal carotid artery. It has been suggested by Purves that the carotid chemoreceptors are important in control of cerebral vasculature both in the response to norepinephrine and in the response to CO_2 ²³⁰. Thus the present results may have been due to

a reflex action of the cerebral vessels involving these receptors. Other workers, however, believe that the chemoreceptors play little part in the cerebrovascular responses to noradrenaline²³¹ and McCalden and Eidelman have shown that there is no change in the response to infused noradrenaline when the bifurcation nerves are removed²³². It therefore seems likely that the results reported here are due to a direct effect of noradrenaline on the cerebral circulation, and not via a reflex mediated by the carotid receptors.

Fisher²³³ feels that it is possible that two forms of hepatic encephalopathy and coma exists. The first, most common, with moderate impairment of hepatic function, is secondary to the presence of toxins that are normally cleared by the liver. It is possible that these toxins may be responsible for the sensitivity of the cerebral vasculature to noradrenaline which was found in this study. This is supported by the finding that alcohol, which plays a positive role in many of the patients with hepatic failure, has been shown to disrupt the blood brain barrier to catecholamines²³⁴. It is possible that the severe coma often associated with alcoholic hepatitis may evolve from complete disruption of the blood brain barrier, the major defense mechanism of the brain. Fisher also feels that there is some evidence that the liver normally produces some substance which is essential to normal brain metabolism. In massive hepatic necrosis these substances are absent and under such circumstances brain function may be even more severely compromised than in the first cited condition²³³.

It must be stated that in this study vascular resistance was calculated from the ratio of perfusion pressure (ΔP) to flow (F). It is pointed out by Purves¹⁹⁶ that this can only be done if R is independent of either P or F. This is unlikely to be seen in vivo because of passive distention or active constriction of blood vessels and the anomalous

viscosity of blood. Measurements of vascular impedance would have been more appropriate but attempts to measure this have not been successful¹⁹⁶. Therefore calculations of vascular resistance was done as mentioned above, bearing in mind that these are only approximate measurements and not accurate.

Summary

These experiments in which the effect of obstructive jaundice on the cerebral circulation was studied confirm the findings found in the kidney. Here, similar to the findings in the kidney, an increased sensitivity to the effect of noradrenaline was found and in addition an attenuated response to the dilator effects of CO₂ was noted. It was suggested that these changes could be explained by a general factor present in the serum which is either not detoxified by the liver, or is present in excess in hepatic disease. The similarity of these findings in jaundice to the findings of McCalden and Eidelman when COMT was inhibited suggest the possibility that this unknown factor acts by inhibiting uptake II processes, thereby allowing more noradrenaline to reach the receptor sites. However this has not been shown directly in this thesis and other explanations are also possible (see final discussion). It is also suggested that the sensitivity of the cerebral circulation to catecholamines may explain some of the features of hepatic encephalopathy.

Page 117.

SECTION IV.
EXPERIMENTS USING AN ISOLATED PERFUSED ARTERY OR KIDNEY
PREPARATION.

THE EFFECT OF JAUNDICED PLASMA ON THE RESPONSES OF A
PERFUSED ARTERY OR KIDNEY TO THE PRESSOR EFFECTS OF
NORADRENALINE.

Introduction

From the experiments presented in Sections II and III it would appear that there is an enhanced sensitivity of blood vessels to the effects of noradrenaline in obstructive jaundice. It would also seem that some blood-borne factor or factors are responsible for this altered sensitivity, since similar effects were found in two different vascular beds; i.e. the kidney and the brain. If this factor, or factors are present in the plasma, then it should be possible to produce similar potentiated responses to the effects of noradrenaline in an artery or kidney perfused with jaundiced plasma in vitro. This possibility is examined in the experiments described below.

Methods and Materials

The arterial model used to assay the vasoconstrictor action of noradrenaline (NA) was similar to that used by De la Lande and Rande²³⁵. The technique involved the measurement of perfusion pressure in a constant flow system. Two preparations were used in different experiments, the isolated perfused artery segment and the perfused kidney.

New Zealand white rabbits were anaesthetised with intravenous pentobarbital sodium. Then either a kidney or a femoral artery was exposed. The preparation was cannulated proximally, removed from the animal, washed through with a heparinised Krebs solution and then perfused using a constant flow peristaltic pump (LAB1000) with Krebs solution at 37°C. The organ bath was also maintained at 37°C temperature using an outer thermostatically controlled water jacket. The Krebs solution contained NaCl 118.0, KCl 4.69 NaH_2PO_4 1.33 NaH_2CO_3 25.0 glucose 5.56 CaCl_2 2.52 and MgCl_2 1.05 mM/l and all perfused solutions were maintained at pH = 7.4 and constant P_{CO_2} and P_{O_2} by bubbling

was containing 5% CO_2 and 94% O_2 through the perfusate reservoir. (Figure 19). Perfusion pressure was monitored using a Statham BP²³ transducer connected to the flow system via a T tube and the perfusion rate was kept constant throughout each experiment at 4-5 ml/min. This rate gave inflow pressures of 80-85 mmHg in the kidney experiments and 5-10 mmHg in the aorta experiments.

The arterial constriction responses to NA were determined by injecting graded doses of NA in warmed Krebs as a bolus into the perfusate just proximal to preparation. The resultant changes in vessel calibre and resistance were monitored as the amplitude of change in perfusion pressure and recorded on a Beckman dynograph.

The system was allowed to stabilise for at least 30 minutes during perfusion with Krebs solution. Then a series of responses to graded NA doses was obtained. The perfusate was then changed to plasma which had been removed from one of ten baboons with surgically induced obstructive jaundice. All of the animals had their bile ducts ligated for at least two weeks and the plasma was removed and then stored in a freezer until it was used. This was allowed to perfuse for 60 minutes and then a further series of responses to NA was determined. The perfusate was again changed to Krebs and 60 minutes later another series of responses was obtained. In all plasma samples the plasma renin, total and conjugated bilirubin, blood urea, alkaline phosphatase, transaminases aspartate and potassium concentrations were measured. Five experiments using kidneys and five using femoral arteries were carried out plus a similar number of controls with each preparation using non-jaundiced plasma from sham operated baboons.

Analysis of results

Method I

The dose response data obtained in each individual experiment was plotted as the increase in perfusion

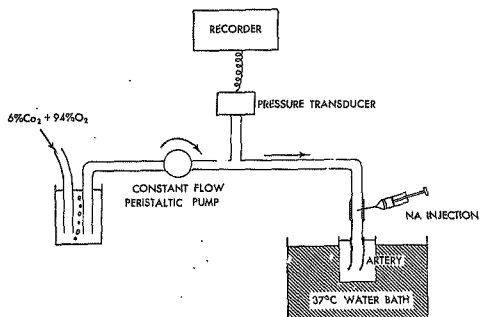


Figure 19. THIN ISOLATED PERFUSED ARTERY PREPARATION.

Page 121.

pressure (response - mmHg) against the logarithm of the dose (μ g NA). This produced three curves for each experiment; one for the control (Krebs) perfusate, one for the jaundiced plasma perfusate and one for the washout with Krebs solution. The responses to noradrenaline at equivalent doses when Krebs was perfused, was compared to the responses when jaundiced plasma was perfused by a Students "t" test for paired data.

Method II

a) Lineweaver Burke.

The dose-response data obtained in each individual experiment was plotted on the reciprocal of the perfusion pressure increase (1/response, mmHg) against the reciprocal of the dose (1/dose of NA μ g). This produced straight line relationships in each experiment as predicted from the Michaelis-Menten kinetics for drug-receptor interaction²³⁶.

$$\frac{1}{R} = \frac{K_{eq}}{R_{max}} - \frac{1}{D} + \frac{1}{R_{max}}$$

where R = response in mmHg

D = NA dose (μ g)

R_{max} = measurement response (mmHg)

K_{eq} = equilibrium coefficient for drug-receptor interaction.

In each experiment values of R_{max} and K_{eq} were calculated from the intercepts of the line of the y and x axes, respectively. When $1/D = 0$, $1/R$ (y intercept) is equal to $1/R_{max}$. When $1/R = 0$ then $1/D$ (x intercept) is equal to $-1/K_{eq}$. Changes in $1/R_{max}$ imply changes in efficiency of the drug receptor complex, while changes in $-1/K_{eq}$ imply changes in drug receptor affinity.

b) Hofstee

Although the Lineweaver Burke procedure provides useful

information on drug receptor inter-relationships it is however a double reciprocal plot, i.e. the reciprocal of response is plotted as a function of the reciprocal dose of the drug. When the responses are small, any error in measurement is magnified by this double reciprocal procedure. In Hofstee procedure the response is expressed as a function of the ratio R/D ²²⁷. Therefore the error is less since only one reciprocal is used. In addition the spacing of the points on the axis R/D are more evenly distributed than in the Lineweaver Burke procedure. In the Hofstee procedure the Michaelis-Menton equation is rearranged.

$$R = -K_{eq} - \frac{R}{D} + R_{max}$$

The slope of the line is $-K_{eq}$ and the y intercept is R_{max} . In the experiments using jaundiced plasma the results were analysed by both the Hofstee and the Lineweaver Burke procedure.

Results

Figure 20 shows three sets of responses obtained from a perfused kidney. The top trace shows the pressure changes in the perfusate after injections of doses of noradrenaline NA ranging from 0.08 - 8 μg as a bolus dose. In some of the kidney experiments this whole range of noradrenaline doses was not used. The middle trace shows the pressure changes after injection of NA when the preparation was perfused with jaundiced plasma and the bottom trace shows the responses after a period of washout with Krebs solution. It is clear that the responses to NA are much larger in the presence of jaundiced plasma. When the plasma was replaced with the Krebs solution the NA sensitivity of the preparation returned towards normal. Similar results were found for the kidney experiments and the femoral artery experiments. These responses in each experiment can be seen in Table 19. Occasionally it was found that the kidney was so sensitive to the effect of noradrenaline

Page 123.

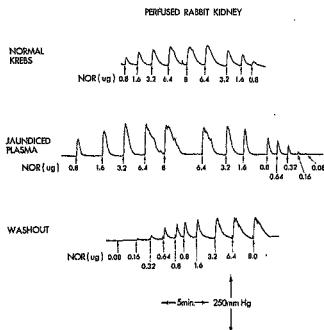


Figure 20. RESPONSES OF ISOLATED PERFUSED KIDNEY

- TOP TRACE - Response recorded during perfusion with Krebs solution.
- MIDDLE TRACE - Response during perfusion with jaundiced plasma.
- BOTTOM TRACE - Response during perfusion with Krebs solution on washout.

TABLE 19

Responses in perfusion pressure (mmHg) with dose of NA ranging from 0.08 - 8.0 μ g. Values are shown during Krebs (K) solution perfusions and during jaundiced plasma perfusion (JP).

Exp. No.		0.08	0.16	0.32	0.64	NA dose (μ g)					3.2	6.4	8.0
						0.8	1.6	3.2	6.0	8.3			
Arteries 1	K	-	-	-	0	0	5.0	6.0	8.3	9	-	-	-
	JP	3	4.6	6.3	8.3	8.6	12.0	-	-	-	-	-	-
2	K	-	-	-	1.0	2.6	4.3	5.3	7.3	8.3	-	-	-
	JP	3	3.3	4.3	5.0	7.8	-	-	-	-	-	-	-
3	K	-	2.0	4.3	5.6	5.3	12.3	18.3	-	-	-	-	-
	JP	9.3	12.0	16.6	18.4	-	-	-	-	-	-	-	-
4	K	-	-	-	0	0	6	6.5	6.6	7.3	-	-	-
	JP	4	6.6	8.3	8.6	8.8	11.3	-	-	-	-	-	-
5	K	-	-	-	-	0.6	2.0	5.3	5.6	6.6	-	-	-
	JP	-	-	-	-	1.3	2.6	7.3	8.6	9.6	-	-	-
Kidneys 1	K	6.2	12.5	43.7	68.7	28.1	62.5	81.2	100	100	-	-	-
	JP	-	-	-	-	84.3	131.2	156.2	159	150	-	-	-
2	K	0	6.2	5.0	17.5	21.2	43.7	75	-	-	-	-	-
	JP	5	12.5	25.0	53.7	67.5	80	-	-	-	-	-	-
3	K	0.7	2.5	5	17.5	10	47.5	65	-	-	-	-	-
	JP	8.7	18.7	38.7	67.5	-	-	-	-	-	-	-	-
4	K	-	-	6.2	15.6	18.7	46.9	46	62.5	-	-	-	-
	JP	12.5	-	25	50	56	118.7	168	-	-	-	-	-
5	K	0	6.2	18.7	31.2	54.1	87	-	-	-	-	-	-
	JP	31	43	43	54	-	-	-	-	-	-	-	-

Page 125.

after perfusion with jaundiced plasma, that it responded with large increases in pressure which did not return to the baseline (Figure 21.)

Figure 22 shows a $\log$ /response plot of the data from a typical kidney experiment. It can be seen that the jaundiced plasma caused the dose response curve to shift to the left of the control (Krebs) curve indicating that a potentiation of the effects of NA had occurred. In order to produce an increase of 50 mmHg in perfusion pressure, approximately 1 μ g of NA was needed when the perfusate was Krebs solution. When the jaundiced plasma was perfused, only about 0.5 μ g of NA produced the same response. When the plasma was washed out of the preparation with Krebs solution, the dose response curve shifted back towards the initial curve, showing that the changes induced by jaundiced plasma are not caused by an increased vascular sensitivity due to the passage of time.

An example of a control experiment using normal baboon plasma is shown in Figures 23 and 24. No significant potentiation occurred when normal plasma was perfused.

Table 20 shows the results of the biochemical investigations carried out on all of the specimens of plasma used. No correlation could be found between the degree of NA sensitivity changes and the degree of jaundiced (bilirubin mg%), urea levels, the level of transaminases, alkaline phosphatase, sodium, or potassium ions. In addition there was no consistent relationship between plasma renin activity and the sensitivity. Unfortunately there was no data for plasma renin activity in any of the isolated kidney experiments or in the kidney controls. All plasma renin assays were carried out in the baboons prior to exsanguination.

Figure 25 shows a double reciprocal plot of data from a typical kidney experiment. It can be seen that both the slope and x intercept of the line obtained with Krebs

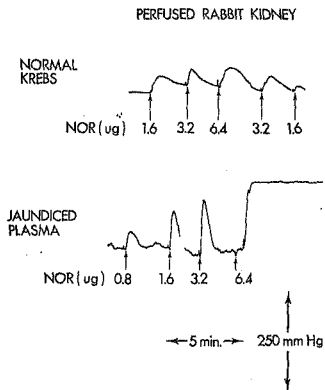


Figure 21. Shows response of an isolated perfused kidney during perfusion with jaundiced plasma (lower trace). It can be seen that at a dose of 6.4 μ g of noradrenaline there was a large increase in pressure. This did not return to the baseline within one hour, although the complete trace is not shown.

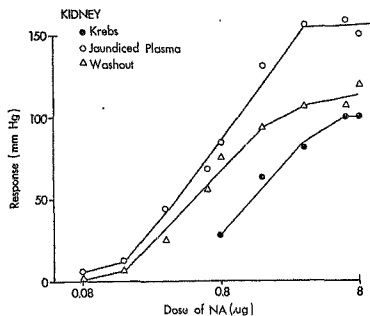


Figure 22. LOG/DOSE RESPONSE CURVE FROM KIDNEY EXPERIMENT.

It can be seen that jaundiced plasma causes a shift to the left of the response curve.

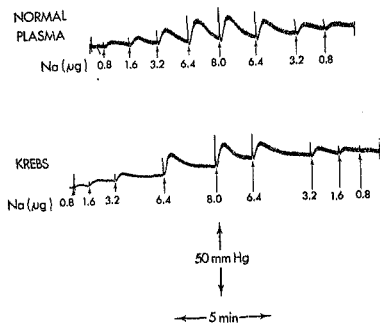


Figure 23. RESPONSES OF ISOLATED KIDNEY DURING PERFUSION
WITH NORMAL PLASMA.

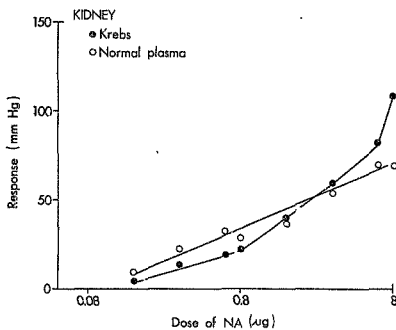


Figure 24. LOG/DOSE RESPONSE CURVE DURING PERFUSION WITH
NORMAL PLASMA.

TABLE 20

The Biochemical Investigations

Page 130.

ARTERIES

(a) Jaundiced plasma

No.	Billi- rubin (Direct) Units	S.G.O.T. Units	S.G.P.T. Units	Alk. Na ⁺ Units. l	K ⁺ Urea meq/l	Phos meq/l	Renin activity pg/ml/hr
1.	11.5 (8.2)	78	76	624	145	3.8	41
2.	16.5 (9.5)	59	78	532	146	4.2	41
3.	9.0 (5.9)	86	195	145	140	4.0	36
4.	10.3 (6.6)	86	99	237	138	3.8	32
5.	22.1 (13.3)	65	50	433	143	4.0	38

(b) Normal plasma

No.	Billi- rubin (Direct) Units	S.G.O.T. Units	S.G.P.T. Units	Alk. Na ⁺ Units. l	K ⁺ Urea meq/l	Phos meq/l	Renin activity pg/ml/hr
1.	0.5 (0.2)	49	37	33	138	3.5	30
2.	0.5 (0.2)	43	40	13	140	3.0	32
3.	0.5 (0.2)	49	39	17	135	4.0	31
4.	0.5 (0.2)	39	25	7	143	3.8	28

KIDNEYS

(a) Jaundiced plasma

No.	Billi- rubin (Direct) Units	S.G.O.T. Units	S.G.P.T. Units	Alk. Na ⁺ Units. l	K ⁺ Urea meq/l	Phos meq/l	Renin activity pg/ml/hr
1.	9.1 (5.7)	65	50	254	141	3.2	32
2.	16.6 (13.5)	78	76	434	140	3.0	48
3.	16.6 (13.5)	86	195	668	138	3.4	36
4.	11.5 (8.2)	51	36	280	142	4.0	40
5.	16.4 (11.2)	96	99	165	145	3.9	30

(b) Normal plasma

No.	Billi- rubin (Direct) Units	S.G.O.T. Units	S.G.P.T. Units	Alk. Na ⁺ Units. l	K ⁺ Urea meq/l	Phos meq/l	Renin activity pg/ml/hr
1.	0.5 (0.2)	43	40	9	143	3.9	25
2.	0.5 (0.2)	49	37	25	140	4.0	30
3.	0.5 (0.2)	86	95	7	138	4.2	36
4.	0.5 (0.2)	29	29	12	142	3.8	32

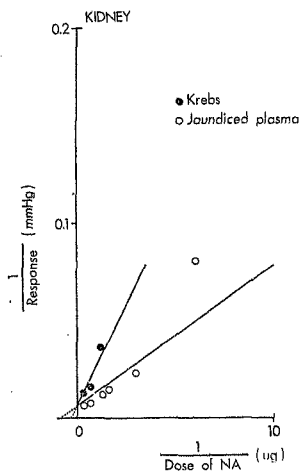


Figure 25. LINWEAVER-BURKE (DOUBLE RECIPROCAL) PLOT OF
DATA FROM ONE KIDNEY EXPERIMENT

perfusion is different from those during jaundiced plasma perfusion, whereas the y intercept in this particular example is virtually unchanged. Thus the maximum response (R_{max}) of this artery had remained almost the same while there was a change in the equilibrium coefficient (K_{eq}). Figure 26 shows a Hofstee plot of the responses in Krebs compared to the responses in plasma using a typical femoral artery experiment. It can be seen that both the slope and x and y intercepts are different when the responses in Krebs are compared to the responses when jaundiced plasma was perfused.

Table 21 shows the values of K_{eq} and R_{max} calculated in all experiments using a Lineweaver-Burke plot both during Krebs and during plasma perfusion. Using a paired "t" test the K_{eq} and R_{max} (jaundiced plasma) was found to be significantly different from the K_{eq} and R_{max} (Krebs) in the artery experiments. In the kidney experiments the K_{eq} (jaundiced) was less than the K_{eq} (Krebs) and there was a tendency for R_{max} (jaundiced) to be increased but these differences were not statistically significant.

These results show that perfusion with jaundiced plasma potentiates the response of both the isolated perfused kidney and the femoral arteries to infused NA, and this change is determined by a modified equilibrium coefficient although the tissues maximum response tended to be increased. Table 22 shows the comparison of the values obtained for K_{eq} and R_{max} using a Lineweaver-Burke plot or a Hofstee plot for the five femoral artery experiments. Although it was mentioned that the Line-weaver Burke plot may be inaccurate when the responses are small, it can be seen that there is essentially very little difference between the values obtained by either method. Therefore for the rest of these experiments using the isolated perfusion system, only the Lineweaver-Burke plot was used.

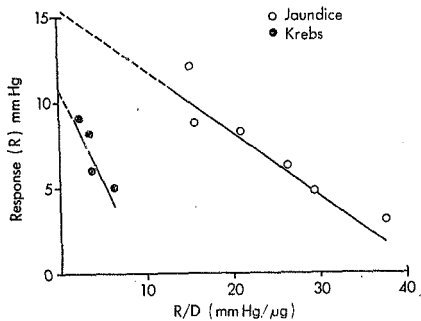


Figure 26. HOFSTEE PLOT OF DATA FROM A KIDNEY EXPERIMENT.

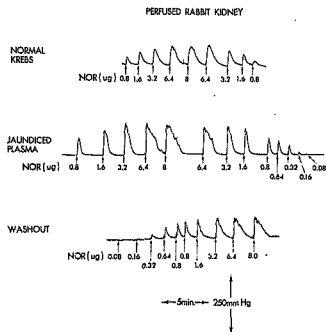


Figure 20. RESPONSES OF ISOLATED PERFUSED KIDNEY

- TOP TRACE - Pressure responses during perfusion with Krebs solution.
- MIDDLE TRACE - Responses during perfusion with jaundiced plasma.
- BOTTOM TRACE - Responses during perfusion with Krebs solution on washout.

TABLE 21

Values of $R_{\max}$ and K_{eq} for all experiments and controls.

ARTERIES

(a) Jaundiced plasma expts.

No.	K_{eq}		$R_{\max}$	
	Krebs	Plasma	Krebs	Plasma
1.	2.5	1.5	7.5	14.4
2.	4.3	3.5	10.5	12.5
3.	0.9	0.3	10.8	14.7
4.	1.9	0.0	9.1	15.0
5.	1.3	0.1	19.6	20.8

p < .01

p < .01

KIDNEYS

(b) Jaundiced plasma expts.

No.	K_{eq}		$R_{\max}$	
	Krebs	Plasma	Krebs	Plasma
1.	30.0	1.2	100.0	200.0
2.	0.1	0.6	11.6	16.7
3.	1.5	0.2	50.0	126.7
4.	9.0	0.05	500.0	526.0
5.	4.3	0.6	125.0	111.0

N.S.

N.S.

(b) Normal plasma controls

No.	K_{eq}		$R_{\max}$	
	Krebs	Plasma	Krebs	Plasma
1.	0.9	0.6	9.8	4.9
2.	1.9	1.8	4.0	2.0
3.	0.6	0.5	5.4	5.9
4.	14.9	9.2	12.3	7.9

N.S.

N.S.

(b) Normal plasma controls

No.	K_{eq}		$R_{\max}$	
	Krebs	Plasma	Krebs	Plasma
1.	1.1	1.0	166.7	100.0
2.	5.3	1.5	166.7	83.3
3.	2.8	3.8	55.5	166.7
4.	6.4	1.2	200.0	125.0

N.S.

N.S.

TABLE 22

Comparison of Lineweaver-Burke Plot and Hofstee Plot for values of K_{eq} and R_{max} .

<u>LINEWEAVER-BURKE</u>				<u>HOFSTEE</u>	
		<u>R_{max}</u>	<u>K_{eq}</u>	<u>R_{max}</u>	<u>K_{eq}</u>
1.	K	10.8	0.9	10.9	1.0
	J P	14.7	0.3	15.5	0.3
2.	K	7.5	2.1	7.5	2.1
	J P	14.4	2.0	13.5	1.7
3.	K	9.1	1.9	9.3	1.9
	J P	15.0	0.1	15.2	0.1
4.	K	10.6	4.3	9.7	3.6
	J P	14.5	3.5	11.6	2.9
5.		19.6	1.3	20.8	1.4
		20.8	6.1	21.5	6.1

Discussion

These results show that jaundiced plasma contains some factor or factors which potentiates the response of isolated vessels to the effects of noradrenaline. It would appear therefore that the increased sensitivity of renal and cerebral circulation to the effects of NA in obstructive jaundice can be explained by a factor or factors present in the plasma and not by some structural damage to the vasculature, although this has not been directly excluded.

Analysis by Lineweaver-Burke or Hofstee methods gave similar results and showed that the equilibrium coefficient was decreased and the maximum response of the vessels increased. It is likely that if more experiments were performed in the kidney experiments that the increase in the maximum response would have been statistically significant. There was however a consistent decrease in the equilibrium coefficient (K_{eq}) for the drug receptor interaction. This is consistent with an increase in affinity of the receptor for the drug i.e. more drug receptor complex has been formed.

The increase in R_{max} can only be explained by an increased efficiency of the drug receptor complex which may be the result of an increase in tissue excitability, or the unmasking of additional adreno-receptors. It is also possible, however, that the drug is having an allosteric effect. To test this possibility Hill proposed an equation by which it is possible to evaluate whether a drug receptor action is due to allosterism or not²³⁷. This equation is:

$$R = \frac{R_{max} (D)^n}{(D)^n + K^1}$$

where n = number of sites binding the substrate or the interaction coefficient, and K^1 replaces K_{eq} in the Michaelis-Menten equation.

If $n = 1$ the equation becomes identical to the

Michaelis-Menten equation and in this case $K^1 = K_{eq}$. When an allosteric effect is produced K^1 is not $= K_{eq}$ and n becomes interally greater than one²³⁷.

Table 23 shows the values for n when the formula was applied to the results obtained from perfusion with jaundiced plasma in the femoral artery experiment. It can be seen that n is almost $= 1$ in all experiments. Therefore the increase in R_{max} is not due to an allosteric effect and is more likely due to unmasking of more receptors or an increased excitability of the tissues from some alteration in membrane characteristics.

Potentiation of responses to NA in isolated perfused rabbit ear artery have been shown when Krebs was replaced with normal rabbit plasma or whole blood²³⁸. This was suggested to be due to the interaction between 5-hydroxytryptamine (which is present in large amounts in rabbit plasma) and injected NA. Since enzymes responsible for 5-hydroxytryptamine (5-HT) breakdown are present in the liver²³⁹, it is possible that an increase in the levels of circulating 5-HT could explain these results. However, 5-HT in other species is normally carried in the platelet fraction of the blood²⁴⁰ with little or none in the plasma. Thus, significant amounts of 5-HT are unlikely to be present in the samples of jaundiced plasma although this has not been measured directly. This would suggest that 5-HT plays little part in the phenomenon.

Thus these experiments show that the jaundiced plasma increases the response of the vasculature to the effects of noradrenaline in vitro similar to those found in vivo in the renal and cerebral circulations. This would appear to be produced by a decreased equilibrium coefficient which is explained by an increased drug receptor complex i.e. an increased receptor affinity for the drug. There is also an increased maximum response of the tissue which could be explained by increased tissue excitability or by an unmasking of more receptor sites.

Shows value of interaction coefficient (n) for results during Krebs perfusion and after perfusion with jaundiced plasma in 5 experiments with an isolated perfused artery.

<u>EXPT. NO.</u>	<u>KREBS</u>	<u>JAUNDICED PLASMA</u>
	n	n
1.	1.32	1.09
2.	1.05	0.91
3.	1.45	1.06
4	1.25	1.02
5.	1.12	1.06

EFFECTS OF HYPOCHOLESTEROLAEMIC PLASMA, CHOLESTEROL AND
LOW-DENSITY LIPOPROTEINS ON THE VASCULAR SENSITIVITY TO
THE EFFECTS OF NORADRENALINE.

Introduction

The experiments both in vivo and in vitro showed that in obstructive jaundice there is a heightened response to NA. McCalden and Ridelman²²⁸ have shown that when uptake of noradrenaline into vascular smooth muscle (Uptake 2) is inhibited there is an enhanced cerebrovascular response to NA, which is almost identical to that obtained in animals following ligation of the common bile duct. Salt and Iversen²⁴¹ have demonstrated that cholesterol (when dissolved in alcohol and added to Krebs solution) is capable of uptake 2 inhibition in cardiac muscle. If the vascular smooth muscle behaves in a similar manner this could explain the results obtained in jaundiced animals and in the isolated artery preparations. An inhibition of uptake 2 processes would result in more NA being available for drug receptor combination and thus a heightened response of the vessel could be expected.

In obstructive jaundice there is a large increase in total serum cholesterol due to an overproduction by the liver²⁸. It is possible that one of the factors responsible for the increased sensitivity to NA is an increase in total plasma cholesterol. This is carried in plasma mainly on the low density lipoproteins although about 20% may be carried on the high density lipoproteins²⁴². A situation analogous to obstructive jaundice exists in Fredrickson's Type IIb hyperlipidaemia where the low density lipoproteins (LDL) are greatly increased²⁴³.

Therefore in the experiments below using the isolated artery preparation described previously, the effects of increased response of the vessels to NA of cholesterol in

saline, cholesterol in plasma, cholesterol in hyperlipidaemic plasma and a low density lipoprotein extract was investigated. The experimental protocol can be seen in Table 24.

Material and Methods

The arterial model used was the same as that described in the previous section. In addition the experimental protocol followed was identical to that described for those experiments. However, only arteries were used in these experiments. In addition it was decided not to use a femoral artery in these experiments but to use the main renal artery in the preparation.

a) Preparation of hyperlipidaemic plasma

60 ml of blood was taken from each of 15 fasting patients attending a special clinic for hyperlipidaemia (from whom informed consent has been obtained). In each case it was confirmed by both ultracentrifugation and electrophoresis that these patients were true Fredrickson's Type IIc hyperlipidaemics. The blood was centrifuged for 3 minutes at 2,500 revs/min. and the supernatant plasma removed. Plasma urea and electrolytes were measured in each sample. In each experiment three plasma samples were selected at random, pooled and analysed for total cholesterol, beta cholesterol (LDL) and triglyceride concentration. This plasma was perfused in exactly the same manner as that described for perfusion of jaundiced plasma. Six experiments using hypercholesterolaemic plasma (HC) were performed and five using plasma from normal patients as controls.

b) Pure cholesterol

Pure cholesterol (B.D.A.) was dissolved either in saline or in plasma. It was found that it was difficult to obtain high concentrations of cholesterol using this method and a maximum concentration of 10 mg/ml was obtained. Cholesterol was also dissolved in propenol and this solution was added

TABLE 24

Experimental protocol for examining the effects of cholesterol on the responses of an isolated perfused artery to the actions of noradrenaline. All responses compared to responses during Krebs solution perfusion.

- (a) Test: Artery perfused with plasma from Type IIA hyperlipidaemic patients - 6 expts.

Control: Artery perfused with plasma from normal subjects - 5 expts.

- (b) Test: Artery perfused with cholesterol in Krebs - 5 expts. Artery perfused with cholesterol in plasma - 10 expts. Artery perfused with cholesterol in propanol - 5 expts.

Control: Propanol alone dissolved in Krebs perfused - 3 expts.

- (c) Test: Artery perfused with low density lipoprotein concentrated from normal subjects - 5 expts.

Control: Artery perfused with bovine albumin - 5 expts.

to Krebs solution and perfused using the technique already described for junctioned plasma. In addition propanol alone was added to Krebs and the responses of the vessel to the effects of noradrenaline were obtained when this was perfused. Five experiments were carried out using cholesterol dissolved in saline, 10 experiments using cholesterol dissolved in plasma and 5 experiments using cholesterol dissolved in propanol in concentrations ranging from 20 mg/1 to 50 mg/1 were used. Three experiments using propanol alone dissolved in Krebs, were also carried out (Table 24).

c) Preparation of low density lipoproteins (beta lipoprotein)

Plasma was removed from 10 normal fasting patients. These samples were spun in a Beckman G265B ultracentrifuge at 19,000 r.p.m. for a period of 30 minutes to remove the chylomicrons. Then the samples were spun at 40,000 r.p.m. for 18 hours with a density solution containing 11.4 gr NaCl; 0.1gr K_2EDTA ; 1 ml NaOH made up to one litre with distilled water and of specific gravity 1.006. Very low density lipoproteins (pre-beta lipoproteins) moved from plasma to this solution and were removed. The remaining plasma was spun further at 40,000 r.p.m. for 20 hours with a second density solution containing 24.98gr NaBr in a 100 ml of first density solution and of specific gravity of 1.182. The low density lipoproteins (β -lipoproteins) moved from plasma into this solution and their presence was confirmed using a Technicon All automatics as well as by electrophoresis. These low density lipoproteins were then dialysed against normal saline for 24 hours to remove the EDTA and bromide. This concentrated lipoprotein solution was added to Krebs solution and was infused into the artery in concentrations ranging from 80-110 mg%. Five experiments were carried out plus an equal number of controls using bovine albumin (MURCK) at doses of 50-250 mg%.

Results

a) Hyperlipidaemic plasma.

Figure 27 shows three sets of responses obtained from a single artery experiment. The top trace shows the pressure

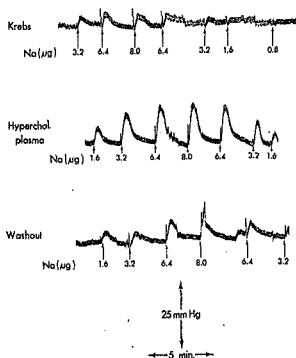


Figure 27. PERFUSION WITH HYPERCHOLESTEROLÆMIC PLASMA

- Upper Trace - During perfusion with Krebs solution.
- Middle Trace - During perfusion with hypercholesterolaemic plasma.
- Bottom Trace - During perfusion with Krebs solution as washout.

changes in the perfusate after injection of NA ranging from $0.8 \mu\text{g}$ to $8 \mu\text{g}$. The middle trace shows that pressure changes after injections of doses of NA ranging from $1.6 \mu\text{g}$ to $8 \mu\text{g}$ when the artery was perfused with hyperlipidaemic plasma and the bottom trace after a period of washout with Krebs. It is clear that the responses to NA are much larger in the presence of hypercholesterolaemic plasma. Table 25 shows the individual responses obtained in each of the six experiments before and during perfusion with hypocholesterolaemia (HC plasma). Using a paired "t" test the response with HC plasma was found to be significantly different from the responses in Krebs in four of the six experiments. Another similar experiment is shown in Figure 28, as a log dose versus response plot. The potentiation of the NA responses in the presence of HC plasma is seen as a shift of the curve to the left of the control curve. After washout with Krebs the dose-responses were shifted back towards (but never quite reached) the initial curve. When normal plasma was perfused no potentiation was seen (Figure 29).

Figure 30 shows a double reciprocal plot of the data from a typical experiment. In this it can be seen that both the slope and the x intercept obtained with the Krebs solution are different from those obtained during perfusion with HC plasma. The y intercept is also changed. Thus the maximum response of R_{max} (of this artery) is increased slightly while there was a large change in the drug receptor equilibrium coefficient (K_{eq}). Table 26 shows the values K_{eq} and R_{max} for all HC experiments. Using a paired "t" test the K_{eq} (HC) was found to be significantly different ($p < 0.05$) from the K_{eq} (Krebs). Although there is a tendency to R_{max} to be increased with the HC plasma this was not significant at the 5% level. There was no significant change in either K_{eq} or R_{max} in the normal plasma control. The assayed values of the total cholesterol and percentage cholesterol were also shown in Table 26. There was no correlation between the degrees of NA potentiation and

TABLE 25

The perfusate pressure changes (mm Hg) in six experiments when noradrenaline was injected in doses ranging from 0.8 to 8.0 μ g. The values are shown during Krebs (K) and during hypercholesterolaemic plasma (HC) perfusion. The results of paired "t" tests are shown on the right.

		<u>NA dose (μg)</u>					
EXPT NO.		0.8	1.6	3.2	6.4	8.0	
1	K	0	1.2	3.4	4.7	5.0	$p < 0.05$
	HC	2.5	5.3	10.6	14.7	17.5	
2	K	0	0.5	0.7	1.3	1.5	$p < 0.02$
	HC	1.0	1.7	3.8	5.0	5.0	
3	K	1.8	2.9	3.7		8.8	$p < 0.01$
	HC	5.1	10.3	16.9	18.8	22.1	
4	K	0.2	1.0	0.7	0.9	1.2	N.S.
	HC	1.1	1.3	2.1	2.7	6.2	
5	K	6.3	7.5	16.9	30.0	-	N.S.
	HC	15.0	33.7	43.7	-	-	
6	K	0.9	2.5	4.7	6.9	6.6	$p < 0.05$
	HC	8.4	13.8	18.8	-	-	

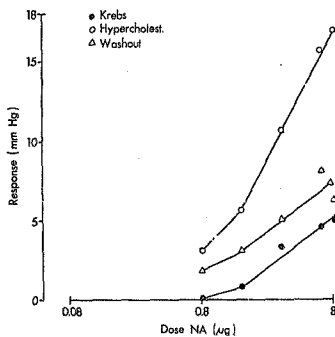


Figure 28. LOG/DOSE RESPONSE CURVES OBTAINED DURING PERIUSION WITH PRESS AND DURING HYPERCHOLESTEROL-EMULSIFIED PLASMA.

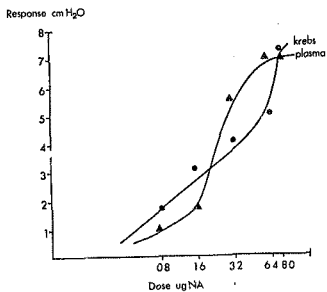


Figure 29. LOG/DOSE RESPONSE CURVE OBTAINED DURING
PERFUSION OF NORMAL HUMAN PLASMA.

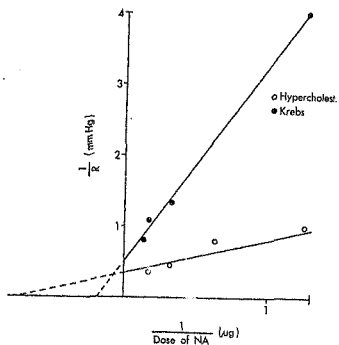


Figure 30. DOUBLE RECIPROCAL PLOT (LINWEAVER-BURKE)
 FROM EXPERIMENT COMPARING RESPONSES IN KREBS
 SOLUTION TO THOSE WITH HYPERCHOLESTEROLAEMIC
 PLASMA.

TABLE 26.

The equilibrium coefficient (K_{eq}) and the maximal possible response (R_{max}) calculated for each hypercholesterolaemic plasma experiment and each normal plasma control. The significance (paired "t" test) of any changes are shown at the base of each column. Also shown are the values of total cholesterol (mg%) and the % β cholesterol for each of the plasmas used.

HYPERCHOLESTEROLAEMIA EXPERIMENTS

Expt. No.	Total Cholesterol mg %	β cholesterol %	K_{eq}		R_{max}	
			Krebs	HC Plasma	Krebs	HC Plasma
1	415	72	7.2	1.0	2.5	14.3
2	370	70	4.5	5.0	12.5	40.0
3	525	75	33.0	20.0	33.3	100.0
4	326	71	5.5	1.3	1.9	2.3
5	300	73	10.0	3.1	166.0	250.0
6	490	70	5.0	0.1	40.0	20.5
			$P < 0.05$		$0.05 < P < 0.1$	

NORMAL PLASMA CONTROLS

1	170	65	15.0	2.9	12.5	1.5
2	165	69	8.9	2.9	33.3	16.6
3	200	70	1.1	2.2	4.3	5.3
4	195	65	2.7	8.0	25.0	27.7
5	201	68	2.9	4.2	6.7	9.1
			N.S.		N.S.	

total cholesterol concentration. In all of the experiments a high correlation coefficient when the dose and response was obtained (0.78 - 0.98).

b) Pure cholesterol

In the five experiments performed when cholesterol was dissolved in saline no potentiation of the response to noradrenaline was found at any of the concentrations of cholesterol used. (10 μ C/ml - 50 μ C/ml). Also using the maximal possible concentration of cholesterol, no potentiation of the effects of NA was found in any of the ten experiments carried out when cholesterol was dissolved in plasma or when perfuser dissolved in propenol. It was also found that propenol added either to Krebs or to normal plasma had no effect on the vascular response to NA.

c) Low density lipoproteins (beta-lipoproteins)

Figure 31 shows the responses obtained to NA in an isolated artery before, during and after perfusion of 100 μ g of low density lipoproteins (LDL). Although the doses of NA used with lipoprotein were 1/10th those in Krebs, much larger responses were obtained. This would indicate a shift of the log-dose response curve to the left during LDL perfusion, as shown in Figure 32. Comparison by a paired "t" test of the responses in Krebs to the responses when lipoproteins were added, shows the response in lipoproteins to be significantly different ($p < .05$) in all five experiments. These responses in each experiment can be seen in Table 27.

Table 28 shows the calculated values of K_{50} and E_{max} (from a Lineweaver-Burke plot) for all five experiments using low density lipoprotein. A decrease in K_{50} and an increase in E_{max} occurred. No significant change in NA sensitivity occurred in any of the controls when bovine albumin was used.

Discussion:

When pure cholesterol was dissolved in either plasma or

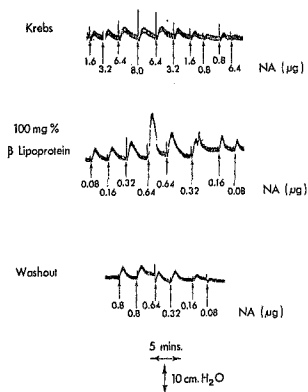


FIGURE 31. PERFUSION WITH LOW DENSITY LIP. PROTEINS
(β - LIPOTROPIC)

Upper Trace - During perfusion with Krebs solution.
Middle Trace - During perfusion with lipoprotein.
Bottom Trace - During washout with Krebs solution.

TABLE 27

Perfusate pressures (cms H₂O) when noradrenaline (NA) was injected in doses varying from 0.08 - 8 μ g. Values are shown during Krebs (K) and during perfusion with β lipoprotein (LDL).

NA Dose (μ g)

Exp. No.		0.08	0.16	0.32	0.64	0.8	1.6	3.2	6.4	8.0
1	K	-	0	0	0	2.5	3.5	3.0	5.0	5.5
	LDL	5.5	6.0	9.0	25.0	-	-	-	-	-
2	K	-	0	0	0	3.0	6.0	7.0	12.5	16
	LDL	-	2.0	3.0	7.5	9.0	18.5	30.5	-	-
3	K	0	0	0	0	1.5	2.5	5.0	7.5	7.5
	LDL	3.5	4.5	7.5	27	29	29.5	-	-	-
4	K	0	0	0	.5	1.5	5.5	7.5	100	10.5
	LDL	1.5	5.5	10		26.0	30.5	29.0	-	-
5	K	-	0			1.5	2.0	10	5.5	20.5
	LDL	4.0	4.0	7.5		35	35	37	-	-

TABLE 27

Perfusate pressures (cms H₂O) when noradrenaline (NA) was injected in doses varying from 0.08 - 8 μ g. Values are shown during Krebs (K) and during perfusion with β lipoprotein (LDL).

Page 18.

NA Dose (μ g)

Exp. No.		0.08	0.16	0.32	0.64	0.8	1.6	3.2	6.4	8.0
1	K	-	0	0	0	2.5	3.5	3.0	5.0	5.5
	L	5.5	6.0	9.0	25.0	-	-	-	-	-
2	K	-	0	0	0	3.0	6.0	7.0	12.5	16
	L	-	2.0	3.0	7.5	9.0	18.5	30.5	-	-
3	K	0	0	0	0	1.5	2.5	5.0	7.5	7.5
	L	3.5	4.5	7.5	27	29	29.5	-	-	-
4	K	0	0	0	1.5	1.5	5.5	7.5	100	10.5
	L	1.5	5.5	10	14.0	26.0	30.5	29.0	-	-
5	K	-	0	0	0	1.5	2.0	10	5.5	20.5
	L	4.0	4.0	7.5	9.5	15	35	37	-	-

TABLE 28

The values of the equilibrium coefficient (K_{eq}) for the NA receptor interaction and the maximal response (R_{max}) to NA in five experiments when Krebs was followed by perfusion with a flipoprotein solution (LDL).

Expt. No.	K_{eq}		R_{max} (cm H ₂ O)	
	Krebs	protein	Krebs	protein
1	0.85	0.19	5.08	16.95
2	5.14	5.86	22.73	71.43
3	2.49	0.75	11.49	15.53
4	2.42	1.44	13.89	55.56
5	13.89	2.23	52.63	58.82
$p < .05$		$p < .05$		

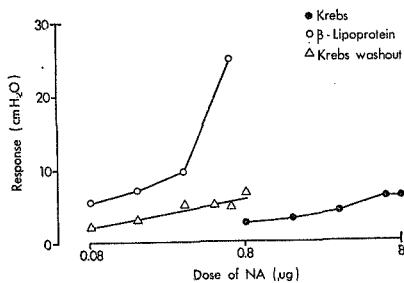


Figure 32. LOG/DOSE RESPONSE CURVES OBTAINED DURING PERFUSION WITH KREBS AND DURING LIPOPROTEIN PERFUSION.

saline no potentiation of the effects of noradrenaline were observed. The concentrations used were in excess of those used by Salt and Iverson in heart muscle but lower than those found in hyperlipidemic plasma. Salt and Iverson report that the extra-neuronal uptake of NA into heart muscle was inhibited²⁴¹. The failure to demonstrate the potentiation of the responses to NA in the vascular preparation may mean that vascular smooth muscle is not as sensitive to cholesterol as heart muscle, and that much larger concentrations of cholesterol are needed.

Large increments of plasma cholesterol are found in Fredrickson's Type IIa hyperlipidemic. This is largely carried in plasma as an increase in the LDL (beta lipoprotein) fraction²⁴³. When plasma from these patients was used potentiation of the responses to NA was observed. This potentiation might have been achieved either because the concentration of cholesterol was much higher than in the previous experiments; or because when cholesterol is attached to its apoprotein it is much more effective in this regard. There may also have been some other factor in the hyperlipidemic plasma which potentiated NA. However, this seems unlikely as the experiments with isolated low density lipoprotein would indicate that this constituent of the plasma was primarily responsible for the potentiation. This would strengthen the hypothesis that cholesterol attached to its apoprotein is more effective than pure dissolved cholesterol. An important control experiment would have been the infusion of the apoprotein alone i.e. separate from the cholesterol moiety. Various attempts were made to separate the cholesterol from its apoprotein but these were all unsuccessful due to the tendency of the protein to denature. The analysis of the data via Michaelis-Menton kinetics indicates that when the vascular smooth muscle is exposed to a high cholesterol concentration there is a decreased equilibrium coefficient (K_{eq}) for the noradrenaline receptor interaction and an

increased maximal response (R_{max}). This increase as already mentioned must be associated with increased intrinsic activity of the tissue and has already been discussed at length when considering the changes found when jaundiced plasma was perfused.

These results would therefore suggest that when vascular smooth muscle is exposed to a high cholesterol concentration, the muscle becomes more sensitive to NA. This hypersensitivity may be mediated by an inhibition of extra neuronal uptake mechanism, similar to that previously reported in heart muscle but other mechanisms are also possible and will be discussed later. These results are also similar to those obtained when jaundiced plasma was perfused. Therefore it is possible that the increase in cholesterol which is carried in the low density lipoproteins is one of the factors involved in producing the increased sensitivity to the effects of noradrenaline found in both vivo and in vitro experiments reported in obstructive jaundice. This possibility will be studied in further experiments to be described below.

EXPERIMENTS WITH EXTRACTS FROM JAUNDICED PLASMA

Introduction

The previous experiments have indicated that low density lipoproteins may be one of the factors responsible for the potentiated responses to noradrenaline which have been found both in vivo and in vitro. In experiments in which lipoprotein was perfused, this protein was a concentrate taken from normal human plasma. In the experiments described below the following was done

- (a) A deproteinized solution prepared from jaundiced plasma was perfused into the isolated femoral artery preparation in a similar manner to that previously described. The responses to injected NA when Krebs was perfused was then compared to those obtained during perfusion of this extract.
- (b) Low density lipoproteins (beta-lipoprotein) was separated from jaundiced plasma and perfused into the isolated artery preparations. The responses to noradrenaline when Krebs was perfused were compared to those obtained during perfusion of this extract in a similar manner to that previously described.
- (c) The solution of the jaundiced plasma obtained after the low density lipoproteins had been removed was perfused into the isolated artery preparation. The responses to noradrenaline obtained during this perfusion were compared to those obtained during Krebs perfusion.
- (d) Other constituents of the jaundiced plasma were perfused into the isolated artery preparation in order to determine whether potentiation of the responses to noradrenaline occurred. These experiments will now be discussed in detail below.

a) Experiments with Denroteinized Jaundiced Plasma

Preparation

Baboon plasma (from animals with induced obstructive jaundice) was denroteinized by adding 20 mls of 3% perchloric acid to 200 mls of plasma at 1°C. The acid was added slowly with constant magnetic stirring while the vessel containing the plasma was cooled in a large volume of ice-water mixture. When the protein precipitation was complete the extract was centrifuged at 10,000 x g for 30 minutes at 0°C after which the supernatant was neutralized to pH 7 with saturated potassium carbonate. The precipitated potassium chlorate was removed by centrifugation at 3,000 x g for 10 minutes at 0°C. The resultant supernatant was adjusted to pH 7.35 with a few drops of concentrated sodium hydroxide. Finally the supernatant was filtered under pressure to remove any trace of perchlorate particles remaining. This supernatant was then used as a protein-free extract of plasma. The dilution of the plasma by adding the perchloric acid was 10%.

Results

No potentiation occurred in five experiments using this solution in a similar manner described already for perfusion of jaundiced plasma into an isolated artery preparation. The responses in each of these experiments can be seen in Table 29.

b) Experiments with Low Density Lipoproteins (LDL) From Jaundiced Plasma

Preparation

The low density lipoproteins were generated from plasma obtained from baboons in whom the bile duct had been ligated two weeks previously. These lipoproteins were generated in an identical manner to that described previously. Low density lipoprotein (LDL) was however perfused back in concentrations ranging from 1 mg - 2.5 mg/ml, which is the same concentration as that found before the extraction.

TABLE 29

Shows perfusate pressure changes (cms H₂O) in four experiments when noradrenaline (NA) was infused in doses ranging from 0.8 - 8 μ g. Values are shown during Krebs perfusion (K) and during deproteinised jaundiced plasma perfusion.

Expt. No.	DOSE NA (μ g)					
		0.8	1.6	3.2	6.4	8.0
1	K	1.5	3.4	6.8	7.0	7.1
	P	1.3	2.7	5.9	8.9	7.0
2	K	3.6	4.8	5.0	6.8	7.0
	P	2.9	5.0	5.5	7.1	7.1
3	K	7.5	7.8	9.4	11.7	12.0
	P	5.6	6.9	11.8	11.0	12.5
4	K	1.0	1.5	2.0	3.5	4.7
	P	2.0	2.5	3.8	4.0	4.5

This is in contrast to the LDL obtained from normal plasma where increased concentrations were used.

Results

Potentiation of the pressor effects of NA occurred. A trace obtained during perfusion of this lipoprotein into an isolated artery preparation can be seen in Figure 33. The top trace shows the responses obtained when Krebs solution was perfused, the middle trace shows the responses obtained when the lipoprotein was perfused. It can be seen that the responses of the artery when lipoprotein from jaundiced plasma was perfused are much greater than those obtained when Krebs alone was perfused. The values obtained for K_{50} and R_{max} from these experiments can be seen in Table 30. It can be seen that here was both a decrease in K_{50} and an increase in R_{max} .

c) Experiments with Jaundiced Plasma from which LDL has been Removed.

The plasma remaining after the low density lipoprotein had been removed by the method described previously was infused into an isolated artery preparation in a similar manner to that previously described. Five experiments with this plasma were performed and it was found that potentiation of effects occurred, although this was not as great as when jaundiced plasma was perfused or when lipoproteins from jaundiced plasma were perfused into the isolated preparation. (Table 31). There was however a significant difference between the result obtained when Krebs alone was perfused and those obtained when this extract was infused ($p < 0.05$).

d) Constituents of Jaundiced Plasma

1) Bilirubin

Cryсталline bilirubin (unconjugated) was dissolved in 0.1 M sodium carbonate, and diluted with saline. This was added to Krebs solution and concentrations ranging from 5 mc to 7 mcg was obtained. Five experiments were carried out comparing the results obtained when bolus doses of

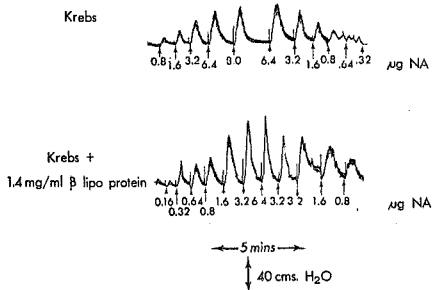


Figure 33. PERFUSION WITH LOW DENSITY LIPOPROTEINS
(β LIPOPROTEIN) FROM JAUNDICED PLASMA.

Top Trace - During perfusion with Krebs solution.

Middle Trace - During lipoprotein perfusion.

TABLE 30

Shows changes in the equilibrium coefficient (K_{eq}) and the maximum response (R_{max}) in five experiments when Krebs was followed by perfusion with low density lipoproteins (β lipoproteins) from jaundiced plasma.

<u>K_{eq}</u>		<u>R_{max} (cm H₂O)</u>	
<u>KREBS</u>	<u>LIPOPROTEIN</u>	<u>KREBS</u>	<u>PLASMA</u>
11.9	2.2	6.8	13.1
23.1	1.5	1.6	99.1
72.9	15.6	23.1	49.4
13.0	1.2	5.5	19.7
17.9	0.2	3.7	23.0
MEAN	27.7	8.1	40.8

$p < .05$

$p < .05$

TABLE 31

Shows perfusate pressure changes (cm H₂O) in five experiments when noradrenaline (NA) was infused in doses ranging from 0.8 - 8.0 μ g. Values are shown during Krebs perfusion (K) and during perfusion with jaundiced plasma from which the low density lipoproteins had been removed (P).

		<u>Dose NA (μg)</u>				
Expt. No.		0.8	1.6	3.2	6.4	8.0
1	K	1.2	1.4	2.6	3.8	4.0
	P	2.8	3.4	5.7	7.4	7.5
2	K	2.0	2.5	3.6	4.0	4.5
	P	3.5	4.5	5.0	8.1	6.5
3	K	1.0	1.1	2.0	2.4	2.7
	P	2.5	3.0	3.5	4.8	5.1
4	K	..9	1.7	2.5	3.0	4.1
	P	1.6	2.9	4.5	6.8	7.2
5	K	3.7	5.7	6.9	7.5	7.9
	P	4.5	6.8	8.5	9.0	8.9

noradrenaline were injected into an isolated artery preparation when Krebs alone was infused and when this was followed by a solution of Krebs containing unconjugated bilirubin. No potentiation occurred. (Table 32). Three experiments were also carried out using sodium carbonate alone and no difference was seen between the responses obtained when Krebs alone was perfused or when Krebs plus sodium carbonate was perfused. (Table 32).

2) Triglyceride

Experiments were performed using concentrations of glycerol dissolved in 2 ml of propenol in concentrations ranging from 50 mg to 150 mg%. Five experiments were carried out using this solution of triglyceride and Krebs and no difference was found between the responses obtained when Krebs alone was perfused and the responses obtained when Krebs plus triglyceride plus propenol was used. (Table 33). It has already been shown that propenol alone has no effect on the responses of the artery to injected bolus doses of noradrenaline.

3) Cholesterol esters

Cholesterol oleate was dissolved in propenol and added to Krebs solution in doses in concentration ranging from 50 mg to 150 mg%. Five experiments were carried out and no potentiation of the response of the vessel to the effects of noradrenaline was seen when these esters were added to Krebs solution. (Table 34).

4) Phospholipid

It has been shown that in obstructive jaundice the concentration of phospholipid is also increased⁸². However, it was not possible to find a suitable medium in which phospholipid could be dissolved so that it could be added to Krebs. Various attempts were made to dissolve the phospholipid in propenol and all of these attempts were unsuccessful. It was therefore not possible to determine whether a phospholipid played any role in potentiating the effects of noradrenaline in the isolated

TABLE 32

Shows perfusate pressure changes (cm H₂O) in five experiments when noradrenaline (NA) was infused in doses ranging from 0.8 - 8 μ g. Values shown during Krebs perfusion (K) and during perfusion with bilirubin dissolved in sodium carbonate (B.S.C.) or sodium carbonate dose (SC).

		Dose NA (μ g)				
Expt. No		0.8	1.6	3.2	6.4	8.0
<u>B.S.C.</u>						
1	K	1.4	1.4	2.6	3.1	4.5
	BSC	1.0	1.2	2.0	1.7	3.6
2	K	2.6	2.5	3.1	4.0	4.1
	BSC	2.7	3.0	3.5	4.1	4.0
3	K	1.9	1.7	2.3	3.2	4.1
	BSC	1.8	1.9	2.1	3.0	4.5
4	K	0.9	1.6	2.5	3.6	3.8
	BSC	1.1	1.7	2.6	3.5	3.6
5	K	3.7	3.6	4.0	5.6	5.8
	BSC	3.0	3.7	4.2	5.0	5.1
<u>S.C.</u>						
1	K	1.1	1.2	3.6	4.0	4.1
	SC	0.9	1.4	3.8	4.5	4.0
2	K	2.8	2.9	3.6	4.7	5.0
	SC	2.0	2.8	4.1	4.6	4.9
3	K	4.6	4.7	6.2	7.8	7.9
	SC	3.9	4.2	5.7	6.5	7.0

TABLE 33

Shows perfusate pressure changes (cm H₂O) in five experiments when noradrenaline (NA) was infused in doses varying from 0.8 - 8 μ g. Values shown during Krebe perfusion (K) and during infusion with triglyceride dissolved in propanol (TP).

Expt. No.	<u>Dose NA (μg)</u>				
	0.8	1.6	3.2	6.4	8.0
1	K 1.1	1.2	2.6	4.0	4.1
	TP 0.9	1.3	3.5	4.3	4.4
2	K 2.8	2.8	3.6	3.8	4.0
	TP 2.9	3.0	3.7	4.1	4.5
3	K 1.0	1.1	2.6	3.0	4.1
	TP 1.3	1.5	2.7	3.6	4.9
4	K 2.7	2.6	3.6	4.0	4.1
	TP 2.6	2.5	3.0	3.9	3.9
5	K 1.6	1.7	2.5	2.9	3.0
	TP 0.9	1.2	2.1	2.7	3.1

TABLE 34

Shows perfusate pressure changes (cm H₂O) in five experiments when noradrenaline (NA) was infused in doses ranging from 0.8 - 8 μ g. Values shown during Krebs (K) and during perfusion with cholesterol oleate (CO).

		<u>Dose NA (μg)</u>				
Expt. No.		0.8	1.6	3.2	6.4	8.0
1	K	4.1	4.3	5.7	6.8	6.9
	CO	4.0	4.4	5.6	5.7	6.7
2	K	1.1	1.3	1.7	2.0	2.1
	CO	1.5	1.6	1.9	2.0	2.3
3	K	1.7	2.0	3.6	3.9	4.1
	CO	2.0	2.5	3.7	4.0	4.3
4	K	1.8	1.8	1.9	2.1	2.3
	CO	1.6	1.9	2.0	2.3	2.5
5	K	3.6	4.0	4.5	5.0	5.9
	CO	3.0	4.6	4.7	5.5	6.1

artery preparation.

5) Bile salts

Solutions of sodium taurocholate and deoxycholate in concentrations ranging from 200 μ M to 400 μ M were infused in Krebs into the isolated artery preparation. No difference was seen between these responses and the responses obtained when Krebs alone was perfused. Figure 34 shows the responses obtained in an isolated artery preparation when sodium taurocholate was diffused. It can be seen that although there is some potentiation of responses when noradrenaline was initially injected these responses were not maintained and the differences between these responses, and the responses obtained in Krebs were not statistically significant - (5 experiments).

Discussion of Results

These experiments show that the low density lipoprotein (LDL) from jaundiced plasma potentiated the effects of noradrenaline on isolated artery preparations. The LDL was infused in similar concentrations to that found in plasma obtained from baboons with obstructive jaundice. Therefore it would appear that this increase in LDL could be at least one of the factors which may be responsible for the potentiation seen in vitro when jaundiced plasma was perfused into either an isolated artery or an isolated kidney preparation. It may also be one of the factors responsible for the potentiation of noradrenaline seen in vivo in the renal circulation as well as in the cerebral circulation.

The remaining plasma from which the LDL had been removed also potentiated the effects of noradrenaline. Therefore the increase in LDL is not the only factor involved in generating this effect. It would seem however, that the factors responsible for the potentiation of the effect of noradrenaline are carried in the protein fragment of the plasma since deproteinised plasma produced no effect

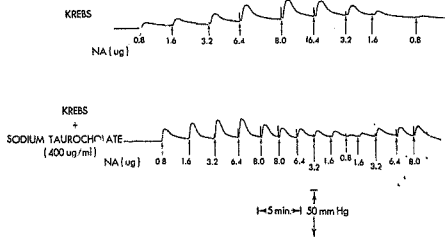


Figure 34. PERFUSION WITH MILE SALT (SODIUM TAUROCHOLATE)

Top Trace - During perfusion with Krebs solution.

Lower Trace - During perfusion with sodium taurocholate.

on the responses of the vessels to noradrenaline. It was not possible to isolate any factor responsible for the potentiation of effects of noradrenaline from the plasma remaining after the low density lipoprotein had been removed. No potentiation was seen when either cholesterol esters, bilirubin, bile salts or triglycerides was perfused into the isolated artery preparation. In the introduction to this thesis it was pointed out that Brum had implicated conjugated bilirubin as one of the factors responsible for sensitizing the kidney to the effects of ischaemia. It was not possible to obtain conjugated bilirubin to perfuse into the isolated artery preparation and it is possible that this may be one of the factors which may be increasing the sensitivity of vessels to the effects of noradrenaline when the LDL is removed.

Aoyagi and Lomenstein⁸ have implicated bile salts as the factor responsible for the increased sensitivity of the kidney to periods of ischaemia. Infusions of bile salts into the isolated artery preparations, however, did not potentiate the effects of noradrenaline. It is possible though, that bile salts in combination with some other factor may be capable of potentiating the effects of noradrenaline.

Also, it was not possible to test whether phospholipid was involved in the production of an increased sensitivity to the effects of noradrenaline. It may be that alterations in phospholipids are also capable of altering membrane characteristics and thereby altering the responses of vessels to the effects of noradrenaline. It is also possible that steroids other than cholesterol such as oestrogens may be involved. Iverson has shown that oestrogens are also capable of inhibiting uptake 2 mechanisms²⁰¹. An oestrogen breakdown is decreased in liver disease²⁸ it may be possible that an increase in these steroids as well as other steroids in liver disease, may be also responsible for producing the increased response to the effects of noradrenaline. This possibility

not investigated in this study.

5

this section it was shown that jaundiced plasma can potentiate the response of an isolated artery or kidney to the effect of noradrenaline. This was mediated by a decreased K_{eq} and an increased R_{max} . Hypercholesterolaemic plasma and low density lipoproteins from normal or jaundiced plasma produced similar effects. None of the constituents of jaundiced plasma which were examined produced similar effects. It is suggested that an increased cholesterol concentration combined with its apoprotein may be one of the factors responsible for the increased sensitivity of vessels to the action of noradrenaline in the presence of obstructive jaundice. That other factors are involved is evident by the fact that jaundiced plasma from which the low density lipoprotein had been removed, still potentiated the arterial response to noradrenaline infusion.

Page 172.

SECTION V

INFUSIONS OF LOW DENSITY LIPOPROTEINS INTO THE CEREBRAL
VASCULATURE

FINAL DISCUSSION AND CONCLUSION

INFUSIONS OF LIPOPROTEINS INTO THE CEREBRAL VASCULATURE

Introduction

From the previous experiments isolated arteries it would seem apparent that LDL which carries cholesterol is one of the factors which may explain the reported results from the experiments on the kidney and brain flow in obstructive jaundice; viz an increased sensitivity to circulating noradrenaline. If this hypothesis is correct then infusions of large amounts of lipoproteins into the circulation of the intact animal should result in a similar increase in sensitivity to the effects of NA. The experiments below describe the effects on NA sensitivity of the cerebral vasculature of infusions of low density lipoproteins into the cerebral circulation of baboons. In this case the cerebral circulation was used because of the greater accuracy of the ^{133}Xe method to measure cerebral blood flow compared with its use for the measurement of renal blood flow.

Material and Methods

The experimental design was similar to that described in Section II. Eight experiments were performed on baboons (*Papio ursinus*) weighing between 10-15 kilograms. The experimental design was similar to that described in Section III. In these experiments, however, each baboon acted as its own control. Two control measurements of cerebral blood flow were made and then cerebral blood flow was measured during perfusions of 4 μCi , 8 μCi and 16 μCi of NA/min. Each concentration of NA was infused for 10 minutes before cerebral blood flow was measured and the flows were recorded for 15 minutes. At the end of the infusions of noradrenaline a further control flow was measured after the animal had been allowed to rest for 10 minutes.

Low density lipoprotein was prepared using an ultracentrifuge in an identical manner to that described

previously. Normal baboon plasma was used. Concentrations of between 3 and 4 mg/min were infused. After the protein had been perfusing into the cerebral circulation for 10 minutes cerebral blood flow was measured during perfusion of the lipoprotein alone and then during perfusion of 4 μ g, 8 μ g and 16 μ g of NA/min. In some of the experiments measurement were only made using doses of 4 μ g and 8 μ g of NA/min because of the paucity of lipoprotein available. Samples of blood were taken from the internal jugular vein for measurement of lipoprotein fractions of the plasma before and after the infusion of lipoprotein into the cerebral vasculature. As previously described only alterations in grey matter flow were calculated because of the decreased accuracy of white matter flow measurements. Grey matter flow (CBF) before and after lipoprotein infusion were compared using a paired students "t" test. Percentage change in flow as well as change in cerebrovascular resistance were compared using a Wilcoxin test for non-parametric data.

Results

In the control period infusions of 4 μ g, 8 μ g and 16 μ g of NA/min caused an increase of 3.9 ± 6.8 , 11.7 ± 3.2 and 6.1 ± 5.5 ml/min/100g tissue respectively. During lipoprotein perfusion there was a mean increase in resting flow when lipoprotein was infused alone of 12.4 ± 4.0 ml/min/100gr tissue. These changes were compared by means of a students "t" test for paired data and was found to be statistically significant. During lipoprotein infusion 4 μ g, 8 μ g and 16 μ g of NA/min caused a reduction of 15.6 ± 5.2 , 6.4 ± 2.9 and 11.7 ± 4.9 ml/min/100gr tissue respectively. This was significantly different from the control values for all doses of NA infused ($p < 0.01$). There was a significant percentage reduction in flow when low density lipoprotein was perfused. This was -13.1, -7.29 and -15.78% after 4 μ g, 8 μ g and 16 μ g of NA/min respectively. The mean values of grey matter flows (CBF) in each animal during noradrenaline perfusion before and during lipoprotein infusion are shown in Table 35. The total mean CBF values for all experiments are

Table 35

Shows grey matter flow ml/min/100gr tissue in eight experiments during doses of noradrenaline (NA) ranging from 4 μ g - 16 μ g per minute was infused.

Experiment No.	Dose NA (μ g)			
	0	4	8	16
<u>Control</u>				
1	85.3	-	92.3	79.1
2	55.6	-	63.9	61.6
3	58.2	-	83.1	79.1
4	62.7	63.9	69.2	66.5
5	56.1	84.1	77.1	-
6	56.4	43.7	59.4	-
7	53.6	48.9	-	-
8	67.4	75.5	79.1	-
Mean difference	-	+3.9+6.8	+11.7+3.2	+6.1+5.5
<u>Experimental</u>				
1	99.1	-	103.9	75.5
2	69.2	-	69.2	55.4
3	66.7	-	59.2	57.3
4	66.5	36.1	55.4	66.5
5	90.0	77.3	-	-
6	75.5	51.1	63.9	-
7	48.2	47.5	-	-
8	79.4	69.2	66.5	-
Mean difference	-	-15.6+5.2	-6.4+2.9	-11.7+4.9

shown in Table 36. The mean changes in CBF values for all animals when lipoprotein was infused alone are shown in Table 37. The mean percentage changes in CBF are shown in Table 38. The mean change in CBF and percentage change in CBF are shown graphically in Figures 35 and 36 respectively.

After infusion of 4 μ g, 8 μ g and 16 μ g of NA/min in the control period cerebrovascular resistance (CVR) altered by $+0.04 \pm 0.3$, $-0.2 \pm .1$ and -0.3 ± 0.2 mmHg/ml respectively. During infusion of the lipoproteins CVR rose to $0.7 \pm .3$, 0.2 ± 0.1 and 0.3 ± 0.1 mmHg/ml and after infusions of 4 μ g, 8 μ g and 16 μ g of NA/min respectively. This difference in CVR after the lipoprotein infusion was significantly different from control values only at the 4 μ g and 16 μ g NA/min level. The percentage increase was 37.7, 10.8 and 17.4 after lipoprotein infusion with 4 μ g, 8 μ g and 16 μ g NA/min respectively. In the control period there was a decrease of 4.7, 11.3 and 13.6% of basal flow with infusions of 4, 8 and 16 μ g NA/min respectively. These differences were statistically significant at 4 μ g and 16 μ g/min infusion rates using a Wilcoxin test for non-parametric data. The mean changes in CVR in each experiment during noradrenaline perfusion, before and after lipoprotein infusions are shown in Table 39. The CVR values for all experiments are shown in Table 36. The mean percentage changes in CVR are shown in Table 38. The mean change in CVR and the percentage change in CVR are shown graphically in Figures 37 and 38 respectively.

The increase in the lipoprotein fraction before and after infusion of low density lipoprotein into the cerebral vasculature can be seen in Table 40. Due to a technical error results of only 5 experiments are shown.

Discussion

These results show that infusions of low density lipoprotein (LDL) increase the sensitivity of the cerebral vasculature to the effects of NA. In the control periods infusions

TABLE 36

Mean values for all experiments of grey matter flow (CBF) and cerebrovascular resistance (CVR) in the control period and after infusion of noradrenaline in doses ranging from 4 μ g - 16 μ g per minute. The changes in grey matter flow (Δ CBF) and cerebrovascular resistance (Δ CVR) are also shown.

Values in CBF in ml/min/100gr.

Values of CVR in mmHg/ml.

CONTROL

DOSE NA μ g	CBF	Δ CBF	CVR	Δ CVR
0	61.9 $\pm$ 3.7	-	2.1 $\pm$ 0.2	-
4	63.2 $\pm$ 7.6	+3.9 $\pm$ 6.8	2.3 $\pm$ 0.2	0.04 $\pm$.3
8	74.8 $\pm$ 4.3	+11.7 $\pm$ 3.2	1.8 $\pm$ 0.1	-0.2 $\pm$.1
16	71.5 $\pm$ 4.4	+6.1 $\pm$ 5.5	1.7 $\pm$ 0.2	-0.3 $\pm$.2

EXPERIMENTAL

DOSE NA μ g	CBF	Δ CBF	CVR	Δ CVR
0	74.3 $\pm$ 5.5	-	1.6 $\pm$ 0.1	-
4	56.2 $\pm$ 7.5	-15.6 $\pm$ 5.2	2.4 $\pm$ 0.4	+0.7 $\pm$ 0.3
8	69.7 $\pm$ 7.1	-6.4 $\pm$ 2.9	1.8 $\pm$ 0.2	+0.2 $\pm$ 0.1
16	63.7 $\pm$ 4.6	-11.7 $\pm$ 4.9	1.9 $\pm$ 0.2	+0.3 $\pm$ 0.1

TABLE 37

Shows mean grey matter flow (CBF) in eight experiments before and after lipoprotein infusion. The mean changes in grey matter flow (Δ CBF) are also shown. The total mean value $\pm$ one standard error are also shown.

<u>CBF ml/min/100gr tissue</u>		
<u>CONTROL</u>	<u>AFTER LIPOPROTEINS</u>	<u>Δ CBF</u>
85.3	99.1	+13.8
55.6	69.2	+13.6
58.2	66.6	+ 8.4
62.7	66.5	+ 3.8
56.1	90.0	+33.9
56.4	75.5	+19.1
53.6	48.	- 5.4
67.4	79.4	+12.0
Mean 61.9 $\pm$ 3.7	74.3 $\pm$ 5.5	- 12.4 $\pm$ 4 (P < 0.05)

TABLE 38

Shows mean percentage changes in grey matter flow (Δ CBF) and cerebrovascular resistance (Δ CVR) after infusion of noradrenaline from 4 - 16 μ g per minute. Control values and those after lipoprotein infusions are shown.

PERCENTAGE CHANGES

CONTROL

<u>Dose NA (μg)</u>	<u>Δ CBF</u>	<u>Δ CVR</u>
4	+ 6.4	- 4.7
8	+16.1	-11.3
16	+11.6	-13.6

AFTER LIPOPROTEINS

<u>Dose NA (μg)</u>	<u>Δ CBF</u>	<u>Δ CVR</u>
4	-13.1	+37.7
8	-7.3	+10.7
16	-15.7	+17.3

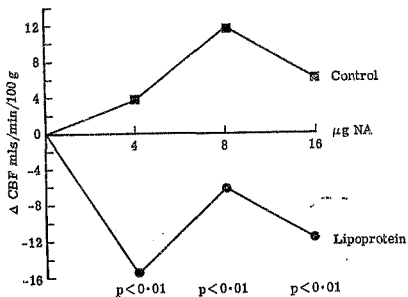


Figure 35. THE MEAN CHANGE IN GREY MATTER FLOW (CBF).

The mean change in CBF during infusions of noradrenaline (NA) in doses varying from 4-16 μg/min are shown before and during lipoprotein infusion. P represents the results of a paired "t" test between the control values and the values after lipoprotein infusion.

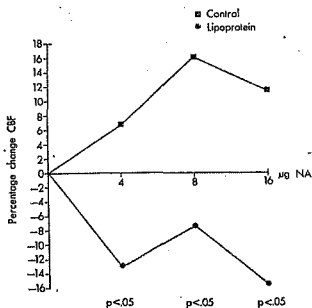


Figure 36. MEAN PERCENTAGE CHANGE IN GRAY MATTER FLOW (Δ CBF)

The mean percentage changes in CBF during infusions of noradrenaline (NA) in doses varying from 4-16 μ g/min are shown before and during lipoprotein infusions.

TABLE 39

Shows cerebrovascular resistance (mmHg) in eight experiments when doses of noradrenaline (NA) ranging from 4 μ g - 16 μ g per minute were infused.

Experiment No.	<u>DOSE NA (μg)</u>			
	0	4	8	16
<u>Control</u>				
1	1.1	-	1.1	1.3
2	2.5	-	2.2	2.3
3	1.8	-	1.1	1.2
4	2.2	2.2	2.0	2.1
5	2.3	1.7	1.9	-
6	2.1	2.9	2.2	-
7	2.7	2.8	-	-
8	1.8	1.7	1.9	1.1
Mean Change	-	+0.04 $\pm$ 0.3	-0.2 $\pm$ 0.1	-0.3 $\pm$ 0.2
<u>Experiment</u>				
1	1.0	-	1.1	1.5
2	2.1	-	2.0	2.4
3	1.5	-	1.5	1.7
4	2.0	3.9	2.5	2.2
5	1.5	1.8	-	-
6	1.5	2.1	1.8	-
7	2.0	2.7	-	-
8	1.5	1.7	1.8	-
Mean Change	-	+0.7 $\pm$ 0.3	+0.2 $\pm$ 0.1	+0.3 $\pm$ 0.1

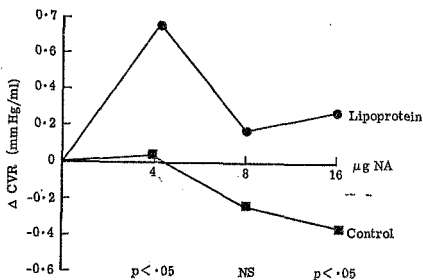


Figure 37. THE MEAN CHANGE IN CEREBROVASCULAR RESISTANCE (ΔCVR)

The mean change in CVR during infusion of noradrenaline (NA) in doses ranging from 4-16 $\mu\text{g/min}$ are shown before and during lipoprotein infusions.

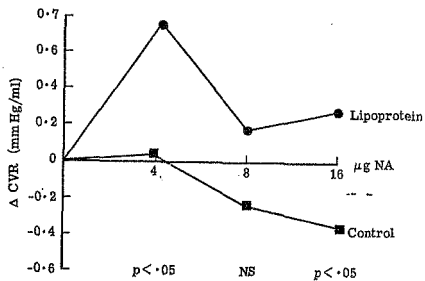


Figure 37. THE MEAN CHANGE IN CEREBROVASCULAR RESISTANCE
(ΔCVR)

The mean change in CVR during infusion of noradrenaline (NA) in doses ranging from 4-16 $\mu\text{g/min}$ are shown before and during lipoprotein infusions.

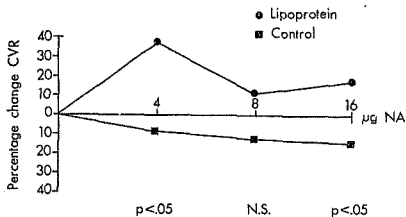


Figure 30. THE MEAN PERCENTAGE CHANGE IN CARDIOVASCULAR RESISTANCE (Δ CVR)

The mean percentage change in CVR during infusions of noradrenaline (NA) in doses ranging from 4-16 μ g/min are shown before and during lipoprotein infusions.

TABLE 40

Shows changes in lipoprotein fractions, before and after infusion of low density lipoprotein.
Results of 5 experiments shown.

HDL = High density lipoprotein

LDL = Low density lipoprotein

VLDL = Very low density lipoprotein

Expt. No.	Total cholesterol mgm%	HDL mm% (=cholesterol)	LDL mgm% (=cholesterol)	VLDL mgm% (=triglycerides)
1	Control 60 After 70	22 13	13 36	124 110
2	Control 68 After 98	12 13	38 65	88 92
3	Control 65 After 95	13 15	40 57	60 68
4	Control 62 After 87	14 17	38 64	100 97
5	Control 61 After 77	12 11	26 61	80 91

of NA reduced vasodilatation similar to that seen in normal animals as described in Section III. Infusions of LDL altered the sensitivity of the vasculature and produced vasoconstriction which was significantly different from the vasodilatation found during the control period.

These results are compatible with the argument presented in Sections I - IV that the increased sensitivity to NA is due to an increase in circulating cholesterol carried by LDL. It is also possible that there are other factors sensitizing the vessels but that these may not be as important as the rise in cholesterol since the potentiation found when the LDL was removed from jaundiced plasma was much less than when lipoprotein was present. The responses to NA seen in the experiments in which pure LDL was infused into the brain are almost as great as those in the jaundiced animals. The smaller response seen at the $8\mu\text{g}$ level is probably due to the increase in flow seen in experiment No. 1 which, influenced the average results of all experiments to a large extent. It is possible that if more experiments were performed the change with $8\mu\text{g}$ perfusions would be greater. It was also mentioned in Section III that the changes found in obstructive jaundice might be due to responses in unhealthy vessels although it was considered that this was unlikely. In these experiments the animals were all healthy and a similar response to that found in jaundice was produced. Thus these experiments show in addition, that the changes found in obstructive jaundice are unlikely to have been caused by damage to the vessels.

The dilatation seen when lipoprotein alone was infused must also be explained. If an unaltered rise in cholesterol carried by LDL causes an inhibition of uptake II mechanism, then when lipoprotein was infused the amounts of endogenous NA reaching the receptor site may be the same as that seen when large amounts of NA are infused in normal animals. This would produce a vasodilatation since it is postulated that small amounts of NA reaching the receptor sites cause vasodilatation and large amounts of vasoconstriction. Thus it seems that the dilatation seen when

Page 187.

LDL was infused alone, reinforces the hypothesis that the increase in cholesterol results in an inhibition of uptake II mechanism thereby allowing NA to reach the receptor sites in sufficient concentration to cause vasodilatation with normal amounts of circulating noradrenaline but vasoconstriction when large amounts of noradrenaline are infused. However, there are other possible explanations of the effects of cholesterol which will be discussed further below.

FINAL DISCUSSION AND CONCLUSION

In the introduction to this thesis the impairment of renal blood flow in cirrhosis of the liver as well as in portal hypertension and malignant disease of the liver was discussed. It has been shown in cirrhosis that although renal failure occurs late in the condition, at a stage when liver function has become decompensated, it is also possible to demonstrate alterations in renal blood flow distribution long before the onset of frank renal failure. Similar alterations in renal blood flow distribution also occur in malignant disease and portal hypertension.

The first aim of this study was to investigate the possibility that changes in renal blood flow occur in obstructive jaundice without the presence of renal failure, similar to that seen in cirrhosis of the liver and other diseases such as malignant disease and portal hypertension. Also it has been demonstrated that renal failure is a frequent complication of surgery in patients with obstructive jaundice. It has been postulated that the cause of this is an increased sensitivity of the kidneys to periods of ischemia. The second aim was to try and find the etiology of this increased sensitivity of the kidneys to ischemic changes. In experiments on baboons with surgically induced obstructive jaundice it was shown that there are changes in renal blood flow distribution and rate before the onset of renal failure. It was found that there is a shift of blood flow distribution to the inner cortical and outer medullary areas without any evidence of renal failure. Urea levels were not significantly raised and although clearance studies were not performed in this study a previous preliminary investigation showed that creatinine clearances decreased in baboons with obstructive jaundice²⁴⁴. Also it was shown in the present study that there was no gross histological change in the kidney two weeks after ligation of the common bile duct. Thus it would seem that in obstructive jaundice there are similar alterations in

renal perfusion as those found in cirrhosis and malignant disease of the liver as well as that found in portal hypertension.

The study in baboons with obstructive jaundice also showed that the baboons were hypersensitive to the effects of noradrenaline when compared with normal baboons. Kew had suggested that in cirrhosis the changes found in inter-renal blood flow distribution may be due to sympathetic over-activity.²⁴ Although this may be so, in obstructive jaundice the renal vasculature seems to be sensitive to levels of noradrenaline which produce no effect in normal animals. Thus when there is an increased level of circulating catecholamine such as would be seen during the trauma of surgery, the renal vessels may be so sensitive that outer cortical vasoconstriction is produced, and acute renal failure may develop. It has been shown that in normal human patients the level of circulating noradrenaline is 0.3 ng/l .^{24,25} In the jaundiced animals the concentration of .25 ng/ml produced vasoconstriction within the kidney. Although it is difficult to extrapolate animal experiments to the human situation it would seem that the findings in this study support the contention by Brown¹⁶ that in obstructive jaundice the kidneys are more sensitive to periods of hypertension, particularly since in hypertension there are increased levels of circulating catecholamines.^{16,25}

The increase in sensitivity could be due to many causes. It was mentioned that Brown feels that conjugated bilirubin is the factor responsible while Aoyagi and Iwamoto²⁷ showed that infusion of bile salts increases sensitivity of the kidneys to ischaemia. Others^{28,29} find increased plasma renin leading to an increase in angiotensin II causes the renal failure seen in these conditions.²⁹ In all these studies no correlation could be seen between serum bilirubin (direct or indirect) and sensitivity to noradrenaline. There was also no correlation between alterations in electrolyte and serum renin activity was not increased. Furthermore Kew¹ believes that any increase

in plasma renin activity is a result of the alterations in renal perfusion and not a cause of them, since when renal perfusion is increased by dilator drugs, in cirrhosis of the liver, plasma levels of renin fall. Further evidence that the changes are due to increased sensitivity to NA was provided by the finding that renal blood flow could be improved by phenoxybenzamine (an alpha blocking agent) and this compound also reversed the changes induced by infusions of norepinephrine. Although this contradicts the work by Epstein¹⁰, who showed in patients with cirrhosis that renal blood flow could not be improved by phenoxybenzamine, his experiments were accompanied by systemic hypotension whereas in this study there was no fall in blood pressure.

That an increase in bile salts may be the factor producing the changes will be discussed further below.

It was also shown in these studies that both the alterations in renal perfusion and increased sensitivity to NA were progressive. This is indicated by the finding that bhecons after two weeks of jaundice showed more marked alterations in intrarenal blood flow distribution and were more sensitive to NA than bhecons who had the bile duct ligated for only one week. This would indicate that the liver is producing some factor which is increasing in concentration, or that some substance is not being detoxified by the liver. Although it was pointed out that it was more likely that some factor is being added to the blood (since there is very little hemocellular source after two weeks of jaundice), in either event the factor causing the alterations, if blood borne, would cause similar alterations in perfusions in other vascular beds.

Investigations of the cerebral circulation using the ¹³³Xe technique showed that in the cerebrovascular bed, there is also an increased sensitivity to NA in the presence of obstructive jaundice. These experiments confirm the hypothesis that in obstructive jaundice there is some factor in the plasma which increases the sensitivity of the vasculature to the effects of circulating norepinephrine.

These experiments also elucidated some of the problems regarding the role of noradrenaline in the control of the cerebral circulation. It has been shown clearly by Rosendorff and Granston¹⁹⁹ that if noradrenaline is placed on the adventitial surface of cerebral vessels, constriction or dilatations of these vessels is produced. The effect of blood borne NA however is uncertain. Small doses produce vasodilation and larger doses vasoconstriction. Infusions of NA via the carotid artery have been shown to produce a vasodilation²⁰³, vasoconstriction²⁰⁴ or no effect²⁰⁵. McCleiden and Bidelman have inhibited the uptake of noradrenaline into the smooth muscle (uptake 2) and have demonstrated that noradrenaline then produces cerebral vasoconstriction²⁰⁶. This work suggests that normally very little noradrenaline is reaching the receptor sites so that when catechol-o-methyl-transferase (COMT) is inhibited, (this then inhibits the uptake of noradrenaline into smooth muscle - Uptake II) enough noradrenaline reaches the receptor sites and a vasoconstriction results. The results of infusing noradrenaline in the presence of obstructive jaundice produces almost identical results to that seen when COMT is inhibited. Thus in the presence of jaundice it would appear that enough noradrenaline is reaching the receptor sites to produce a vasoconstriction. This would indicate that the factor or factors present in the plasma of animals with bile duct ligation increases the sensitivity of the vessels to noradrenaline by inhibition of the normal smooth muscle uptake mechanism, although other mechanisms are possible.

It has been mentioned in Section III that it is well established that changes in P_{CO_2} of arterial blood profoundly affect the cerebral blood flow. A rise in P_{CO_2} increases cerebral blood flow while a fall produces a decrease. This is thought to be due either to alteration in pH around the vessel or due to a direct effect of CO_2 on the blood vessels or both. While this mechanism may be the major contribution to this cerebral blood flow response to P_{CO_2} in a quantitative sense, evidence is accumulated that some neurally mediated reflex mechanism

Page 192.

is involved. It is thought that this reflex is mediated via the sympathetic nervous system.

It has been shown that the cerebrovascular response to CO_2 is altered by sympathetic nerve stimulation or section¹⁸⁰. Also alpha-adrenergic blockade has been shown to potentiate the cerebral vasodilator response to increased $PaCO_2$ ¹⁸⁷. Complete interruption of sympathetic nerves in tetracaine man reduces the vasoconstrictor response to lowered $PrCO_2$ ¹⁸⁸.

The studies in jaundiced animals show attenuated responses to $PrCO_2$ and an exaggerated response to hypercarbia reversed by alpha blockade. This finding together with the findings when NA is infused, demonstrate that in a situation where the actions of the sympathetic nervous system are potentiated, the cerebral CO_2 reactivity is altered by an alpha-receptor mediated vasoconstriction. Furthermore the present studies provide evidence that an increased $PrCO_2$ induces cerebral vasodilatation partly via a neurally mediated mechanism via a decrease in sympathetic tone. Also these studies would indicate that a decreased $PrCO_2$ may cause cerebral vasoconstriction partly by an increase in sympathetic activity.

The increase in cerebral blood flow with alpha blockade rules out the possibility that reduced response to $PrCO_2$ in the jaundiced animal may be due to some pathological change in the cerebral vessels. The reduced response to $PrCO_2$ and the increased response with hypercarbia therefore further support the finding that in obstructive jaundice there is a hyper-responsiveness to sympathetic activity and no structural damage to the blood vessel.

The findings in the brain and the kidney suggest that there is a factor in the plasma which increases the vascular responses of the vessels to the effects of noradrenaline. The results using our isolated artery preparation confirms this. Jaundiced plasma infused into an isolated perfused artery or kidney caused a large increase in the responses of the vessels to injected noradrenaline.

In some cases the vasculature of the kidney became so sensitive that an irreversible constriction of the vessels occurred which would be expected to cause acute renal failure if a similar situation occurred in vivo. This could explain the high incidence of post-operative renal failure found in man by Dawson¹⁶, when increased circulating catecholamines, produced by the stress of surgery and periods of hypotension, may be having the same effect on the kidney as that found in the isolated perfused organ.

The isolated organ preparation also provided an explanation of the mechanism whereby the jaundiced plasma produces this potentiated effect in response to injected noradrenaline. By application of a Lineweaver-Burke plot and Hofstee plot it was shown that the equilibrium coefficient is decreased. This could imply that increased amounts of drug receptor complex had been formed. A tendency for the maximal response of the vessels to increase was also shown. By application of the Hill plot it was demonstrated that this was not due to an allosteric effect but probably due to an unmasking of more receptors or some alteration in post-receptor membrane activity. It is apparent, therefore, that obstructive jaundice is producing some change in the membrane characteristic. One of the consequences of such change could be a reduced uptake of noradrenaline into the smooth muscle. The similarity of the findings when noradrenaline was infused into the cerebral circulation to that produced when uptake 2 mechanisms are inhibited would suggest that this in fact may be occurring. This would also explain the decrease in equilibrium coefficient since noradrenaline would then become available for drug receptor combination.

The finding by Inversen that cholesterol is capable of inhibiting uptake II²⁰² and a large increase in cholesterol in cholestatic jaundice suggests that an increase in cholesterol is the factor responsible for producing the heightened sensitivity to noradrenaline found in these studies. Hypercholesterolaemic plasma produced similar effects to jaundiced plasma but pure cholesterol had no

effect. This suggests that cholesterol must be present in the normal physiological state to produce any effect on smooth muscle uptake mechanisms; i.e. attached to apoprotein. When low density lipoprotein was separated from jaundiced plasma a large decrease in $K_{0.5}$ and an increase in R_{max} was produced. This would imply that the increase in cholesterol found in obstructive jaundice is at least one of the factors responsible for the increase in sensitivity to norepinephrine found in these experiments. It has also been shown that in obstructive jaundice there is an abnormal lipoprotein produced, that moves with the beta lipoproteins on electrophoresis. This is called lipoprotein X²⁴⁷. It is possible that this abnormality of lipoprotein in obstructive jaundice may be responsible for the changes reported here. It would seem however that this is unlikely since the plasma from Fredrickson's Type III hyperlipidaemic patients produce similar increase in sensitivity to HA in the isolated artery preparation as that found with beagons with obstructive jaundice. No abnormal protein has been found in this disease, and in addition when low density lipoprotein was concentrated from normal plasma similar effects were found via a decrease in $K_{0.5}$ and an increase in R_{max} .

Although it has been suggested that the results reported here in obstructive jaundice and in hypercholesterolaemia could be explained by an inhibition of uptake mechanisms, an increased serum cholesterol level may produce other effects. It has been shown recently that when the first shell of the lipid bilayer surrounding a calcium transport protein, is replaced by cholesterol, there is a complete and reversible inactivation of ATPase activity²⁴⁸. This would have profound effects on energy production and calcium transport within the smooth muscle cell. Also, other steroids such as cortisol have been shown to affect intracellular potassium concentrations²⁴⁹, which would be expected to profoundly alter the membrane potential of cells. Thus although the uptake of HA into the smooth muscle cell may be inhibited by the high cholesterol levels, it can be seen that there are many other effects of cholesterol and

steroids on cellular physiology which could explain the results reported here.

An increased sensitivity to noradrenaline produced by an increased cholesterol may also provide an explanation for the alterations in renal perfusion found in cirrhosis and malignant disease of the liver. In cirrhosis the free cholesterol content of the plasma rises although the total cholesterol is decreased²⁸ and in malignant disease of the liver it has recently been shown that hypercholesterolaemia may be found²⁵⁰. This hypercholesterolaemia in primary cancer of the liver is probably due to an increased synthesis of cholesterol due to lack of normal feedback mechanisms. The finding of altered renal perfusion in portal hypertension cannot be explained by alterations in cholesterol and it is here perhaps that some other mechanism presumably is operating.

The finding that plasma from animals with obstructive jaundice is capable of potentiating the effects of noradrenaline even when the low density lipoprotein is removed, indicates that an increased cholesterol level is not the complete explanation. Bilirubin has been shown to increase the damage caused by ischaemia but it seems unlikely that changes found in this study are due to this substance, as no correlation was found between the levels of bilirubin either conjugated or unconjugated and the degree of noradrenaline sensitivity either in the in vivo or in vitro experiments. In addition unconjugated bilirubin infused into the isolated artery preparation produced no change. Although conjugated bilirubin was not infused and it is theoretically possible that this is one of the factors responsible, the lack of correlation between the sensitivity of the vessels to noradrenaline and the levels of conjugated bilirubin found in the experiments make this hypothesis unlikely. Similarly Aoyagi and Lowenstein have suggested that bile salts are responsible for the increased sensitivity of the kidney to ischaemia¹⁷.

No alterations in noradrenaline sensitivity of the vasculature to NA was found when bile salts were perfused. It was mentioned that it was possible that bile salts may be acting in combination with other factors and may in fact play some part in the perfusion changes seen in jaundice. It was also not possible to isolate any factors from the plasma remaining, after the low density lipoprotein had been removed, that could account for the increased sensitivity of the vessel to noradrenaline. It was pointed out that since in liver disease the breakdown of steroids is inhibited, and some steroids such as oestrogens are shown to decrease uptake II mechanisms, that an increase in plasma steroid levels may be one of the other factors causing an increased sensitivity to noradrenaline in jaundice.

Finally when low density lipoprotein was perfused into the intact cerebral circulation similar vasoconstriction was produced during infusions of noradrenaline. This supports the hypothesis that the changes seen with infusions of noradrenaline into the cerebral circulation, in the presence of obstructive jaundice was due, at least in part, to an increase in serum cholesterol. In addition the increase in flow which occurred when lipoprotein was infused alone would tend to reinforce the theory that an increase in low density lipoprotein levels inhibits Uptake II processes although as described, other effects may be occurring. Noradrenaline when infused in large doses into normal animals produces vasodilatation. As already explained this is probably due to the beta effect produced when only small amounts of noradrenaline reach the receptor sites, the rest being taken up into the smooth muscle. When lipoprotein is infused alone, if inhibition of Uptake II is occurring then sufficient of the normal endogenous noradrenaline is reaching the receptor sites to produce vasodilatation similar to that seen when large amounts of endogenous noradrenaline are infused into the normal animal. Thus the vasodilatation produced with lipoprotein infusions confirms the theory that inhibition of normal smooth muscle uptake mechanisms has occurred thereby allowing noradrenaline to reach the receptor sites. When noradrenaline

Page 197.

is then infused in large amounts or is present in increased amounts in the plasma, sufficient amounts reach the receptors so that an alpha receptor mediated vasoconstriction is produced.

Conclusion:

This thesis has shown that in obstructive jaundice similar changes occur in renal blood flow distribution as that which occurs in liver diseases such as cirrhosis. It has also been demonstrated that alterations in renal perfusion are due at least in part to increased sensitivity to norepinephrine. This altered response of blood vessels is also present in other vascular beds such as the cerebral circulation. It has been postulated that in the cerebral circulation norepinephrine does not normally reach the receptor sites in sufficient quantities to affect cerebrovascular resistance but in obstructive jaundice inhibition of normal smooth muscle uptake mechanisms occur so that norepinephrine when infused into the carotid artery reaches the receptor sites to produce a vasoconstriction. In addition it was demonstrated that in obstructive jaundice an attenuated response to increasing levels of PGO_2 was produced and an exaggerated response to hyperoxemia was found. This is consistent with the theory that the responsiveness of the vessels to CO_2 is at least in part due to a neurally mediated reflex since this attenuation could be reversed by an alpha blocking agent.

Further confirmation of the theory that in obstructive jaundice there is an increased sensitivity of the vasculature to the effects of norepinephrine, was provided by experiments using an isolated perfused artery or kidney. When plasma from beagons with obstructive jaundice was perfused into this preparation there was an increased sensitivity of preparation to the pressor effects of norepinephrine. Analysis by Lineweaver-Burke Plot or Hofstad Plot showed that this was due to a reduction in the equilibrium coefficient and an increase in the maximum response of the vessel. A reduction in equilibrium coefficient is explained by an increased drug-receptor affinity which could be

produced by inhibition of normal uptake 2 mechanisms. It was concluded that the increase in the maximum response of the preparation was due to either a change in membrane characteristics or an uncovering of more receptor sites. It was also demonstrated that no allosteric effect had occurred.

It is further concluded that one of the factors producing the changes found in this study was an increase in cholesterol which when attached to its apoprotein was shown to potentiate effects of noradrenaline both in vivo and in vitro. It was also mentioned that other factors may play some part in the production of an increased sensitivity to noradrenaline since jaundiced plasma with the low density lipoproteins removed was also capable of increasing the responses of the vessels to noradrenaline although to a lesser extent than that found when low density lipoprotein was present. It was postulated that this factor may be due to increased level of steroids which also occurs in obstructive jaundice. It is acknowledged that there are numerous questions which have not been answered in this thesis which would provide interesting fields for further research. Some of these are

- a) What are the other factors producing the potentiation of the effects of noradrenaline when jaundiced plasma is perfused without low density lipoproteins present?
- b) What effects are low density lipoproteins producing on cellular membranes and intracellular physiology?
- c) In obstructive jaundice is there an increased responsiveness of blood vessels to circulating adrenaline as well as to noradrenaline; and in addition is there a heightened response to other vasoactive amines such as serotonin?
- d) Is there a potentiated response of blood vessels to the effects of noradrenaline in other liver diseases such as cirrhosis?

It may also be argued that in the experiments using an isolated artery perfused with jaundiced plasma, it would have been better to compare this plasma directly with normal plasma and not with Krebs solution. This was not done as it was felt that all comparisons should be made against a standard reference solution to which the constituents of jaundiced plasma could be added in known concentrations. It is acknowledged that it might be considered more correct to have used normal plasma as the standard solution, but this would have introduced numerous technical difficulties and therefore Krebs solution was used throughout.

Nevertheless this study has shown that in obstructive jaundice there are alterations in renal perfusion similar to that found in cirrhosis of the liver and other diseases of that organ. It has been shown that there is an increased vascular sensitivity to the effects of noradrenaline which may explain the high incidence of post-operative renal failure found so frequently in patients with obstructive jaundice. It was also shown that an increase in cholesterol carried on its apoprotein as low density lipoprotein is at least one of the factors responsible for this altered reactivity of vessels to the effects of noradrenaline. Finally this thesis provided some explanation of the normal control of the cerebral circulation.

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