THE EFFECTS OF

POST-HAEMORRHAGIC ANAEMIA

on

THE RENAL CIRCULATION

GORDON S. STOKES MB.BS.

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School of Physiology,
University of New South Wales,
SYDNEY.

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CHAPTER 1

INTRODUCTION

Anaemia is a condition in which the blood lacks its normal content of red cells or haemoglobin per unit volume. Since most studies of this condition in animals have involved preliminary haemorrhage to produce the required reduction in haematocrit, another variable, i.e. blood volume, may be introduced into the experimental situation. Thus in assessing the possible factors which play a part in the response of the vascular bed of a given tissue to anaemia, it is important to consider the contributions of reduced circulating red cell concentration, reduced oxygen transport and systemic hypovolaemia. In addition the effects of anaemia on circulation through other tissues must be examined, as these may alter extrinsic regulatory factors acting upon the region under consideration and also determine the arterial pressure at which it is perfused. In the present study the effects of acute and chronic anaemia on the renal circulation are analysed, and an attempt made to separate the several factors contributing to these effects. No information is provided about the effects of anaemia on other parts of the peripheral circulation, on cardiac output or systemic O_2 transport. However these aspects have been previously

investigated in some detail in the unanaesthetized rabbit (41-43), and in other species (96).

In general, the compensation for reduced oxygen transport by the blood in anaemia appears adequate in the resting animal; tissue oxygenation is maintained by increased utilisation of arterial oxygen and increased cardiac output. There is a progressive fall in the oxygen saturation of mixed venous blood as the haematocrit and oxygen capacity are reduced (41), and in severe anaemia with an arterial oxygen content of 5 ml per 100 ml, oxygen utilisation is about 60%, compared to approximately 25% when arterial oxygen content is normal (96). The arterial oxygen saturation in anaemia has been reported as within normal limits (19,41, 43,161). Ryan and Hickam (150) have found a significantly increased alveolar-arterial O_2 gradient in anaemic patients, but this appeared to be due to admixture of pulmonary venous blood with systemic venous blood from the Thebesian and bronchial veins, rather than to decreased oxygenation in the alveolar capillaries.

Increased cardiac output in anaemia has been reported by numerous workers in man (e.g.19,161), in the anaesthetized and unanaesthetized dog (15,45,78,132) and in the unanaesthetized rabbit (41,43) using either the direct Fick method or indicator dilution techniques. In these studies total peripheral vascular resistance was decreased, and stroke volume was increased; changes in heart rate played little or no part in the increase in cardiac output. The

reasons for the hyperdynamic circulation in anaemia are not well understood; the contributions of reduced blood viscosity, increased right atrial pressure and blood volume changes have been examined.

Whittaker and Winton (186) and Levy and Share (109) have shown that a change of haematocrit from 40 to 15% produces only a small increase in the relative viscosity of blood flowing in the isolated hindlimb of the dog. If these in vivo findings are applicable to other regions of the circulation, it is unlikely that reduced blood viscosity is quantitatively an important factor in the reduction of peripheral resistance in anaemia.

An elevation of mean right atrial pressure in anaemic patients was reported by Sharpey-Schafer (161), who postulated that this effect was the result of active venoconstriction and was the determinant of increased cardiac output. Darian Smith and Simmonds (41,43) found a significant relationship between increased cardiac output and mean right atrial pressure in unanaesthetized anaemic rabbits. However Hatcher et al (78) found no association in time between the onset of increases in right atrial pressure and the onset of the hyperdynamic phase, after bleeding dogs; indeed in some of their animals the maximum right atrial pressure was observed at the time of the minimum cardiac output. Also, it has been reported by Davis et al (45) that in a series of anaemic unanaesthetized dogs, elevated cardiac output was associated with an increase in right

atrial pressure only in animals which had limitation of right ventricular function due to experimental pulmonary stenosis, and not in those which were simply anaemic. From these results it appears that elevated right atrial pressure in anaemia is more likely to be a consequence of cardiac failure than a determinant of increased cardiac output.

During the experimental production of anaemia it has been demonstrated that cardiac output was reduced immediately after bleeding, but increased as blood volume recovered towards its previous level (41,43,78). In chronic hypovolaemic anaemia in rabbits the cardiac output was higher in those animals which had less marked reduction in total blood volume (43). After bleeding and replacement of dextran-saline in dogs, cardiac output had a significant inverse relationship to haematocrit in both normovolaemic and hypervolaemic animals, but was much higher in hypervolaemic experiments than in normovolaemic experiments at any comparable haematocrit (132). It thus appears that blood volume and haematocrit have independant regulatory effects on the circulation, but some degree of restoration of blood volume may be necessary before elevation of cardiac output can occur in post-haemorrhagic anaemia. It has also been argued that the delayed rise in cardiac output in post-haemorrhagic anaemia results from the slow elaboration of a humoral factor which acts on the heart or peripheral vessels (78). Some support for this view comes from assay experiments in which infusion of blood from anaemic dogs with high

cardiac outputs caused an increase in cardiac output in normal recipient animals (88).

Since the increase of cardiac output in anaemia is associated with a relatively slight reduction in arterial blood pressure, values calculated for total peripheral resistance have indicated widespread vasodilatation. However the vascular response is not uniform throughout all tissues. Thus there is evidence of vasodilatation in the coronary, splanchnic and cerebral circulations and in muscle, whereas vasoconstriction may occur in skin (96,184). There is considerable disagreement about the effects of anaemia on the renal circulation; the recent literature on this subject is reviewed in detail later in this report.

It is pertinent first to trace the development of modern techniques for measuring renal haemodynamics and to discuss their limitations. Much of the confusion about the behaviour of the renal circulation in this as in other pathological states appears to have been caused by inappropriate application of direct and indirect methods of blood flow measurement, and by insufficiently rigid criteria as to what constitutes controlled, stable conditions in the experimental animals used. Thus the approach to the literature has been guided by the conclusion which the late H.W. Smith reached in his monograph "Principles of Renal Physiology", 1956:- 'Perhaps the most important lesson to be gleaned from our present knowledge of the renal circulation is how to go about studying that circulation'.

Review of Techniques in Measuring Renal Blood Flow.

Interest in the physiological control of renal blood flow was first aroused by the observation of Claude Bernard in 1859 (12) that section of the splanchnic nerves in the anaesthetized animal results in an increased urine flow on the operated side. Bernard attributed this "denervation diuresis" to a unilateral increase in renal blood flow on the same side, a supposition which led to numerous attempts over the next seventy years, to determine renal blood flow by actual measurement after section of the splanchnic and/or renal nerves.

In 1883, Cohnheim and Roy (33) used an instrument they called the "oncometer" to measure changes in kidney volume after splanchnic nerve section, and related these changes to variations in renal blood flow. Early attempts to measure renal blood flow directly employed Ludwig's "stromuhr" - a device to collect, measure and then return small aliquots of blood, inserted into the renal artery (Landergren and Tigerstedt, 1893- (1o6)) or later, into the renal vein (Burton-Opitz and Lucas, 1908- (27). In 1927, Rein (143) developed the "thermo-stromuhr", which utilises a sensitive thermocouple to detect temperature changes induced upstream in a blood vessel: the greater the blood flow past the heat source (a high-frequency alternating current applied to the vessel wall), the smaller is the temperature change produced (81). In that it does not involve interruption of blood flow, Rein's method was a significant

advance, and has been applied extensively to the measurement of renal blood flow (163). The electromagnetic flowmeter (92), which utilises the voltage induced by blood flowing through a magnetic field, was a further development which allowed more accurate assessment of rapid fluctuations in blood flow.

The most serious disadvantage of most of these methods, as pointed out by Homer Smith in 1940 (163), was that they were applied to animals which were anaesthetized, and/or recently laparotomized with gross interference to renal vessels and nerves. The "denervation hyperaemia" which was a finding in many of these studies, is in itself an index of the unphysiological nature of the preparations, as it shows the presence of a degree of renal autonomic nervous activity foreign to the intact resting animal.

More recent methods of direct determination of renal blood flow have included the use of an optically-recording bubble flow-meter in the renal vein (154,156), and various means of temporarily diverting renal vein outflow into a measuring receptacle (10,14,26,116,155). In studies on dogs shocked by haemorrhage and dehydration, Balint, Kiss, and Sturcz (10) measured the rate of renal venous outflow by periodically tapping it off from a catheter connected between the renal vein and external jugular vein. They found a large discrepancy between renal blood flow determined in this way, and by renal clearance methods. In further studies using the renal-jugular venous shunt,

Bull and Metaxas (26) and Selkurt and Elpers (155) demonstrated similar discrepancies between direct and indirect measurement of renal blood flow. Balint et al., and Selkurt and Elpers have attributed these discrepancies entirely to the inapplicability of renal clearance methods during hypotension and oliguria. However, Bull and Metaxas also doubt the validity of the direct method during hypotension. They point out that the renal interstitial pressure, which at normal arterial blood pressure is higher than and relatively independent of renal venous pressure, decreases markedly when the arterial pressure falls below about 60 mm. Hg., and becomes sensitive to changes in venous pressure such as might be produced in these venous outflow preparations. Since renal interstitial pressure has been shown to be of great importance in the regulation of renal blood flow (82), any procedure which changes it, such as the draining or siphoning of blood from a renal-jugular venous shunt, may provide a fallacious measurement of renal blood flow.

Indirect Methods for Determination of Renal Blood Flow.

In 1870, Adolph Fick put forward a simple principle (61) which forms the basis for most of the reliable methods used in determining regional blood flows today. This principle may be stated in its general form as follows:- If an indicator substance x be added to or eliminated from the blood stream in its flow through a tissue, then the rate of blood flow through the tissue, $(F) = \frac{Qx}{Ax - Vx}$, where Q

is the amount of x added or eliminated per unit time, and A_x-V_x is its arterio-venous concentration difference.

The specific example of a regional flow used by Fick to illustrate his principle was the pulmonary circulation, the indicator substance added being oxygen, and the arterio-venous difference that produced by normal respiration. In 1898, Zuntz and Hagemann (188) put this theoretical example to the test: measuring the oxygen (and carbon dioxide) contents of mixed venous and arterial blood samples, and the respiratory gas exchange, they calculated the cardiac output in horses during rest, exercise and digestion.

The application of the Fick principle to the determination of renal blood flow took over 30 years more to eventuate. In 1931, Dunn, Kay and Sheehan (51) measured the endogenous urea concentrations of arterial and renal venous blood in the rabbit, in order to determine the renal extraction ratio for urea. As an incidental observation, they calculated renal blood flows from the Fick principle, using as a crude measure of the rate of elimination of urea, the average urinary urea excretion over several days prior to each experiment. These workers obtained samples of renal venous blood in the laparotomised animal, by digital compression and direct needling of the renal vein. While acknowledging that these manoeuvres might have reflex vasomoter effects, and result in changes in renal function, they relied on speed in obtaining the samples (the mean time from first abdominal incision to completion of renal

vein aspiration was 40 seconds), to provide blood urea levels representative of the steady state.

A more elegant method of obtaining renal venous blood in the unanaesthetized dog, was developed by Rhoads (145) in 1934, and first used in determining renal blood flow from urea extraction ratios and urea clearance. At a preliminary operation the left kidney was mobilised and "explanted" so that the left renal vein lay in a subcutaneous position, accessible to repeated puncture and aspiration. Another approach was used by Mason, Blalock and Harrison (116) who obtained renal venous blood in the unanaesthetized dog by means of a cannula inserted into the inferior vena cava via the external jugular vein; the orifice of the cannula was positioned at the point of entry of the renal vein, and during sample collection small balloons were inflated to occlude the inferior vena cava proximal and distal to this point. Mason, Blalock and Harrison used this preparation to determine renal blood flow indirectly (again using urea as the indicator substance), and directly by measuring the rate of renal venous outflow. They found approximate agreement (of the order of 20-30% difference) between renal blood flows determined simultaneously by these direct and indirect methods.

A disadvantage of the use of urea, and other naturally occurring metabolites such as creatinine, in the determination of renal blood flow, is their low renal extraction.

Any errors in measuring the arterial and renal venous concentrations of the indicator substance, may be additive in their effect on the renal arterio-venous difference; the smaller this difference, the larger becomes the percentage error produced in the renal extraction ratio, and in the renal blood flow determined by the Fick principle. For example, as pointed out by Van Slyke, Rhoads, Hiller and Alving (178) the amount of urea removed by the kidney in the series of Dunn, Kay and Sheehan was in some experiments so small as to be within the limits of analytical error. The next phase in the development of the Fick method in measuring renal blood flow, was the recognition of certain substances which by virtue of their rapid excretion by the kidney, were characterised by a high renal extraction ratio. These substances were studied initially in a variety of species mainly because of the light they could throw on the controversy then current regarding the existence or non-existence of tubular excretion in the kidney.

In 1910, Rowntree and Geraghty (149) introduced phenol red for use in assessing renal function clinically, after finding that it was the most rapidly excreted of a large number of dyes investigated. The urinary excretion of phenol red during successive time periods after its administration, was measured as an empirical test of renal function, without consideration of its mode of excretion. Cushny in 1917, reviewed existing theories of renal function (40), and rejected the concept of tubular section, which had originated

with Heidenhain's observations on the uptake of circulating dyes by renal tubular cells (79). Cushny maintained that the function of the tubules was to reabsorb a fluid of fixed composition from the glomerular filtrate; the rate of urinary excretion of a substance would be determined only by its plasma level, its filterability at the glomerulus, and the rate of glomerular filtration. However in 1923, Marshall and Vickers (115) observed that 60% of circulating phenol red is bound to plasma albumin and thus is non-filterable at the glomerulus. They calculated that if the dye were excreted, according to the Cushny theory, by glomerular filtration alone, then it would have to be filtered at an impossibly high rate to provide the observed rate of its urinary excretion. Marshall and Crane (114) showed that the rate of urinary excretion of injected urea in the anaesthetized dog is approximately proportional to the plasma concentration, whereas for phenol red this is true only at low plasma concentrations; at higher concentrations the rate of urinary excretion of phenol red does not increase in direct proportion, but levels off and approaches a maximal level, as if an active secretory mechanism were being saturated.

This evidence pointed strongly to the occurrence of tubular excretion of phenol red in the mammalian kidney. Shannon (158) and Smith, Goldring and Chasis (167), demonstrated the relatively high renal clearance values of this substance and suggested its suitability for determining

renal blood flow. However the renal extraction ratios found for phenol red, which in the dog have ranged from 49% to 70% (113,162,170) are not as high as those reported for other substances subsequently shown to be excreted by the renal tubules. The relatively low renal extraction of phenol red, which may be due to extensive plasma protein binding (164), has led to its being little used in the measurement of renal blood flow in practice.

The search for a suitable substance as indicator in the Fick method, led next to a group of organic iodine compounds, which were found to be so concentrated in the urine as to be opaque to X-rays. These compounds included diodrast and hippuran, whose clearances were shown in 1936 by Elsom, Bott and Shiels, (55) to be considerably higher than that of creatinine at low plasma levels. In the case of hippuran, it was also shown by Elsom, Bott and Walker (56), that not only was the clearance much greater than could be accounted for by glomerular filtration alone, but that it even slightly exceeded the renal plasma flow determined simultaneously in the anaesthetized rabbit by thermostromuhr.

In 1938, Smith, Goldring and Chasis (167) examined the applicability of diodrast clearance at low plasma levels, to the measurement of human renal blood flow. Although they did not measure the renal extraction ratio for diodrast, they suggested that the term "effective renal plasma flow" could be applied to the diodrast clearance, the implication being that the extraction of diodrast by functioning renal tissue

is complete. Two years later, White (185), using the explanted kidney preparation (145) in unanaesthetized dogs, reported an average diodrast extraction ratio of 74%. He found that diodrast added to drawn blood, passes slowly from plasma into red blood cells so that after 24 minutes (and 20 minutes centrifuging) the diodrast concentration of red cells was 13% of the plasma concentration. He also observed that the red cell concentration of diodrast in renal vein blood is always considerably lower than that in arterial blood, apparently due to loss of diodrast from the red cells as the accompanying plasma is cleared of diodrast in its passage through the kidney. It appears then, that diodrast passes rapidly between plasma and red cells in dog's blood in vivo, but slowly in vitro. Accordingly White applied a correction factor in calculating renal plasma flow, to diminish the observed diodrast clearance by an amount equal to the clearance from red cells, but he did not apply any correction to the diodrast extraction ratio to allow for exchange between plasma and red cells before centrifugation of blood samples. These observations were confirmed by Corcoran, Smith and Page (37), who found an average diodrast extraction ratio of 84%, using the explanted kidney preparation. Because of abnormal stresses to which the explanted kidney is subject, and since the exchange of diodrast between plasma and red cells in man appears to be less than in the dog, Smith (164), reviewing the use of diodrast in man, believed that its renal extraction ratio should be taken as

about 90%.

The organic iodine compounds suffered from two disadvantages as indicators in the routine determination of renal blood flow: their analysis was difficult, and they caused unpleasant side effects in human subjects. Thus in 1944 Smith, Finkelstein, Aliminosa, Crawford and Graber (168) investigated the possibility that the presence of iodine in hippuran is not essential to the process of tubular excretion. They gained indirect proof that the parent molecule, hippuric acid, is indeed also excreted by this process, and then went on to show that a number of substituted hippuric acid derivatives (which unlike hippuric acid, could be determined very accurately by coupling reactions) had clearances essentially identical to those of hippuran and diodrast. Of these hippuric acid derivatives, para-amino hippuric acid (PAH) was chosen as the most suitable indicator in the determination of renal blood flow in man by the Fick method, for the following reasons (164, 168): -

"(1) At low plasma levels, the clearance is identical with that of diodrast, and hence equally valid as a measure of the effective renal plasma flow.

(11) The chemical determination is extremely simple.

(111) The endogenous plasma and urine blanks are negligibly small.

(1v) It does not penetrate the human red cell in vivo, even on prolonged infusion, and hence any possible error

attributable to extraction from the blood cell during passage through the kidneys is obviated.

(v) It is non-toxic.

(vi) It is less extensively bound to plasma proteins than is diodrast, and hence errors involved in the estimation of the filterable fraction in the plasma (in the use of PAH for measuring Tm-see p. 61) are of less practical importance."

Numerous measurements of the renal extraction ratio for PAH in man indicate a normal average value of 92% (164), except in the first few months of life, when considerably lower values are found, according to a recent report (28).

In the dog, Phillips, Dole, Hamilton, Emerson, Archibald and Van Slyke (138) found that PAH exchanges rapidly between red cells and plasma, so that diffusion from cells to plasma in renal venous blood samples decreases the PAH arterio-venous concentration difference by 5%, even if the blood is immediately centrifuged. When a correction was applied for this in calculating renal PAH extraction ratio, they obtained an average result of 87% in anaesthetized dogs. Block, Wakim and Mann (16) found the same average value; Kinter and Pappenheimer (94), Thompson, Kavaler, Lozano and Pitts (173), and Selkurt et al (152,155) found slightly lower values. As no measurements of renal PAH extraction ratio in the unanaesthetized dog have been reported, it is not known whether the apparently lower results obtained in the dog indicate a true species difference from man.

In the rabbit, Montague and Wilson (126) found an average renal PAH extraction ratio of 90.0% (9 animals) during anaesthesia, and Korner (97) an average of 95.5% (16 animals) several hours after recovery from pentothal anaesthesia. Korner examined the distribution of PAH in rabbit blood, and found that the latter behaves more like human blood than dog blood, in that PAH does not readily enter red cells. It appears that in the rabbit, PAH is as suitable for determination of blood flow as in man. Throughout the present study it has been used as an indicator in the determination of renal blood flow by the Fick method.

The "Indirect" Fick Method Using Inert Gases.

A method whereby the Fick principle is applied to the diffusion of an inert gas from the bloodstream into a tissue, was introduced for the measurement of cerebral blood flow by Kety and Schmidt (93) in 1945. When an inert gas is used as indicator substance, it is only possible to determine the amount absorbed by the tissue indirectly, employing calculations based on the inert gas content of the venous blood. The inert gas used by Kety and Schmidt was nitrous oxide. In 1952, their technique was applied to the measurement of renal blood flow in man, by Galinier (70), and in the dog, by Conn, Anderson and Arena (34). The latter workers compared their results using nitrous oxide, with the renal blood flow determined directly by bubble flow meter and found a difference of 1% $\pm$ 6.8% S.D..

In 1955, radioactive krypton (Kr^{85}) was employed

instead of nitrous oxide to measure renal blood flow in an anuric human subject, by Brun, Crone, Davidsen, Fabricius, Hansen, Lassen and Munck (24). Kr^{85} was found to be a more convenient and accurately measurable indicator than nitrous oxide.

The "indirect" Fick method, using Kr^{85}, provides a more exact measurement of renal blood flow during oliguric states due to renal failure, than does the "direct" Fick method using PAH (130). Not only are no urine samples required, but under the conditions of low renal blood flow usually prevailing, the extraction of krypton is high, leading to large arterio-venous differences that can be accurately determined. PAH extraction ratio in the diseased kidney is on the other hand usually very low, and not amenable to accurate determination, leading to errors in calculating renal blood flow. This argument applies in converse to the measurement of blood flow in the normal kidney under conditions of moderate diuresis, where the PAH method is to be preferred.

Determination of Intrarenal Distribution of Blood Flow.

In 1947, Trueta, Barclay, Daniel, Franklin and Prichard (176) published their monograph on the renal circulation, in which they produced much histological evidence to prove that the renal medullary circulation in the rabbit and in man, gains its blood supply almost entirely from the efferent arterioles of juxtamedullary glomeruli. By injection of india ink and radiopaque substances (thorotrast) into the

renal circulation of the anaesthetized rabbit, they showed that there appeared to be an increased distribution of blood to the medulla at the expense of cortical flow, when the sciatic nerve was stimulated, or the animal subjected to various types of circulatory shock. They attributed this apparent redistribution of blood flow to spasm of the intralobular arteries, with shunting of blood through the juxtamedullary pathway they had described. Numerous other workers have attempted to study changes in intrarenal blood flow by thorotrast radiographs (73,126), and by examination of dye distribution in kidneys post-mortem (6,17,73,84,89, 127,135,171).

However, as pointed out by Scher (151) in 1951, dye distribution indicates blood content rather than blood flow. He developed a method for measuring focal renal tissue blood flows, utilising heated thermocouples implanted into the cortex and medulla, in anaesthetized and unanaesthetized dogs, rabbits, and cats. With this preparation he found that changes in cortical and medullary blood flow due to adrenaline and renal nerve stimulation always ran parallel, lending no support to the concept of a "juxta-medullary shunt".

Another ingenious technique for measuring intrarenal blood flows, was developed by Kramer, Thurau and Deetjeen (102) in the course of their studies of the relationship of medullary blood flow to the renal countercurrent concentrating mechanism, in dogs. They inserted a photo-electric cell into the renal pelvis via the ureter so that it lay against a renal

papilla, and positioned a small electric light bulb in medullary tissue by piercing it through the cortex. Blood flow through the inner medulla was calculated from the passage time of Evans blue dye injected into the renal artery, assuming a value for the volume of the medullary vascular system. Cortical blood flow could be similarly determined, by means of a reflector placed on the surface of the renal cortex. In measuring changes of intrarenal blood flow, this method suffers from the disadvantage that it cannot distinguish between a change in blood flow and a change in vascular volume.

Recently Thorburn, Kopald, Herd, Hollenberg, O'Morchoe and Barger (174) applied the principle of the krypton method previously described, to the determination of intrarenal flows. They measured the rate of disappearance from renal tissue of Kr^{85} injected into the renal artery of the unanaesthetized dog, by means of an external scintillation detector. The decay curve obtained could be separated into a number of exponential components, each of which was shown by comparison with renal autoradiographs made during the time course of Kr^{85} disappearance, to correspond to flow through a different region of the kidney.

The Measurement of Glomerular Filtration Rate by Renal Clearance Methods.

The development of chemical methods for measuring the rate at which the kidney's vital processes operate, began with the attempts of clinicians to evaluate renal function

by certain test substances. Thus in 1912, Ambard (4) attempted to obtain an index of renal function from the rate of urea excretion and its plasma concentration. On the basis of studies of urea excretion in the rabbit, Thomas Addis (1) in 1917 introduced the "urea excretion ratio" as a means of characterising the renal function of individual patients. This ratio, the quantity of urea excreted per unit time divided by the blood urea concentration, expressed virtually the same relationship as the renal clearance formula, although the concept of clearance was not expressed until 1928, by Moller, McIntosh and Van Slyke (125). Addis revealed later (164 p.65), that only after publication of his paper on the urea excretion ratio, was its functional significance as a measure of "the volume of blood freed from urea", pointed out to him.

Moller, McIntosh and Van Slyke defined the urea clearance as, "the volume of blood which one minute's excretion suffices to clear of urea". Jolliffe and Smith (85) applied the concept to the excretion of creatinine, also showing that the clearance of creatinine was considerably greater than that of urea.

The first attempt to measure the glomerular filtration rate was made by Mayrs and Watt (121) in 1922, using sodium sulphate. They had previously shown that the ratios of urinary to plasma concentration for sulphate, phosphate and creatinine, differed only slightly in the anaesthetized

rabbit. On the basis of this finding, and the high urinary concentration of these substances compared to that of urea, they considered that "The concentration ratios of certain very different substances are so nearly the same that it requires a stretch of the imagination to suppose they are secreted independently of each other. It is simpler to assume that they are concentrated by removal of water, and to account for possible slight differences in their concentration ratios by admitting that small quantities ..... may be absorbed".(120)

The first use of creatinine to measure filtration rate was made by Rehberg (142), who used the exogenous creatinine clearance to assess renal function in nephritis (83). It has subsequently been shown that man and other primates are exceptional among the mammalian species, in that they eliminate creatinine partly by tubular secretion. The search for a reliable reference substance, excreted by glomerular filtration alone in all species, continued until 1934, when Richards, Westfall and Bott (146) and Shannon and Smith (159) suggested inulin as such a substance. The lines of evidence by which these two teams of investigators arrived independently at the same conclusions, have been reviewed exhaustively elsewhere (164) and will not be discussed here.

Kaplan and Smith (91), and Josephson and Kallas (87) have demonstrated that in the rabbit, creatinine clearance is identical with the clearance of inulin over a wide range of plasma concentrations (creatinine 5-185 mg.per cent,

inulin 40-465 mg.per cent), and during varying degrees of diuresis.

The Validity of Renal Clearance Measurements in the Rabbit.

Since 1935 when Kaplan and Smith (91) reported an apparent relationship between glomerular activity and water excretion in rabbits, there has been controversy about the use of this animal in studies of renal function. As the spontaneous urine flow of the rabbit is only 0.05 to 0.2 ml/min (97), most investigators (21,47,66,87,91,97) have found diuretics and/or large water loads necessary to obtain accurate renal clearance measurements. However, if glomerular filtration rate varies with urine flow, then the interpretation of any changes in glomerular filtration rate in such studies would be complicated by the increase and subsidence of diuresis.

The techniques used by Kaplan and Smith, and by Dicker and Heller (47) in similar experiments showing alterations of both glomerular filtration rate and renal plasma flow with changes in urine flow, are subject to two criticisms. Firstly the magnitude of the water loads used in these studies was such * that circulatory embarrassment or electrolyte imbalances may have occurred, and prevented the achievement

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* The water load given was equivalent to the ingestion of 7 litres in one hour by a 70 Kg. man. Kaplan and Smith (91) reported the frequent occurrence of convulsions and death, apparently due to water intoxication, among rabbits used in their studies.

of a steady state. The importance of avoiding unphysiological waterloading was recognised by Forster (65), who found no correlation between urine flow and renal circulatory indices (GFR, RPF and glucose Tm), when diuresis was obtained only by a preparatory green feed diet and administration of diuretic drugs (intravenous mannitol and theophylline). Secondly, the use of an intragastric tube to administer water before commencing clearance measurements, suggests the possibility that these measurements were made during a rising diuresis following "emotional oliguria" (21).

This possibility is lent credence by the observations of Brod and Sirota (21), who demonstrated marked reductions in urine flow, renal blood flow and glomerular filtration rate after stressful procedures in the rabbit, in contrast to oliguria without renal circulatory changes, after injection of pitressin. Brod and Sirota, with water loads approximately half the size used by Kaplan and Smith, also showed that urine flow could vary 15-fold without a change in GFR, provided that emotional disturbance was avoided. They suggested that a relationship between urine flow and GFR, especially in the experiments of Dicker and Heller where measurements were begun shortly after intubation, might be fortuitous and due to recovery from the effects of adrenaline release. In assessing this interpretation, Dicker and Heller have further analysed their experiments (48) and shown that much the same relation holds between GFR and urine flow at falling, as at rising rates of diuresis. This finding, together with the

fact that urine flow and GFR are related in amphibians and newborn mammals, leads them to view that the relationship is physiological, and not a pathological response.

In conclusion it may be stated that whatever the response of the renal circulation may be to very large water loads, it appears to have little relevance to experiments in which moderate water loading or diuretics are used. Under these conditions both renal blood flow and GFR are stable despite variations of urine flow, for at least 3-5 hours (65,97).

The Renal Circulation in Anaemia.

A. In Man.

In 1947 Bradley and Bradley (18) examined the effects of anaemia on the renal circulation in unanaesthetized man, using clearance methods to measure renal blood flow and glomerular filtration rate. Their subjects suffered from several types of chronic anaemia, the majority having pernicious anaemia. Accepting diodrast clearance as a measure of "effective renal plasma flow" in anaemia, they found a reduction of ERPF averaging 25% in males and 10.5% in females. The reduction in renal blood flow calculated from ERPF and arterial haematocrit values averaged about 50% for males at haematocrits between 12 and 28. Glomerular filtration rate and filtration fraction were also decreased. That diodrast Tm was depressed in the presence of normal values for glucose Tm suggested a dysfunction of renal tubular cells. Similar results were obtained for renal blood

flow and glomerular filtration rate in pernicious anaemia, by Levin, Gregory and Bennett (108), although in this study as in that of the Bradleys, no measurements of renal diodrast extraction were made. As disturbances in cellular metabolism due to severe vitamin B_{12} deficiency could have interfered with diodrast secretion by the renal tubular cells in these studies, their assumption that diodrast extraction was normal may possibly have caused renal blood flow values to be underestimated.

However, the more recent findings of Whitaker (184) and Reubi, Vorburger and Keller (144) using PAH clearance, confirm the direction of change in renal blood flow reported in the earlier studies. Whitaker determined the renal extraction ratio for PAH in one of his ten patients, and obtained values of 86% before, and 87% after treatment of the anaemia. On the basis of this result, and the similar values reported in two other anaemic patients by Warren, Brannon and Merrill (181), he concluded that renal plasma flow in anaemia was decreased to the same extent as the PAH clearance. Reubi et al. determined E_{PAH} in their four subjects, and found three values (75%, 81% and 83%) lower than the range of a control series: renal blood flow determined by the Fick method, was also below normal, but the reduction was not as marked as in the earlier reports.

In contrast to the fairly uniform results obtained for the renal circulation in anaemic adults, the findings in children with sickle cell anaemia (Etteldorf, Tuttle and

Clayton (59) and Cooley's (Mediterranean) anaemia (Bruck (23)) appear anomalous. In most subjects, PAH clearance and GFR were increased significantly above normal, and were not reduced in any case. Effective renal blood flow and $T_{m_{PAH}}$ were either normal or increased. Etteldorf, Smith, Tuttle and Diggs (60) showed that these normal and supranormal values did not persist into adult life, but perhaps because of progressive renal pathology, decreased progressively.

B. In Dogs.

Paterson (137) made serial determinations of PAH clearance and creatinine clearance in two trained unanaesthetized dogs, rendered anaemic over a period of five months by repeated bleeding and a special diet. At haemoglobin levels below 5 gm. per 100 ml, effective renal plasma flow and GFR began to decrease, reaching 75% of initial values at the lowest haematocrits recorded (16% and 20%). A fall of 40-50% in renal blood flow was inferred. There were no appreciable changes in Tm for glucose or PAH, or in filtration fraction.

In the series of Davis, Goodkind and Ball (45), a more acute anaemia was produced by daily bleeding in six normal dogs. There was an increase in cardiac output, accompanied in four animals by an increase in PAH clearance of from 20 to 40%. GFR was unaltered or increased.

Using anaesthetized dogs, Share (160) and Thompson, Kavaler, Lozano and Pitts (173) showed that when acute anaemia was produced by bleeding with replacement of plasma or dextran, there were no appreciable changes in renal blood flow or GFR. It is interesting to contrast the findings in these experiments where total blood volume was maintained, with those of Paterson, where both renal blood flow and total blood volume were markedly decreased. Korner (96) made a similar comparison between the elevated renal blood flow and blood volume reported in the congenital anaemias, and the decreased RBF and blood volume reported in pernicious anaemia, and suggested changes in renal blood flow may be more closely related to changes in total blood volume than to the level of anaemia.

C. In Cats.

In acute severe anaemia (mean haematocrit 7%) produced by bleeding with plasma replacement, Kinter and Pappenheimer (95) have reported renal blood flows averaging 2½ times the values at normal haematocrit (42%). Reservations must be made however, in assessing this result, as the animals were anaesthetized (chloralose) and subjected to bilateral denervation and other operative interference. Renal blood flow was calculated in each experiment from the clearance and extraction ratio of PAH or diodrast: the clearances were only moderately increased and much of the increase in renal blood flow after bleeding reflects a marked depression

in extraction ratios (94). Although it was shown that this depression was reversible by blood transfusion, it is possible that it represents the effect of renal cortical ischaemia, recoverable by virtue of the increased amount of dissolved oxygen in the perfusing plasma (the animals were ventilated with 100% oxygen throughout each experiment). Black and Saunders (14) found low renal PAH extraction ratios in anaesthetized cats after operative trauma alone.

In summary, studies of the renal circulation in anaemia have revealed a diversity of effects in several species. For example, renal blood flow has been reported to be decreased, unchanged or increased. Associated disease and blood volume changes may be responsible for some of these differences. Only in the anaesthetized cat and dog (94,173) is there detailed information about the behaviour of the PAH extraction ratio in anaemia. The marked effect that has been observed in these preparations suggests that in any study in anaemia utilising PAH clearances, PAH extraction ratio should be measured and used in calculations of renal blood flow.

The Renal Circulation in Post-Haemorrhagic Hypotension.

Most studies of the effects of post-haemorrhagic hypotension on the renal circulation have been carried out in anaesthetized dogs (8,36,74,80,138,152,155). It has been found in general that after haemorrhage sufficient to lower the arterial pressure to levels of 50-70 mm Hg (i.e.

about 20-30 ml/Kg), there is a fall in renal blood flow and in glomerular filtration rate which is at least as great as the fall in pressure. Subsequently as the blood pressure rises again RBF and GFR tend to recover, although not necessarily to control values (138); even after replacement of lost blood, renal haemodynamics may not be restored completely (36,152).

However there is great dissimilarity between series in the details of the renal response to haemorrhage. Using clearance methods with moderate saline diuresis, the apparent depression of renal blood flow during the hypotensive period has in all studies been much greater than the fall in arterial pressure, indicating the occurrence of marked renal vasoconstriction. Using direct methods for the measurement of RBF, Balint et al (8,10) found that RBF fell only in proportion to the arterial pressure and cardiac output, implying that there was no increase in renal vascular resistance or the renal fraction of the cardiac output. They postulated that clearance - based determinations of RBF during hypotension are inaccurate due to storage of PAH in the renal interstitium (9). Selkurt (152) also found a disparity between direct and indirect measurements of RBF, but in his experiments renal resistance calculated from direct-measured flows was still increased. The validity of techniques employed to measure renal blood flow directly has also been questioned; this

point was discussed earlier in the present report (p. 8). If hypertonic mannitol is infused during the period of hypotension after haemorrhage, there appears to be a striking modification of the renal response as judged by clearance data; instead of the anticipated extreme reduction in PAH clearance, it is reduced only to about the same extent as the directly measured renal blood flow (8). The apparent amelioration of the renal response may be due to a direct relaxing effect of mannitol on the renal arterioles (20) or it may be due simply to mannitol diuresis preventing storage of PAH in the medullary interstitium (131). If it is accepted that renal vasoconstriction does occur during post-haemorrhagic hypotension in the anaesthetized dog, as is probably true if the haemorrhage be severe or repeated, then it appears likely that it is predominantly post-glomerular (138) and is mediated by humoral factors rather than neurogenic (36).

Results for the renal extraction of PAH in the period after haemorrhage have also been variable. Phillips et al (138) found that acute haemorrhagic or traumatic shock did not alter the percentage extracted unless hypotension was so severe that renal blood flow was decreased to less than 3% of normal. However Selkurt (152) and Corcoran et al (36) reported marked depression of the renal extraction ratios for PAH and diodrast respectively, at rather higher levels of flow during hypotension.

In man, evidence of increased renal vascular resistance during haemorrhagic shock has been obtained using clearance methods (107). The filtration fraction decreased, instead of increasing as in the dog; this may indicate a different site of action of vasoconstrictor factors.

In summary, it is probable that reduction in renal blood flow during post-haemorrhagic hypotension is not merely a passive response to the fall in blood pressure but is due in part to renal vasoconstriction. However the interpretation of clearance data regarding this effect is vexed by the question of whether PAH is stored in renal tissue. The nature of possible vasoconstrictor agents acting on the renal circulation after haemorrhage is largely unknown.

The Plan of the Present Study.

The effects of post-haemorrhagic anaemia may be attributable to reduced red cell concentration and blood viscosity, to reduced haemoglobin and O_2 transport or to changes in blood volume. Three types of experimental anaemia were studied. In the first type, blood removed from the animal was replaced by plasma so as to investigate the effects on the renal circulation of reduction in haematocrit uncomplicated by decreased blood volume. The changes observed in this type of anaemia were compared with the effects of carboxyhaemoglobinaemia in animals with a normal haematocrit, and the findings in anaemic animals whose blood O_2 transport was increased by breathing 100% O_2. Since

carboxyhaemoglobinaemia results in reduction of tissue oxygenation without decreased blood viscosity, and inhalation of 100% O_2 increases tissue oxygenation (when arterial O_2 content is low) without increasing blood viscosity, these comparisons aided in differentiating the local effects of renal hypoxia in anaemia from those due to reduced blood viscosity.

In the second type of anaemia studied measurements were made during a period of 150 minutes after haemorrhage; the results suggested that reduction in total blood volume was producing extrinsic effects on the renal circulation. It was demonstrated that the "ureter" animal with one kidney denervated and the other intact could serve as a useful experimental model for testing for the presence of nervous and certain humoral effects on the renal circulation. The contributions made by nervous and humoral factors in the renal response to acute hypovolaemic anaemia were then investigated using this experimental model.

In the third type of anaemia studied, the animal's own volumeregulating mechanisms were allowed time to operate following repeated haemorrhage. Thus renal measurements in anaemia were carried out at a stage when the haematocrit was severely reduced and blood volume adjustments were nearly complete. The results in this type of anaemia were compared to the results in the other two anaemias investigated. An attempt was made to determine whether the local effects of reduction in haematocrit or the central

effects of reduction in blood volume had brought about the changes in the renal circulation in more chronic post-haemorrhagic anaemia.

Unanaesthetized animals were used in all experiments. Where anaesthesia was needed in preparatory operations, as much time as possible was allowed for the animal to recover before it was used in an experiment. Renal blood flow and glomerular filtration rate were measured by PAH and creatinine clearance methods; all clearance measurements were carried out during moderate mannitol diuresis. In most of the animals studied, the renal PAH extraction ratio was determined and used in the calculation of renal blood flow.

CHAPTER 2

METHODS

SELECTION, CARE AND FEEDING OF ANIMALS.

Throughout this investigation, hutch-bred rabbits, ranging in weight from 1.9-4.0 Kg were used. Uncastrated male animals of the New Zealand Gi ant strain were preferred because of their leaner habitus, and were used in the great majority of experiments. Castrated male and female rabbits of mixed breed were employed in a few experiments; the female was found unsuitable in general, due to the great difficulty in introducing a bladder catheter through its urethral orifice.

The animals were housed one to a cage in air-conditioned surroundings. The diet consisted of dry animal pellets, with greens twice a week. Special care was taken to ensure that the animals had access to an adequate supply of water up to the time of doing experiments. In addition, all animals which were not given water by indwelling oesophageal tube, were encouraged to drink ad libitum during the course of experiments. A small detachable metal trough was attached just beneath the animal's mouth inside the wooden cage (see fig.1) in such cases, and a moistened pipe cleaner used to prompt drinking. Addition of small amounts of sucrose was also found to promote drinking.

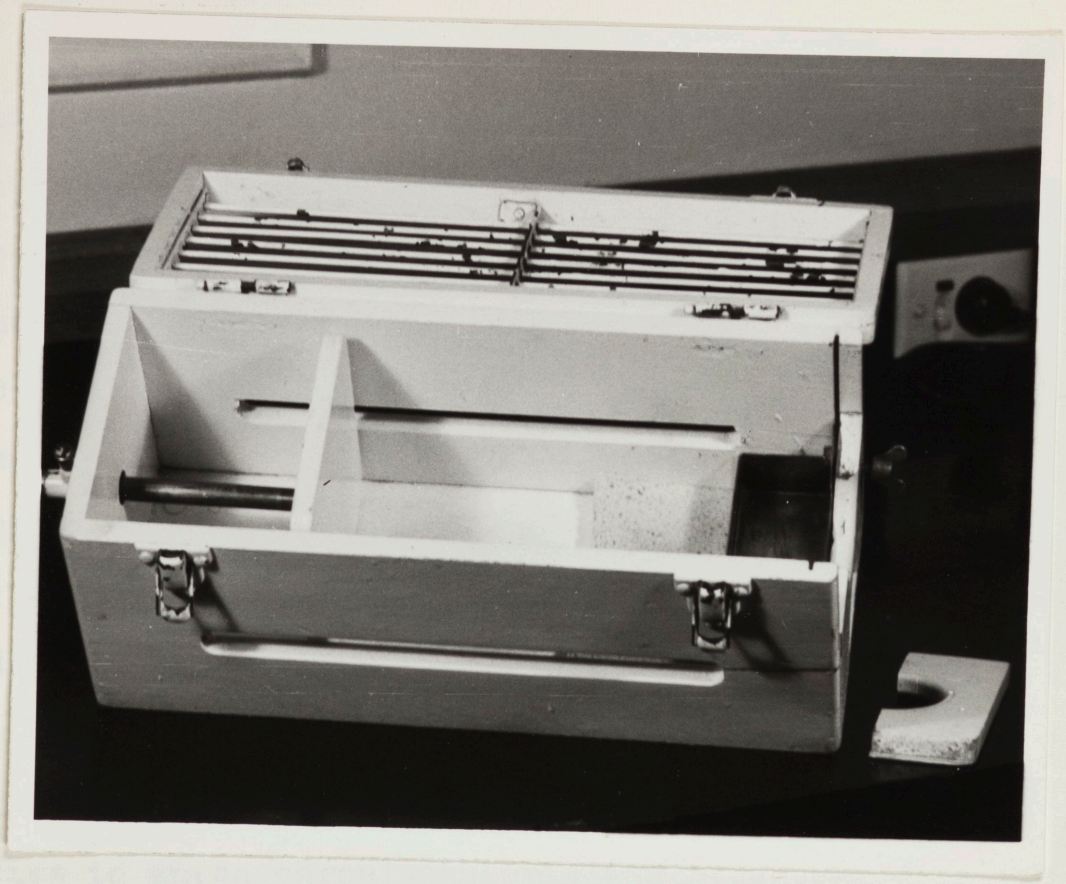

Figure I. Rabbit box with lid open and headpiece removed. Partition can be slid back to allow animal to sit with its head inside the box.

SURGICAL PROCEDURES.

A. Minor Procedures:

1. Bleeding.

In experiments on the effects of post-haemorrhagic anaemia, animals were bled either from the marginal ear vein, or from the catheterized central ear artery. When bleeding was carried out from the marginal ear vein, the animal was held comfortably in a wooden cage with its head protuding (Fig.2). Dilatation of the ear vessels was produced by warming the ear with a 15-watt globe, and gently stroking over the central ear vessels. The marginal ear vein was nicked with a sharp scalpel so as not to produce complete transection, and blood allowed to drip into a calibrated beaker containing several drops of concentrated heparin solution (5,000 units/ml). In studies in which chronic anaemia was produced, this procedure was repeated daily (sometimes twice daily) on 1 or 2 occasions, the cuts in the marginal ear vein being made progressively more distal to the initial venesection. This method allows a maximum of about 20 ml/Kg. of blood to be removed from the animal over a period of 20-30 minutes, at which stage pronounced vasoconstriction slows bleeding to a standstill. In these studies, a venesection of 15 ml/Kg per day for 2-3 days was found to produce a haematocrit between 18 and 25% (normal haematocrit 35-45%) and to be compatible with long-term survival. A small metal spring clip was placed over the cut vein to prevent

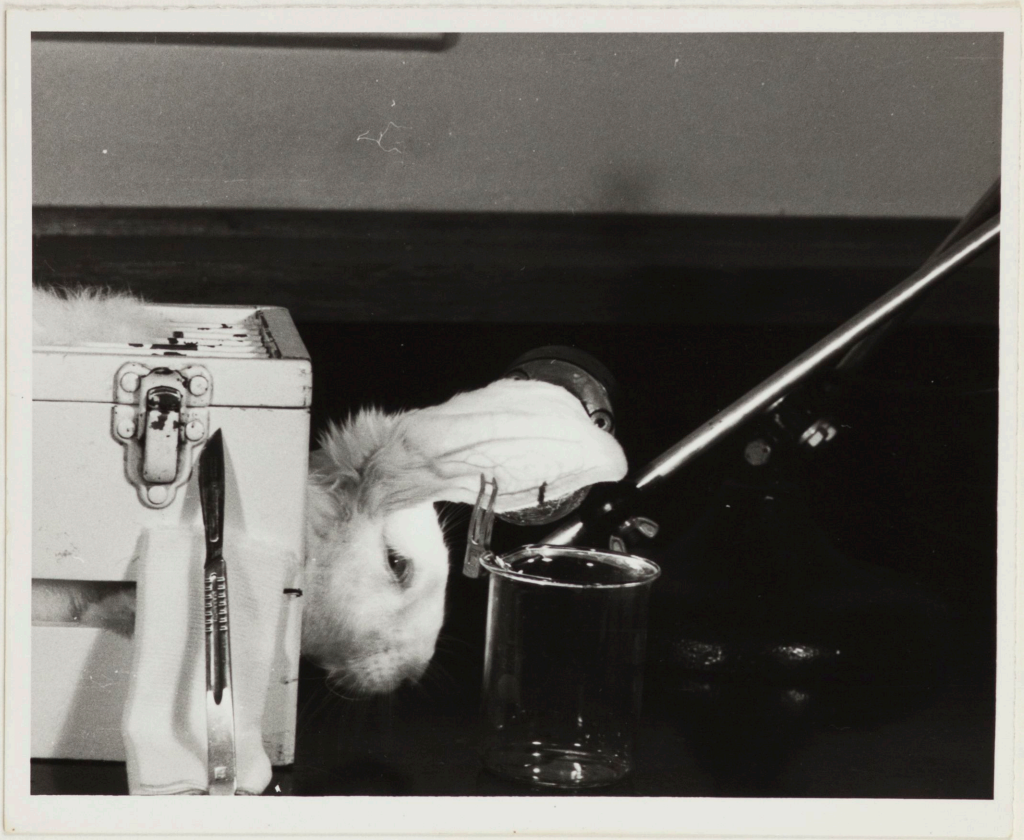

Figure 2. Bleeding procedure. The marginal ear vein has been nicked with a scalpel. The ear is warmed by a 15 watt globe.

inadvertent oozing between venesections (Fig.2)

2. Catherization of the Central Ear Artery and Vein.

The technique followed was essentially as described by Edwards, Korner and Thorburn (52). The animal was held in a wooden box with its head protuding. The skin over the central ear vessels was infiltrated with 0.5% lidocaine hydrochloride (Xylocaine), which does not interfere with the para-amino hippurate colorimetric reaction. A piece of stout plastic tubing 5 cm. long was sutured along one ear to serve as an anchorage for the arterial and venous catheters. The skin over that part of the central ear vessels proximal to the junction of the marginal and central ear veins, was incised, and the vessels separated and dissected free of nerve and fibrous tissue for a distance of 2 cm.. The central ear vein was catheterized for a distance of 2-3 cm. with a bevel-pointed 12 cm.length of 22-gauge Transflex tubing (Irvington Division, Minnesota Mining and Manufacturing Co., Freehold N.J., U.S.A.). After application of several drops of 0.5% Xyocaine to produce dilatation of the central ear artery, it was tied off, and catheterized for a distance of 2-3 cm. with a bevel-pointed piece of polythene tubing (1.2 cm. O.D.) filled with dilute heparin solution. The polythene tubing was tied in position and cut to leave 1 cm. protruding from the artery. This was quickly inserted into a close fitting size of Transflex tubing (20-gauge), and glue applied to the joint. Both catheters were led through the anchoring tubing and

protected from the animal's interference by an enveloping sleeve of adhesive tape. The catheters were flushed with a 1 in 200 dilution of heparin (5000 units/ml) in Ringer-Locke solution, at ½ hour intervals. Great care was taken to avoid the introduction of even very small air bubbles into the arterial catheter, as cerebral embolism with severe convulsions usually terminated the experiment when this inadvertently occurred.

In earlier experiments involving a repeated use of the ear vessels for blood sampling, infusions and arterial pressure recording, first one and then the other ear was utilized. It seemed likely that by the time the second ear's vasculature was interrupted, that of the first ear, operated upon several days earlier had established a collateral circulation sufficient to avoid gross disturbance in the animal's body temperature regulation. However to test the possibility that these procedures disturbed body heat regulation, the central ear vessels of an animal were ligated first on one side, and then one hour later, on the other side. Environmental temperatures (on a hot summer day) and rectal temperatures (using a rectal thermistor, and tele-thermometer), were recorded before interference, and until 3 hours after operation on the second ear. (See Appendix 1.). Rectal temperature remained constant throughout. As further tests, tracheotomy was performed, and the environmental temperature raised to 43° C. These further

challenges to heat regulation raised the rectal temperature only 0.5° C.. It thus appears that bilateral ligation of the central ear vessels did not seriously disturb the rabbit's body temperature regulation.

In later experiments, it was found possible by use of soft polyvinyl catheters (1mm.O.D.) inserted 4-6 cm. into each vessel, and filled with concentrated heparin solution when not in use, to maintain their function for several days, thus leaving one ear always intact. These catheters were folded inside the anchoring tubing and plugged with copper wire between experiments.

3. Bladder Catheterization.

An assistant held the animal on its back by one hand around its forepaws and the other across its lower abdomen. A Jacques size No.7 rubber bladder catheter lubricated with petroleum jelly was inserted through the urethra for a distance of 10 cm., and held in place by a brass cylinder and screw (Fig.3) which was stitched to the perineum. The catheter was then tested by flushing with saline, for return of urine-coloured fluid. In several cases, re-insertion was necessary, apparently due to the catheter tip lodging in the seminal vesicles. In larger, more mature rabbits, a patulous urethra occasionally allowed a slight leak of urine from around the catheter. In these cases a small filter funnel with a curved spout (Fig.3) was used to trap these leaks without seriously enlarging the dead space.

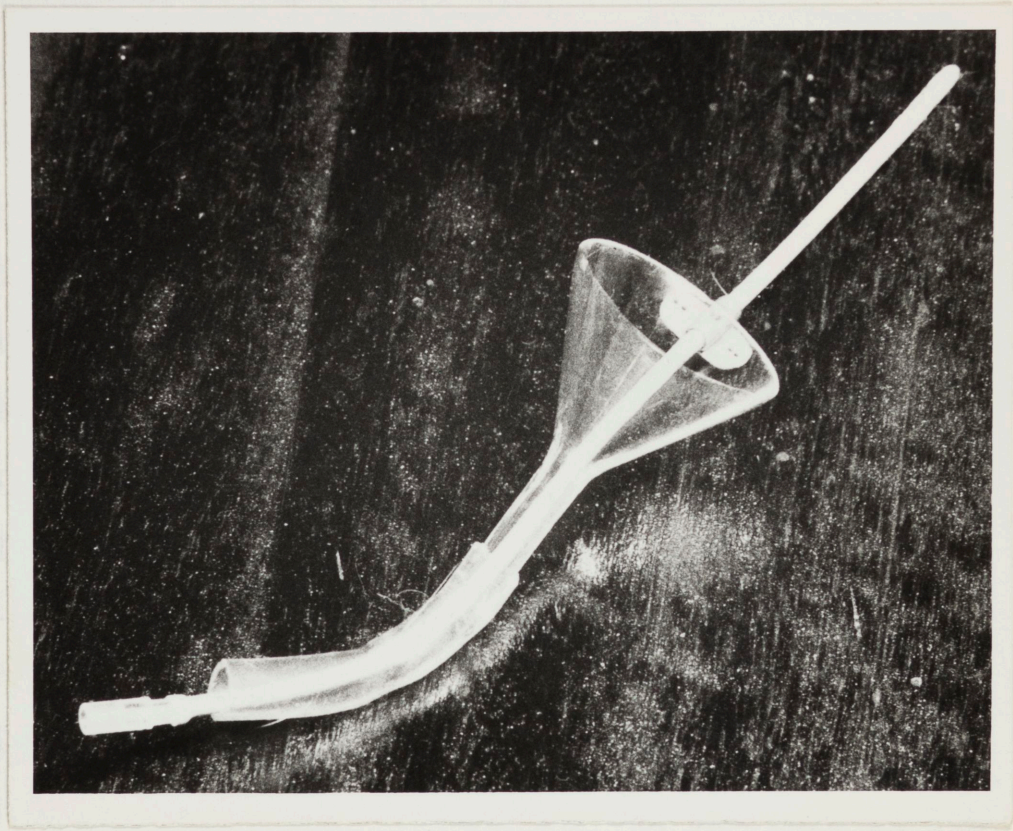

Figure 3. Urine-trapping funnel. Used only in experiments in which a patulous urethra allowed leakage of urine around the rubber bladder catheter. Urine was washed from the spout of the funnel by a jet of saline from a syringe.

B. Procedures Carried Out Using General Anaesthesia.

Except where otherwise indicated intravenous sodium pentobarbital (Veterinary "Nembutal", Abbott) in a dose of 40 mg/Kg, was used as an anaesthetic agent. The duration of surgical anaesthesia was about 1 hour, and complete recovery took 4-6 hours. It was found that the dose of pentobarbital required to produce complete hypnosis and muscular relaxation, was often very close to the dose producing depression of the respiratory centre, and apnoea. Any hypoxia resulting from partial airway obstruction, potentiated the respiratory depressant effect of the drug. Thus it was essential to maintain a patent airway with neck fully extended, and to secure a rapid induction to avoid coughing and accumulation of pharyngeal secretions. The routine adopted was as follows.

(1) With the animal held in a wooden box with its head protruding, two-thirds of the above dose was given rapidly into the marginal ear vein.

(11) The animal was removed from the box and laid on its side. The tongue was pulled out with forceps, and a pharyngeal airway inserted (5mm.O.D. curved polythene tubing, 7cm. in length).

(111) When rhythmic respiration had commenced, the remainder of the anesthetic dose was given over 1-2 minutes, until the corneal reflex had disappeared. If apnoea occurred, rhytmic squeezing of the thorax usually restored respiration promptly.

Once induction was completed, further doses of the anesthetic agent were rarely needed.

During procedures involving dissection in the vicinity of the renal artery, it was noted that this often became markedly constricted, with visible decrease in the oxygen saturation of renal vein blood. Anaesthesia alone was also found to cause a decrease in the normally high (80%) oxygen saturation of renal venous blood. The administration of 100% O_2 per pharyngeal tube, was followed by a prompt return of bright red colour in the renal vein stream. For these reasons, 100% O_2 was given routinely throughout these operations to protect the kidneys from possible ischaemia.

For all operations the following aseptic and antiseptic precautions were observed:

(1) Sterilisation of instruments by boiling for 20 minutes.

(11) Cleansing of skin at site of incision with a local antiseptic ("Zephiran" Bayer, 1/1000).

(111) Crystalline penicillin 500,000 units intravenously, prior to induction of anaesthesia.

(1v) Spraying of wound prior to closure with an antibiotic spray containing neomycin, neosporin and bacitracin ("Neotracin" Andrews Laboratories).

Signs of bacterial wound infection, such as post-operative morbidity or pus formation, were not found in any experiment in this series.

Renal Vein Catheterisation.

In pilot experiments, this preparation was carried out early in the day on which renal clearances were determined, using the method described by Korner (97). A 22-gauge Transflex catheter was inserted via the right external jugular vein, right atrium and inferior vena cava into the right renal vein. The results for PAH extraction ratio in 6 control preparations (appendix A) agreed closely with previous results for the rabbit, and with those obtained subsequently in this study. However this method was found to be unsuitable for anaemic animals since they recovered too slowly from the thiopentone anaesthetic employed; this in turn led to the following difficulties:-

(1) Uncertainty as to when a steady state had been reached in a given preparation. Tachypnoea, troublesome pharyngeal secretions, abnormally low blood pressure and clearance values often persisted over 3 or 4 hours after operation.

(11) There was an increasing risk with the passage of time after operation, that the animal's movements would cause the renal vein catheter either to slip out into the inferior vena cava, or impact into a renal vein tributary resulting in renal infarcation.

Thus a method was evolved for direct introduction of a catheter into the left renal vein, 1 to 4 days before all experiments in which renal blood flow was determined from clearance measurements (Figs.4,5,6). The animal was

Figure 4. Exposure of the left renal vein through an incision in the left hypochondrium. The forceps mark the junction of the renal vein with the lumbar vein which is to be catheterized. The left adrenal gland, left kidney and inferior vena cava can be identified by referring to figure 6.

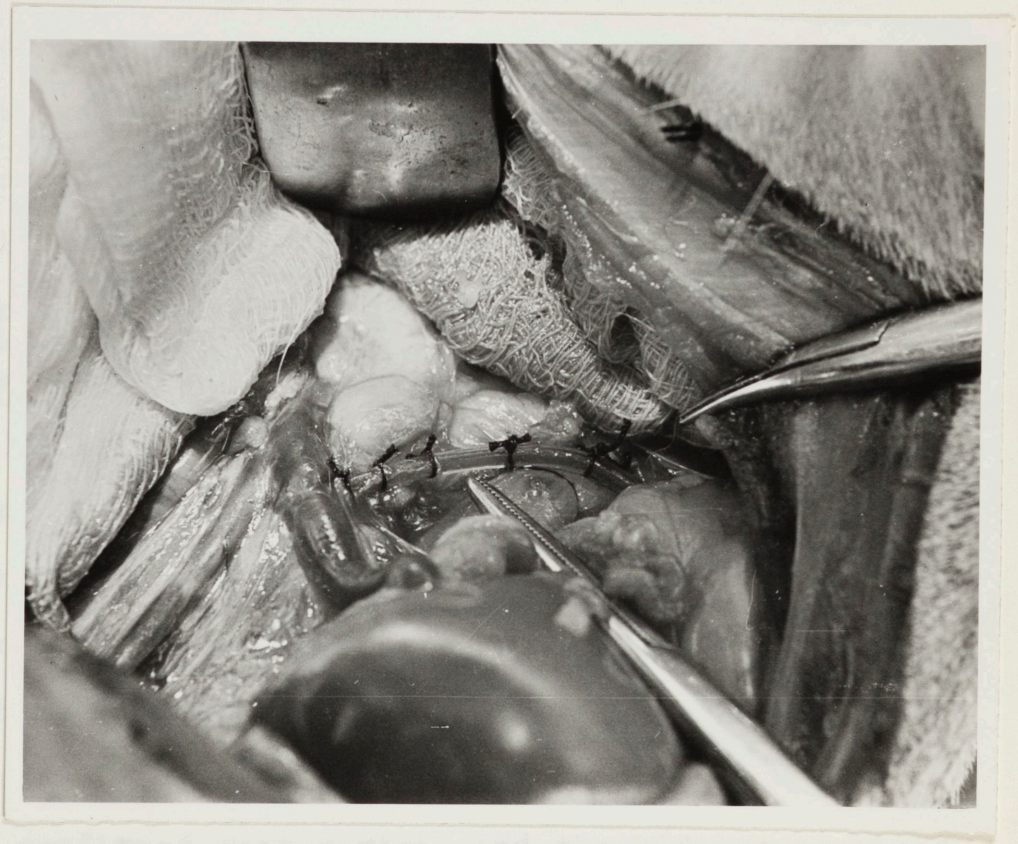

Figure 5. Renal vein catheter in situ. Two sutures anchor the catheter to the left psoas muscle.

anaesthetized with sodium pentobarbital, and a transverse muscle-splitting incision was made in the left flank to give trans-peritoneal access to the left kidney and left renal vein. In most cases, the latter has a lumbar vein entering it superiorly 1-2 cm. from its junction with the inferior vena cava; animals in which this distance was less than 1 cm. and animals which had an anomolous renal venous drainage, were rejected. The lumbar vein tributary was catheterized with 20-gauge Transflex tubing, which was anchored firmly to the left psoas muscle by one or more sutures, so that its siliconed + tip lay within the lumen of the renal vein, and sampled only renal venous blood (see Fig.6). The catheter was brought to the exterior through a stab wound high in the left flank, and protected from the animal's interference by strapping it to the skin and binding a light cloth harness around the abdomen. The catheter was filled with concentrated heparin solution (5000 units/ml), which was replenished daily. Its position in the renal vein was always confirmed post-mortem, and the kidneys examined for microscopic evidence of infarction.

The reproducibility of results for renal PAH extraction ratio was very high in this preparation. The average control E_{PAH} of 95% ± 0.43% (S.E.) in 43 animals was higher

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+ The tip of the catheter was siliconed by immersing it in "Drifilm", SC-87,(American Electric Company)for 10 minutes.

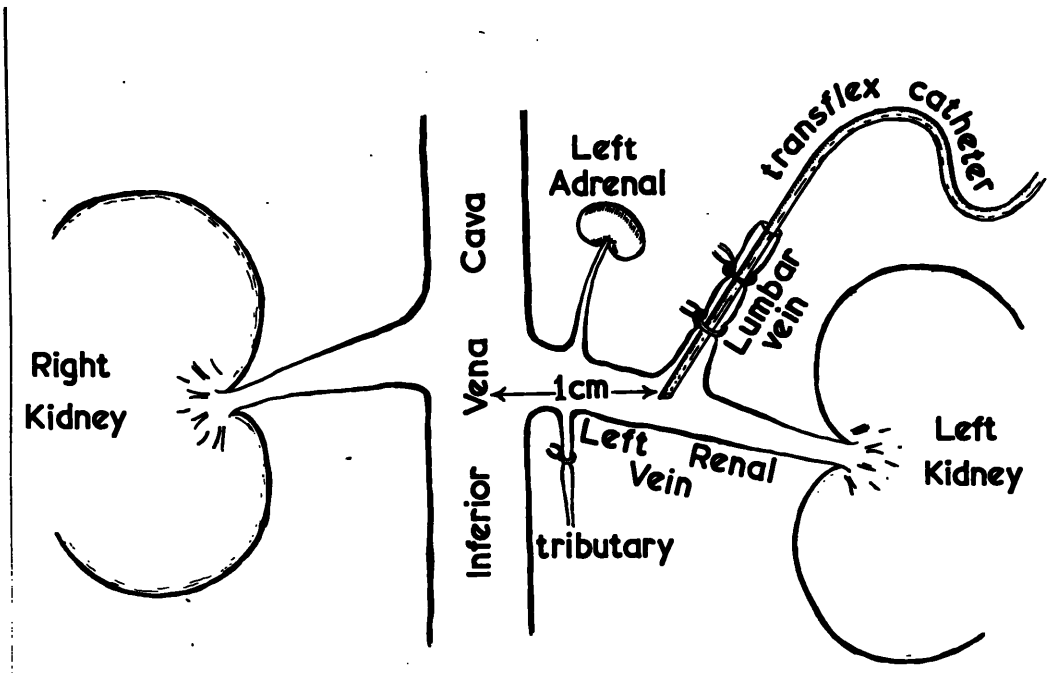


Figure 6. The anatomy of the left renal vein and its tributaries in the rabbit shown schematically with the renal vein catheter in position.

with a smaller standard error, than most reported results in a variety of species (14,28,29,94,97,126,138,144,155, 164,173,181). One obvious advantage of the method is the direct visualisation of the renal vein which permits ligation of all tributaries carrying blood from tissues other than kidney. However in about 5% of preparations the control E_{PAH} value was well below the narrow range of apparent normality. In some of these, marked variability in the renal venous levels of PAH suggested that turbulent blood flow at the caval orifice of the renal vein might have resulted in the sampling of blood from the lower extremities by the renal vein catheter. In other cases with low E_{PAH} values, infarction of the kidney was found post-mortem. Accordingly, the rule was adopted that the results of any experiment in which a control renal PAH extraction ratio of less than 85% was obtained, were rejected, including those cases in which no obvious abnormality was found post-mortem.

Denervation of the Renal Pedicle.

In 16 experiments designed to test the role of the renal vasomotor nerves in the response the renal circulation to various treatments, unilateral denervation of the renal pedicle was carried out. Using sodium pentobarbital anaesthesia, the left renal vessels were exposed by an anterior approach, and dissected apart. Usually several identifiable nerve trunks lay between the artery and vein; these were divided and a length of each

excised. A careful stripping of the adventitia of the renal artery over 1-2 cm. of its length was then carried out, the vasoconstriction which tended to accompany this procedure being prevented by application of 2% Xylocaine to the artery. Between 4 and 10 days were allowed to elapse before differential renal clearances were determined using bilateral ureteric catheters.

The possibility that tubular dysfunction might result from the denervation procedure was tested in 2 normal animals (with chronic left renal vein catheters). Measurements were made before renal denervation, and on the two succeeding days (Table 1). It was demonstrated that there was no appreciable change in renal PAH extraction ratio as a result of renal denervation. In addition E_{PAH} in the denervated kidney of another animal was shown to decrease only slightly after hemorrhage (20 ml/Kg) resembling results obtained in the normal kidney (Table 18).

Bilateral Ureteric Catheterization. (Figs.7-9)

The method followed was essentially as described by Korner. Following catheterization of the ear vessels, the animal was lightly anaesthetized with sodium thiopentone (initial dose 18mg/Kg) and a midline lower abdominal incision made to display the lower portion of each ureter. Both ureters were ligated 2-3 cm. from their insertions into the bladder, and catheterized with 22-gauge Transflex tubing, using a 14 gauge hypodermic needle as cannula. The

TABLE 1

EFFECT OF RENAL DENERVATION ON PAH EXTRACTION

ANIMAL	E_{PAH} % *		ARTERIAL PAH (mg %)		HAEMATOCRIT %	
	1	2	1	2	1	2
Day 1	93.8	93.3	1.7	2.6	26.7	42.0
	88.4	93.5	1.8	2.3		
	Mean 91.1	93.4				
Denervated at end of Day 1						
Day 2	93.4	96.6	1.5	1.8	34.1	38.1
	94.2	95.6	1.5	1.6		
	Mean 93.8	96.1				
Day 3	93.4	95.1	1.8	1.4	28.2	32.5
	88.1	97.5	1.7	1.2		
	Mean 90.8	96.3				

*Each value based on 2 samples of arterial blood and 2 samples of renal venous blood. The 2 values shown for each day were measured one hour apart.

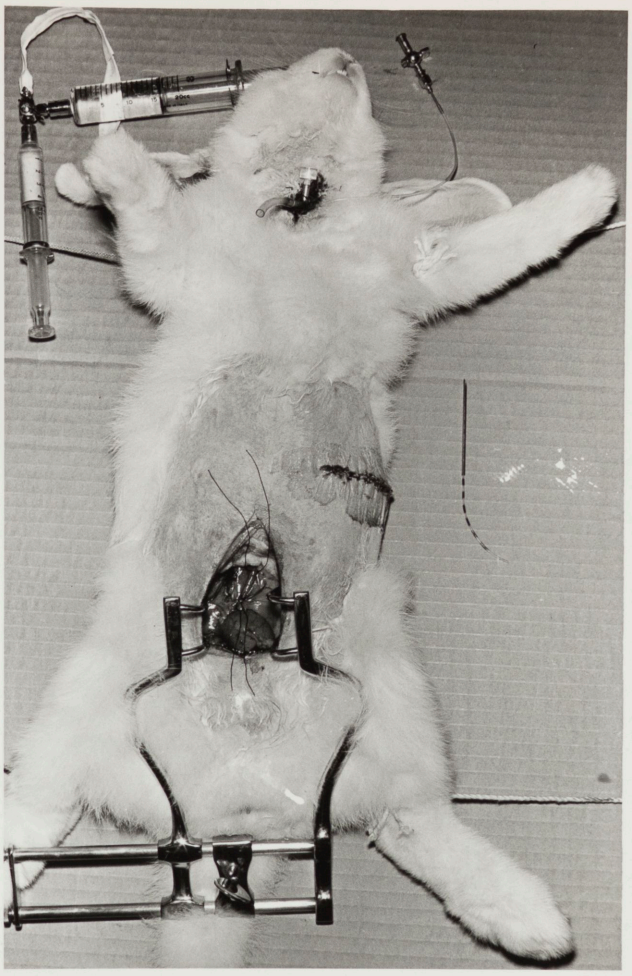


Figure 7. Rabbit prepared for catheterization of the ureters. One syringe connected to the ear vein catheter contains 5% mannitol, the other contains thiopentone solution. The tracheotomy tube and oesophageal catheter are in position. The healed incision for the left renal denervation performed one week earlier is seen in the left hypochondrium, and in the hypogastrium the bladder and right ureter are exposed. One ureter catheter, and the cannula used for its insertion, are shown beside the animal.

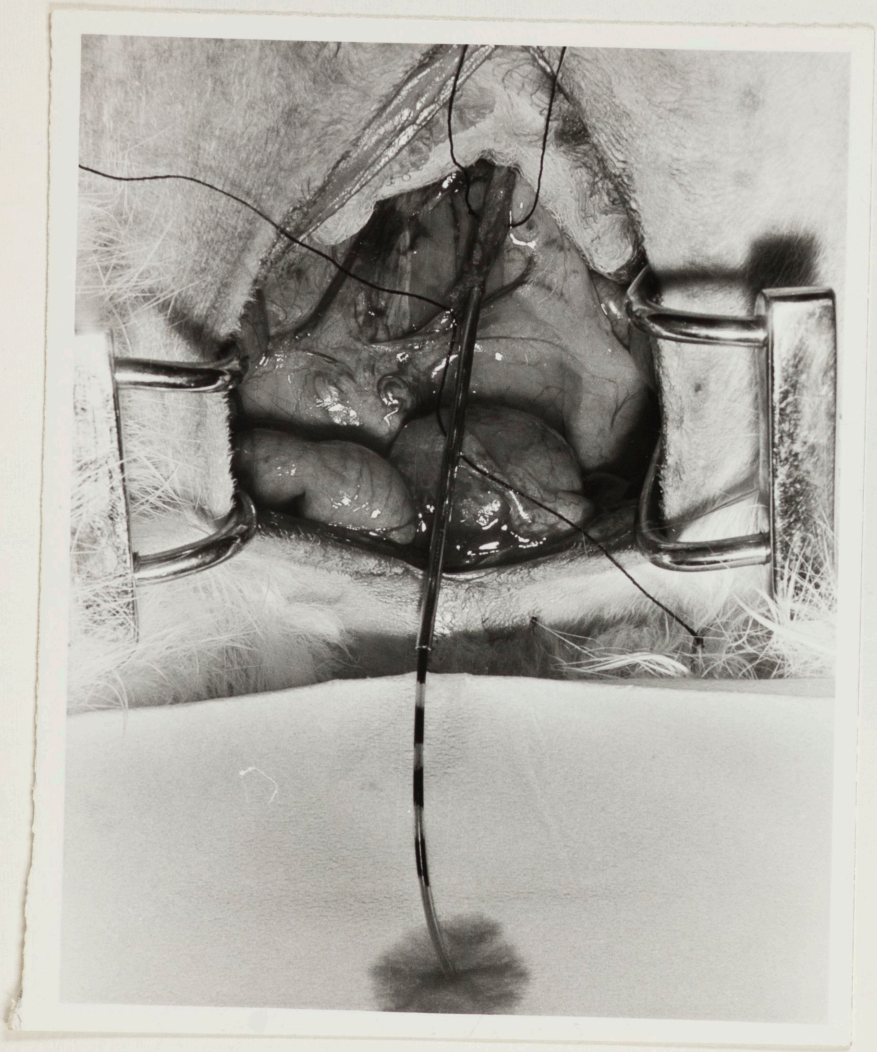


Figure 8. Catheterization of the right ureter.

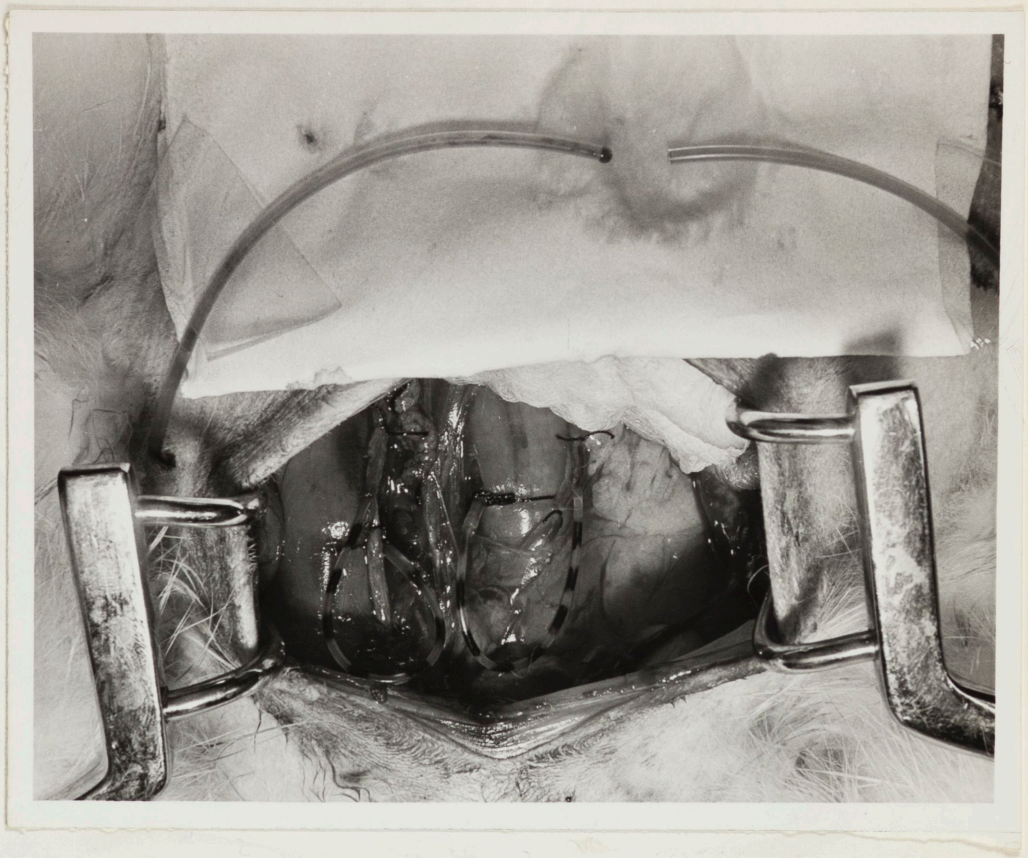


Figure 9. Ureter catheters in position. A suture anchors the junction of the narrow and wide calibre Transflex tubing to the psoas muscle. The wide tubing is brought to the exterior through a stab incision.

catheters were inserted for a distance of 4 cm. into the right ureter and 3 cm. into the left. The free end of each catheter was then formed into a loop and inserted into a 15 cm. length of 18 gauge Transflex tubing using methyl ethyl ketone as an adhesive; this was sutured to the psoas muscle, and brought to the exterior through a stab incision. The inclusion of a slack loop of tubing between the free-lying catheterized ureter and the point of fixation to the psoas muscle, allowed the ureters mobility when the animal regained consciousness and assumed its normal crouched position; without the loops, kinking of the ureters accompanied these changes in position. During this operation and the recovery period of at least 2 hours allowed after its completion before starting renal clearance measurements, a diuresis of 0.3-0.6 ml/min/ kidney was maintained by infusion of 5% mannitol in Ringer-Locke solution at a rate of 0.7 ml/min. Dehydration of the animal was prevented by administering water by oesophageal tube, 20 ml initially and 10 ml. per hour thereafter.

Tracheotomy; Catheterization of the Oesophagus.

During experiments in which 100% oxygen was administered, and experiments in which a low oxygen gas mixture was used as a test procedure after renal denervation, the animals breathed through a tracheotomy tube.

A vertical skin incision was made over the upper portion of the trachea, the strap muscles separated, and an oblique transverse incision made in the trachea. The

widest possible of 3 diameters (4.5, 5 and 5.5 mm. O.D.) of L-shaped brass tubing (see Fig.23) was selected and inserted 2-3 cm. into the lumen.

The oesophagus was cannulated with a 14 gauge hypodermic needle, and 22 gauge Transflex tubing inserted for a distance of 10 cm..

In experiments requiring tracheotomy without the use of general anaesthesia, a preliminary exposure of the trachea was carried out under sodium pentobarbital anaesthesia 3-4 days before the experiment (52). The strap muscles were tied behind the trachea, so that it lay just beneath the skin incision and could be cannulated when required quickly and with little dissection.

Post-mortem Examination:

At the termination of all experiments, the animal was sacrificed by an overdose of Nembutal, and the abdominal contents examined. The kidneys were removed and contained blood allowed to drain from them for a ½ hour. They were then weighed, and sliced for detection of any infarcts.

In experiments in which tracheotomy was performed, the thorax was opened and the lungs examined.

The results from experiments in which abnormal post-mortem findings were made were rejected. Such findings were uncommon in this series, and in decreasing order of frequency included:-

1) Renal infarction.

2) Retroperitoneal haematoma.

3) Coccidial cysts in liver and/or kidneys.

4) Solitary kidney. A single left-sided kidney of approximately double the normal size and weight, but otherwise normal appearance and vasculature was found in 2 animals. In both cases there was a complete absence of renal tissue, ureter and renal blood vessels on the right side. In one case there was an undescended testis also, on the right side. In one of these animals completely normal control renal clearance and extraction ratio values were obtained, and it is included in the chronic anaemia series, with annotation.

RENAL CLEARANCE METHODS:

Introduction:

Throughout these studies, indirect methods were used to measure renal blood flow and glomerular filtration rate. These methods each involved the introduction into the animal's general circulation of a non-toxic indicator substance whose rate of excretion provided a sensitive index of renal perfusion, called the renal clearance of that substance. For the reasons already discussed (pp. 15-23), the renal clearance of para-aminohippurate was used in estimating renal blood flow, while creatinine clearance and inulin clearance were used as indices of glomerular filtration rate.

Accurate measurements of renal clearances over short periods of time in the rabbit require conditions of diuresis, as this animal's normal rate of urine flow is low, and could result in significant delay of excreted indicator substances in the renal pelves and ureters. Therefore in all experiments

involving renal clearance measurements, an intravenous infusion of 5 per cent mannitol provided moderate osmotic diuresis, which has been shown (65) to cause no alteration in renal blood flow or GFR in the rabbit. During experiments in which the animal recovered from anaesthesia in the period immediately prior to measuring renal clearances, moderate water loads were administered by oesophageal tube, to prevent dehydration. Animals which were not intubated had free access to water, and were conscious for at least 18 hours prior to experimental observations; they were also permitted to drink ad libitum during the course of experiments.

Some Properties of Para-amino hippurate (PAH).

The binding of PAH to plasma proteins, its distribution between red blood cells and plasma, and its extraction by the normal kidney, have been discussed (pp15-17).

The effect of PAH on the renal circulation.

Although there is no evidence which suggests that low concentrations of PAH in the circulation cause any alterations in renal haemodynamics, McDonald at al (133) have reported that high plasma concentrations of PAH (33-96 mg %) lead to significant changes in renal blood flow and GFR in man. In the course of Tm measurements in ten subjects, they demonstrated an average reduction of 12% in GFR, and an average increase in RBF of 14%.

In the present investigation, GFR and RBF were determined in ten normal rabbits at plasma PAH

concentrations of 1-3 mg %, and then in the same animals at levels of 20-100 mg % (Table 2A and 2B). Glomerular filtration rate decreased from an average of 21.8 ml/min to 17.1 ml/min, a reduction within animals of 22 ∓3.2 (SE)% ($p = 0.001$). There was no significant change in renal blood flow, which averaged 146 ml/min both before and after the change in PAH concentration, and a reduction in filtration fraction ($p = 0.001$). The effects of high plasma concentrations of PAH (25 - 105 mg %) were also investigated in 8 anaemic animals, whose hematocrit values averaged 12.7% (range 7.7 - 18.3%). Glomerular filtration rate decreased from 18.4 ml/min to 14.7 ml/min, a reduction within animals of 19 ± 6.2 (SE)% ($p = 0.02$). Renal blood flow averaged 124 ml/min at low PAH levels and 141 ml/min at high levels. The effect on RBF was not significant ($p = 0.2$) and filtration fraction was reduced ($p = 0.001$). There were no significant differences between the changes observed in normal and anaemic animals.

It is concluded that high plasma concentrations of PAH in the rabbit depress glomerular filtration rate and filtration fraction, and that these effects are not increased during anaemia. Pyrogens and certain drugs have been shown to have similar effects on the renal circulation (164). The preparation of PAH used in this study was such that its effects could not have been due to contaminating pyrogen.

TABLE 2A (CONTINUED)

THE EFFECTS OF PAH ON RENAL CIRCULATION.

A. Normal Rabbits

B. Anaemic Rabbits

		HAEMATOCRIT %		RBF (ml/min)		GFR (ml/min)		FILTRATION FRACTION		ARTERIAL PAH (mg %)
		C	T	C	T	C	T	C	T	
Animal	1			163	131	26.8	19.5	.239	.212	20.4
	2			117	110	17.7	13.4	.230	.165	37.3
Animal	1	12.2	3	144	149	21.4	18.9	.228	.187	26
	2	9.5	4	177	165	22.6	19.0	.220	.179	25
	3	12.7	5	164	148	26.2	20.9	.238	.205	35
	4	9.0	6	172	133	23.7	19.6	.217	.159	32
	5	15.9	7	107	133	20.7	15	.312	.210	60
	6	12.6	8	130	134	17.9	15.4	.221	.166	74
	7	18.3	9	146	139	21.2	16.2	.233	.179	51
	8	18.0	10	142	155	18.5	15.5	.194	.096	101
					96				.130	73
MEAN				146	146	21.8	17.1	.233	.175	46
MEAN		13.5		12.0	124	141	18.4	14.7	.174	.119	53
% DIFFERENCE										
% DIFFERENCE (within animals)				0.6%		-22.1%		-25.1%		
S.E.				±4.0%	+ 16.3%	± 3.2%	19.1%	±3.6%		
S.E.					± 9.2%		± 8.2%		± 3.1%	
P				0.9		0.001		0.001		
P					0.3		0.0%			

TABLE 3B (CONTINUED)

THE EFFECTS OF PAH ON RENAL CIRCULATION.

B. Anaemic Rabbits

	HAEMATOCRIT %		RBF (ml/min)		GFR (ml/min)		FILTRATION FRACTION		ARTERIAL PAH (mg %)
	C	T	C	T	C	T	C	T	T
Animal 1	12.2	10.3	126	168	22.6	15.9	.204	.105	37
2	9.5	8.2	90	109	10.6	9.5	.131	.095	105
3	12.7	11.0	139	146	17.4	14.0	.144	.106	25
4	9.0	7.7	141	128	15.7	9.8	.123	.083	80
5	15.9	12.8	134	131	22.2	15.7	.198	.138	24
6	12.6	11.2	134	133	19.2	12.6	.164	.107	29
7	18.3	17.3	129	146	24.4	22.9	.232	.189	51
8	18.0	17.8	96	164	15.5	17.6	.197	.130	73
MEAN	13.5	12.0	124	141	18.4	14.7	.174	.119	53
% DIFFERENCE (within animals)			+ 16.3%		-19.1%		-31.4%		
S.E.			± 9.2%		± 6.2%		± 3.1%		
P			0.2		0.02		0.001		

The renal excretion of PAH.

PAH is excreted by the kidney in two ways, by glomerular filtration and by tubular secretion. Glomerular filtration of PAH is limited only by hemodynamic factors, and by the binding of PAH to plasma proteins which is normally accepted as a constant factor. Tubular secretion of PAH involves its transfer from the peritubular blood to the cells of the proximal tubules, concentration within these cells and diffusion into the tubular humen. The first of these processes occurs by diffusion, probably involving combination with a carrier molecule at the cell membrane. The rate of dissociation of the complex formed, and the availability of the carrier system, limit the speed with which PAH can be transferred into the cell. Other substances, including diodrast, phenol red, penicillin and probeneced, appear to employ the same carrier system as PAH. In their presence, the utilisation of the carrier is increased and the excretion of PAH is slowed down.

The nature of para-amino hippurate concentrated within the tubular cell has been investigated by Foulkes and Miller (67), using C^{14} - labelled PAH and rabbit kidney slices. They have demonstrated the presence of two distinct intracellular fractions of PAH. One fraction was freely diffusible; the other, which was highly concentrated with respect to the surrounding medium, was slowly diffusible. It appears that during the process of concentration, PAH is sequestered within some special compartment of the renal

tubular cell.

The concentrating step is an energy-requiring process, and has been shown to involve oxidative metabolism and phosphorylation. In a series of studies in the unanaesthetized dog, and using rabbit kidney slices, Taggart, Cross and Mudge (38,128,129) have shown that acetate, certain other intermediates of the tricarboxylic acid cycle, and dinitrophenol have marked effects on the rate of PAH accumulation by tissue slices in vitro, and on the maximum rate of renal tubular transfer of PAH in vivo. Acetate appears to be a rate-limiting cellular component in the aerobic manufacture of phosphate bond energy; increasing its concentration has a stimulatory effect on PAH transport. Administration of dinitrophenol reduces PAH transport, apparently because it uncouples phosphorylation from the oxidative cycle. There is little knowledge about the processes whereby phosphate bond energy is utilised in building up the concentration of PAH within the tubular cell.

There are several lines of evidence which indicate that PAH secretion occurs only at a localized proximal site in the renal tubules. The renal tubule of the aglomerular fishes, which in its entirety resembles the proximal tubule of mammals, can secrete phenol red (164). Phenol red secretion can also occur in proximal segments of chick mesonephric tubules, but not in distal segments (30). During stop-flow studies in the dog and other mammals, a

proximal site of PAH secretion has repeatedly been demonstrated.

There appears to be no significant storage of PAH in the renal tubule cells (164), despite early reports to the contrary (56). Smith, Goldring and Chasis (167) have utilised competitive inhibition of phenol red transport by hippuran to conclusively establish this point. They demonstrated that the relationship between the plasma concentration of hippuran and the simultaneous phenol red clearance was the same whether the plasma level of hippuran was rising slowly or falling rapidly. Any significant storage of hippuran in the tubule cells while its plasma concentration increased would have continued to depress the phenol red clearance as the plasma level fell rapidly.

Conjugation of PAH in the kidney.

The colorimetric determination of PAH depends on a coupling reaction involving the amino group. In certain species, notably the guinea pig (157), acetylation of the amino group during renal excretion may prevent the determination of significant amounts of urinary PAH, and lead to falsely low estimates of clearance. Very little renal conjugation of PAH occurs in the rabbit (38,157), and it would appear that no important error occurs trough failure to determine p-acetylaminohippurate in the urine.

PAH in renal lymph.

Because of its high content of urea relative to the plasma, renal lymph appears to be derived in part from

reabsorbed tubular fluid (90). However, it has been shown to contain inulin and PAH in concentrations 50-100% as great as plasma, indicating a contribution from the peritubular blood. If sufficient PAH and inulin escaped in the lymph stream, an important error would be introduced into the clearance formulae normally used in estimating RBF and GFR. Bull and Metaxas (26) have examined this possibility in the anaesthetized dog, and have demonstrated that neglect of lymph flow results in no appreciable error in the indirect determination of renal blood flow. The same conclusion was reached by Smith (164), who presented evidence to show that renal plasma flow calculated from clearance and extraction ratio appeared to be greater using inulin than using PAH, and argued that the converse result would be expected if much renal interstitial fluid drained into the lymph.

Details of stock solution of sodium para-amino hippurate.

PAH used in the present study was supplied as a dry powder by Eastern Chemical Co., U.S.A., and prepared in 5 ml. ampoules as a pyrogen-free, neutral, sterile 20% solution, by Baxter - D.H.A. (Laboratories,) Sydney.

Determination of Renal Blood Flow and Glomerular Filtration Rate.

All animals in which renal clearance measurements were carried out had been subjected to one of two operative procedures, i.e. catheterization of the left renal vein or bilateral catheterization of the ureters. Animals with a renal vein catheter were allowed 18 hours or longer to

recover from general anaesthesia; at the end of the recovery period, and after insertion of a bladder catheter and ear vessel catheters they were able to move about freely, drink or eat, their general condition resembling that of normal unoperated animals. For the purposes of the following description they are referred to as "normal" animals, whereas the animals with both ureters catheterized are referred to as "ureter" animals.

A. Measurement of RBF and GFR in "normal" animals.

Before experiments the animal was placed into one of two types of experimental cage (figs.10,21) where it sat undisturbed and without any kind of restraint throughout subsequent procedures. Renal vein, ear vein and ear artery catheters were firmly anchored to the skin, enveloped with adhesive tape and brought slackly to the exterior. The bladder catheter hung through a slit (fig.10) or a seal (fig.21) in the floor of the cage. Drinking water was provided in a small metal trough.

Before commencing infusions, an arterial blood sample was taken for "blank" determinations of PAH, creatinine and inulin. A priming solution (see Table 3) was injected into the ear vein catheter over a period of one minute, and the sustaining infusion commenced at a rate of 0.7 ml/min. The sustaining infusion was delivered from a 50 ml syringe, which was driven at a constant speed by a Palmer slow infusion pump. The plasma levels obtained in different experiments varied between 15-50 mg% for

TABLE 3.

COMPOSITION OF INFUSIONS USED IN DETERMINING RBF AND GFR.

Priming Solution.

Creatinine	250 mg.
*Inulin	250 mg.
Para-amino hippurate 20% (w/v)	0.1 ml
Ringer-Locke solution to	10 ml

Sustaining Infusion.

Creatinine	1 G
*Inulin	1 G
Para-amino hippurate 20% (w/v)	1.0 - 1.5 ml**
Mannitol	5 G
Ringer-Locke solution to	100 ml.

Ringer-Locke Solution (modified)

Sodium chloride	42.5 G
Potassium chloride	1.25 G
Calcium chloride	1.25 G
Sodium bicarbonate	1.0 G
Distilled water to	5 litres.

*Omitted in most experiments.

Inulin was dissolved at 80° C, before adding other constituents to the cooled solution.

**According to the weight of the animal or its expected clearance.

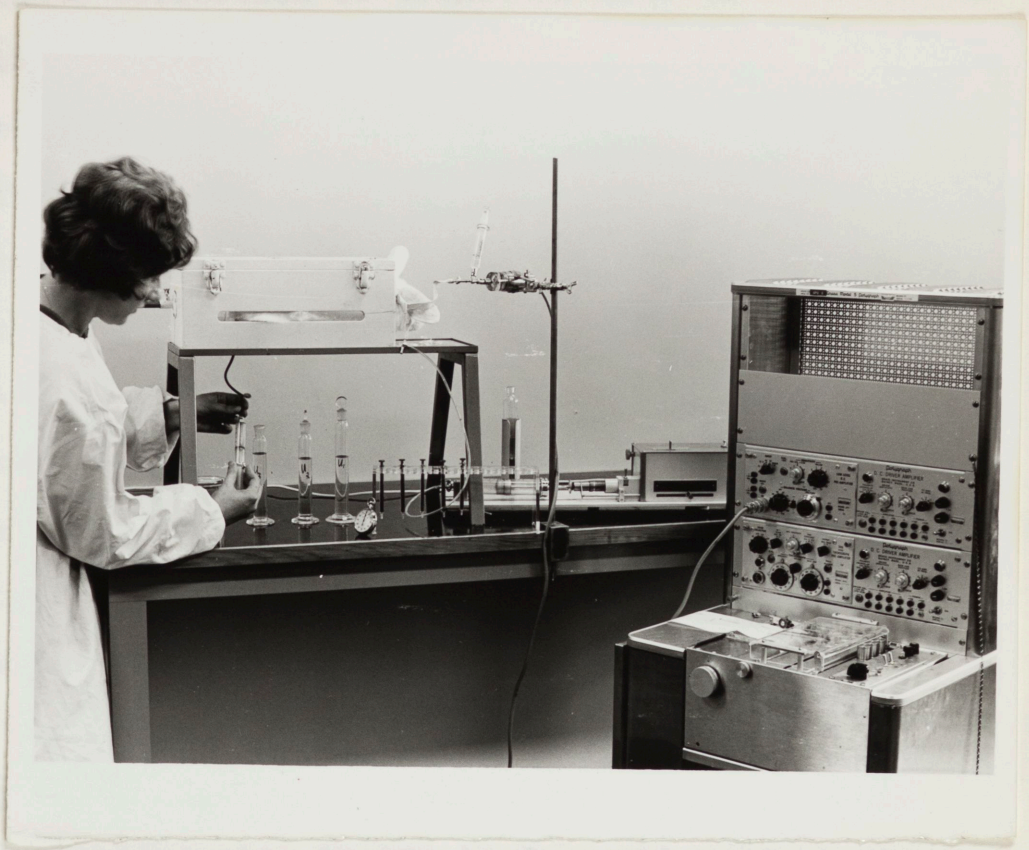


Figure 10. Apparatus used in measurements of renal clearance and blood pressure, showing infusion pump, blood-sampling syringes, pressure transducer and polygraph. A urine collection is being completed by irrigating the bladder with normal saline. In other experiments, in which the ureters were catheterized, the catheters were brought through the slits on either side of the rabbit box.

creatinine, 30-90 mg% for inulin and 1-4 mg for PAH.

After the infusion had been administered for 1 hour, a 10 minute collection of urine was made to estimate the approximate rate of urine flow, and then clearance measurements were started. In the majority of experiments, each single estimate of renal blood flow and glomerular filtration rate was based on three consecutive 10 - minute urine collections. One sample each of arterial blood and renal vein blood was withdrawn at the midpoint of the first urine collection, and again at the midpoint of the third urine collection. Estimates of clearance in two experiments (see Fig.44) were based on two 15- minute urine collection periods, and paired arterial and renal vein samples taken at the midpoint of each collection period. Before commencing each urine collection, the bladder was gently washed out for 1-2 minutes using 10-20 ml of normal saline. Also during the last 2 minutes of each collection, the bladder was irrigated with normal saline so that the urine returns of the last 20-30 seconds became very dilute, and any inadvertent residuum at the end of 10 minutes caused minimal errors. Devices to obtain complete emptying of the bladder, such as suprapublic pressure and air insufflation, were avoided because they disturbed the animals. The volume of urine plus saline washings in each collection was made up to 50 ml, and then diluted before colorimetric determinations of PAH, creatinine and inulin.

Arterial and renal vein blood samples were withdrawn simultaneously into 1 ml all glass tuberculin syringes moistened with concentrated heparin solution. For each arterial sample, 1.3 ml of blood was taken: 0.2 ml was used for a hemoglobin estimation and the remainder centrifuged at 1,500 rev/min for 10 minutes, within 30 minutes of sampling. The plasma was removed from the red cells by Pasteur pipette and its protein content precipitated before colorimetric determination (see p 65). The plasma of each 1.1 ml sample of renal venous blood was similarly separated and treated.

An extra 0.7 ml of blood was taken with alternate arterial samples for determination of haematocrit ratio. Haematocrit ratio was read from Wintrobe tubes after centrifuging for 30 minutes at 3,000 rev/min and at 13.5cm. radius; no difference in the reading was detected if the time of centrifugation was extended to one hour. Gregerson et al (75) have shown that centrifugation for 30 minutes at 3000 rev/min of blood comtaining a known amount of T-1824 leaves 4% of the dye unaccounted for in the plasma above the red cells, and that this is due to trapping of plasma among the cells. Chaplin and Mollison (31) found a smaller degree of plasma trapping after centrifuging at 3000 rev/min for 1 hour, and advocate correction of the haemotocrit reading to 0.975 - 0.985 of its observed value (between values of 10-40%). In the present study no

corrections were applied for plasma trapping. Applying the findings of Chaplin and Mollison to the present study, the error in calculating renal blood flow which may have been incurred through neglect of plasma trapping was 1% at a haematocrit of 40, and 0.15% at a haematocrit of 10. This error is small in contrast to the errors inherent in clearance determinations.

The following functions were used in the calculation of PAH clearance, renal PAH extraction ratio (E_{PAH}), renal plasma flow (RPF), renal blood flow (RBF) and glomerular filtration rate (GFR):-

(i) PAH Clearance (ml/min)= $\frac{U_1 + U_2 + U_3) \times 266.6}{A \times 10}$; where U_{1-3} and A are the values (in optical density units) for the PAH concentration of diluted urine and arterial plasma samples respectively, 266.6' represents dilution factors, and 10 is the number of minutes in each urine collection period. Where the value for a single urine sample was outside a range of ± 15% from the mean value for the three samples, the clearance estimate was rejected (with exceptions indicated in the appendix); variation of this degree was usually due to inadequate bladder washing or to a non-steady state.

(ii) $E_{PAH} = \frac{A - V}{A}$, where V is the value for the PAH concentration of renal venous blood.

(iii) RPF (ml/min) = $\frac{\text{PAH clearance}}{E_{PAH}}$. Wolf's corrected formula for renal plasma flow, which takes into account the

the difference between arterial and venous flow caused by urine formation (182), was not used here, since the urine flows were low relative to RPF, and the extraction ratio of the indicator substance was high.

(iv) $\text{RBF (ml/min)} = \frac{\text{RPF}}{\text{1-haematocrit}}$

(v) GFR (ml/min) = creatinine clearance, derived similarly to (1), except dilution factor is 166.6.

During 11 estimates of GFR in 4 animals, creatinine and inulin clearances were measured simultaneously. Table 4 shows that under normal conditions, and during conditions which tend to depress GFR (anaemia, high plasma PAH levels), creatinine and inulin clearances were approximately equal.

B. Measurement of RBF and GFR in "ureter" animals.

In 21 animals ("ureter" animals) the ear vessels were catheterized; then using light thiopentone anaesthesia, cannulation of the oesophagus, trachea, and ureters was carried out. During the operation 40 ml of warm water was administered in divided doses through the intra-gastric tube, and thereafter the water load was maintained by giving 10 ml per hour. Immediately after the operation, an arterial blood sample was taken for plasma blank determinations, and the sustaining infusion containing Mannitol, PAH and creatinine, (Table 3) was commenced. Renal clearance measurements were not carried out until the animal was sitting up fully conscious and the rates of urine flow from the right and left ureter had been approximately equal

TABLE 4.

COMPARISON OF CREATININE AND INULIN CLEARANCES.

	Haematocrit %	Arterial PAH (mg %)	Creatinine Clearance (ml/min)	Inulin Clearance (ml/min)
Animal 1	37.6	2.5	17.9	16.7
	36.3	74.3	15.4	16.3
	18.0	2.5	15.5	16.0
	17.8	72.9	17.6	17.9
Animal 2	37.5	2.5	21.2	19.7
	35.4	50.8	16.2	16.5
	14.3	99.2	8.0	8.4
Animal 3	33.0	2.4	18.5	19.3
	29.0	101.0	10.2	12.4
Animal 4	18.3	1.8	24.4	22.4
	17.3	59.7	22.9	22.8
		MEAN	17.13	17.07

PERCENTAGE DIFFERENCE (within animals) = 0.3 $\pm$ 2.1 (SE)% N.S.

(within 10-15%) for a period of 30 minutes or more.

During the early post-operative period in 8 animals which were not denervated, it was observed that urine flows from right and left ureters were approximately equal. However, in 21 out of 23 animals previously subjected to chronic left renal denervation, there was a greater urine flow from the left kidney than from the right kidney during the first 30-60 minutes after catheterizing the ureters. This observation resembles many others which have been made during bilateral ureter studies since the time of Claude Bernard (12), and is attributable to increased renal vasomotor tone resulting from anaesthesia and laporotomy (163). In 13 experiments the difference between innervated and denervated sides gradually decreased, and ureter flows were approximately equal during control clearance measurements. The remainder of the denervated animals were not used in clearance studies, but it is recorded that in 9 out of 10, ureter flows became temporarily equal, and then a reversed effect was seen, the left flow becoming slower than the right. At the time of recording these observations, they were attributed to undetected mechanical obstruction of the left ureter, but in view of results obtained in later experiments (see p 129) it seems possible that they were associated with a hypersensitivity of the denervated kidney to circulating catecholamines.

In "ureter" animals used for clearance studies, left/right ratios for renal blood flow and glomerular filtration rate were obtained from PAH and creatinine concentrations of 10 minute urine collections. Estimates of absolute RBF and absolute GFR were based on two consecutive urine collections and a 2.0 ml sample of arterial blood taken at the midpoint. Haematocrit ratio and haemoglobin were also determined from the arterial blood sample. Calculations of PAH clearance and GFR were made in the same way as for normal animals. In calculating absolute renal blood flow in "ureter" animals, a value of 95% was used for renal PAH extraction ratio, which was derived from results in a previous investigation (97), and from results in "normal" animals in the present series.

Determination of the Tubular Maximum for PAH.

For the purposes of this discussion, the "tubular excretory mass" (167), or maximal rate of tubular excretion of PAH (165), will be referred to as PAH tubular maximum or Tm_{PAH}. This measurement represents the amount of PAH which is excreted by the renal tubules per minute (the "tubular transfer") when the tubular transport mechanism is saturated at high plasma PAH levels. The renal excretion of PAH, and its dependence in the processes of oxidation and phosphorylation in the tubular cell have already been discussed (p 51). Although Tm_{PAH} measures only one aspect of tubular function, it is evident that reduced values may

result from a general impairment of tubular function or a loss of functional tubular tissue.

Tm_{PAH} was determined in 10 "normal" rabbits, in 8 of which measurements were repeated subsequently during chronic anaemia. After estimations of renal blood flow, renal PAH extraction ratio and glomerular filtration rate had been made at low plasma PAH levels, the plasma PAH concentration was raised to a high, constant level, varying in different experiments from 25-105 mg %. The sustaining infusion contained 15-22.5 ml of 20% para-amino-hippurate (3-4.5 g) per 100 ml, but otherwise was as described in Table 3. It was found that without a priming infusion, steady levels of plasma PAH could be obtained after one hour.

Each estimate of Tm was based on three 10 minute urine collection periods, and the details of sampling were exactly as described for clearance measurements in "normal" animals. PAH tubular transfer (T) was calculated from the function:-

$T\ (mg/min) = U_{PAH} - f \times A_{PAH} \times GFR$, where U_{PAH} is the urinary excretion of PAH in mg/min, f is the filtered fraction of PAH in plasma, A_{PAH} is the arterial concentration of PAH in mg/ml, and GFR the creatinine clearance in ml/min. Values for the filtered fraction of PAH in plasma have been reported from several species: for the dog, 0.92 (164), for the cat, 0.91 (53), for man, 0.78 - 0.83 (32,164), and for the rabbit, 0.92 (average in 4 animals (86)). In calculations of T and Tm in the present study, a value of 0.91 was used

for f. If any error was produced by this assumption, it is more likely that Tm values were slightly underestimated rather than falsely high.

To determine in a given experiment that tubular transfer was maximal, and therefore equivalent to Tm, it was necessary to show that the plasma PAH levels produced had indeed saturated the tubular PAH transport process. Theoretically, if the "load" of PAH being presented to the renal tubules is greater than the amount they are currently secreting, i.e. the Load/T ratio exceeds unity, then T is equal to Tm. Tubular load (L) is calculated from the function

$$L(mg/min) = A_{PAH} \times RPF - f \times A_{PAH} \times GFR.$$

The value used for RPF is often determined at low arterial PAH levels, to avoid measuring renal PAH extraction ratio. Homer Smith has pointed out (164) that this practice may lead to falsely high estimates of tubular load and Load/T ratio, as RPF could be decreased during the vasomotor disturbances associated with high plasma concentrations of PAH. Thus he suggested that it was necessary to demonstrate a Load/T ratio of 1.5 or greater before it could be argued that the tubules were saturated. In the present study, the value used for RPF was determined from the PAH clearance and renal PAH extraction ratio during Tm measurements, and there was no reason to suspect that tubular load was overestimated. Fig.11 shows the relationship between Load/T ratios and PAH extraction ratio determined at different

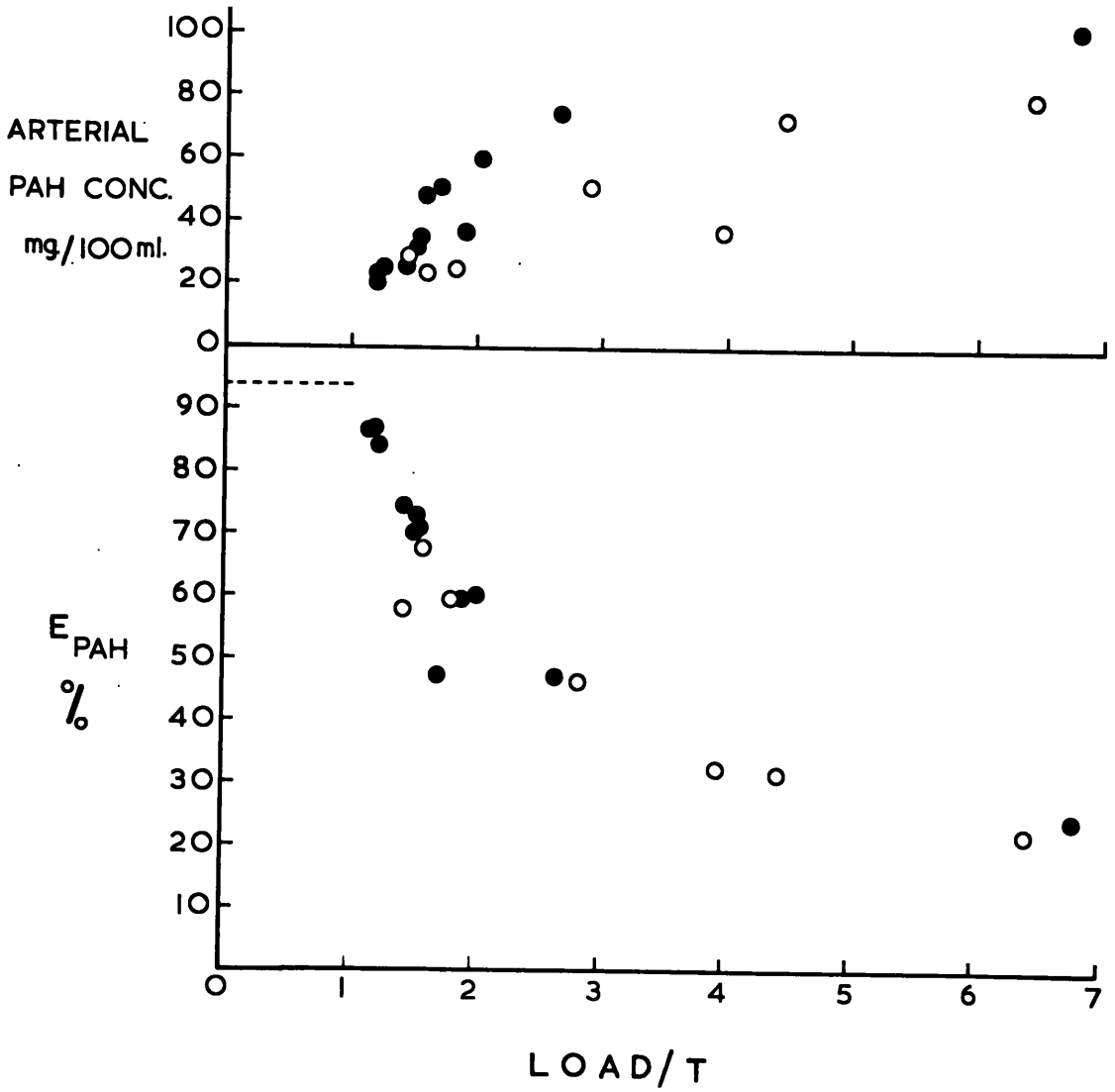


Figure 11. PAH tubular load/PAH tubular transfer (Load/T) in relation to renal PAH extraction ratio (E_{PAH}) and arterial PAH concentration in 12 animals. Black circles - normal haematocrit. Open circles - severe anaemia (haematocrit ratios 17.8 - 7.7%). The dashed line indicates their average E_{PAH} value at normal haematocrit and low arterial PAH concentration (1-3 mg %).

arterial plasma PAH levels. It appears that tubular saturation in the normal or anaemic rabbit, as evidenced by increased PAH concentration in the renal vein blood, was produced by plasma PAH concentrations above 20-25 mg%, and Load/T ratios greater than 1.2. Further evidence regarding the minimal arterial PAH level required for Tm conditions was provided by experiments which are discussed on p. 100 . For the purposes of the present study, T was regarded as equivalent to Tm at arterial PAH concentrations greater than 25 mg%, and with Load/T ratios from 1.25 to 9.3, (mean 2.93 ± 2.2 (SD)).

Titration of E_{PAH} with Increasing Arterial PAH Levels.

The effect of changes in arterial PAH concentration on renal PAH extraction ratio and renal creatinine extraction ratio was investigated in 4 animals at several levels of haematocrit. Although these experiments did not actually involve urine collection and clearance measurements, they are mentioned at this point for convenience. After catherization of the animal's renal vein and ear vessels, its plasma PAH concentration was increased stepwise by a sustaining infusion containing para-amino-hippurate 2 G% and creatinine 3G%, delivered at rates of 0.1 - 1.4 ml/min. Simultaneous arterial and renal venous blood samples were taken when the PAH level had become relatively stable at each infusion rate.

Methods used in Colorimetric Determination of PAH, Creatinine and Inulin.

Protein Precipitation of Plasma Samples.

The method used was the cadmium sulphate technique described by Smith, Finkelstein, Aliminosa, Crawford and Graber (168), with slight modifications to produce a filtrate with a pH of approximately 8.0. Because of the limitations imposed on blood sampling by the physical size of the rabbit, it was necessary to carry out colorimetric determinations of PAH, creatinine, and in some cases, of inulin, on the protein-free filtrate of one small plasma sample. No single precipitation method is ideal for this purpose (165). However, if the filtrate pH was kept slightly on the alkaline side of neutral, no detectable precipitation of inulin occurred, and the addition of strong alkali used in creatinine determination caused little or no precipitation of excess cadmium sulphate.

Reagents:- (a) 1.1 sodium hydroxide. During its preparation this is standardized using a 0.1N solution of potassium hydrogen phthalate, and phenolphthalein as indicator.

(b) Acid cadmium sulphate. 34.68 gm of 3 $CdSO_4.8H_2O$, and 169.1 ml of 1N.H_2SO_4 are made up to 1 litre with distilled water. The H_2SO_4 is titrated against a 1N solution of (a) and made exactly equivalent.

Reaction. 0.5 ml of plasma are added to 1.5 ml acid cadmium

sulphate and 5 ml distilled water. Then add 0.5 ml of 1.1N NaOH, stopper and shake gently for 10 seconds. Leave for 10 minutes, shake again and centrifuge for 20 minutes at 3000 rev/min. The supernatant is removed using a Pasteur pipette, and recentrifuged. This method yields 6.5 mls of clear fluid, and avoids the use of ordinary filter paper which may introduce chromogenic material and interfere with the subsequent determination of creatinine. Table 5 shows that the final pH of one series of filtrates, read in a pH Meter against two different buffer solutions, was about 8.2.

Estimation of Sodium Para-amino Hippurate.

The method has been fully described by Smith et al (168), and is a modification of the method of Bratton and Marshall (1939) for the determination of sulphanilamide. The technique of Smith et al was followed, except that the dye coupling reaction was carried out at a temperature of 0°C.

Reagents: (a) 1.2 N HCl

(b) 100 mg sodium nitrite to 100 ml water

(c) 500 mg ammonium sulphamate to 100 ml water.

(d) 100 mg N-(1-naphthyl) ethylene diamine dihydrochloride

(BDH) to 100 ml water.

Reagents (b), (c) and (d) are made up fresh each day.

TABLE 5

pH OF FILTRATE AFTER PROTEIN PRECIPITATION METHOD.

	TEST 1	TEST 2
pH of Sample 1	8.3	8.6
2	8.2	8.6
3	8.25	8.7
4	8.1	8.6
5	8.0	8.6
6	8.3	8.8
MEAN	8.2	8.6

For TEST 1, the pH meter was standardized with 0.05 M Borax (pH = 9.20) and for TEST 2 with 0.05 M KH phthalate (pH = 4.01).

Preliminary steps.

(1) Plasma filtrates:- Pipette 4 ml each, plus 1 ml water, into 30 ml Erlenmayer flasks. For samples taken during Tm determination use 1 ml plus 4 ml water.

(2) Urine samples:- Stored in the cold overnight, to allow sediment to settle. Pipette 5 ml of 1/100 dilutions into 30 ml flasks. For Tm samples the dilution used is 1/1000.

(3) Water standards and blank. Dilute 1 ml 20% PAH stock solution to 1000 ml.. The three standards used are 1/250, 2/250 and 3/250 dilutions of the diluted stock solution. Pipette 5 ml of each standard into 30 ml flasks. The blank is 5 ml distilled water.

Colorimetric reaction.

(1) Suspend 30 ml flasks containing samples in a brass tray filled with ice and water.

(2) Add 1 ml of reagent (a) to each flask in turn and shake it gently. Then add 0.5 ml each of (b), (c), and (d) in turn, allowing not less than 5 minutes or more than 20 minutes between additions of successive reagents to any one flask. Confusion is avoided if the flask stoppers are used as markers while each reagent is pipetted and mixed with the sample.

(3) Remove flasks from tray and stand for 1 hour before reading at a wavelength of 540 mμ.

Optical density readings were made on a Beckman model DU spectrophotometer with photomultiplier.

The rate of colour development after the completion of the PAH reaction is illustrated in figure 12. The absorption curve is shown in figure 13, and the calibration curves for PAH in water and plasma in figure 14. Plasma recoveries after cadmium sulphate precipitation ranged from 95-100%. The optical density reading of the plasma blank varied from 0.000 to 0.008. The average value in 20 consecutive determinations of normal plasma was 0.003. The value for anaemic plasma, which was often turbid before plasma precipitation, averaged 0.004. In early experiments, no difference was found between blank determinations of arterial and renal venous plasma, thus only the arterial plasma blank was determined in most animals.

Determination of Creatinine.

The method of Folin and Wu (63) was used.

Reagent:- 5 parts saturated picric acid in water
1 part 2.5N NaOH
6 parts distilled water.

The reagent was made up just before use.

Preliminary steps.

Urine specimens were diluted as for the PAH reaction. Water standard were 1/100, 2/100, and 3/100 dilutions of a 0.1 gm% creatinine stock solution (creatinine, Eastman Kodak U.S.A.) 1 ml of each plasma filtrate plus 1 ml water,

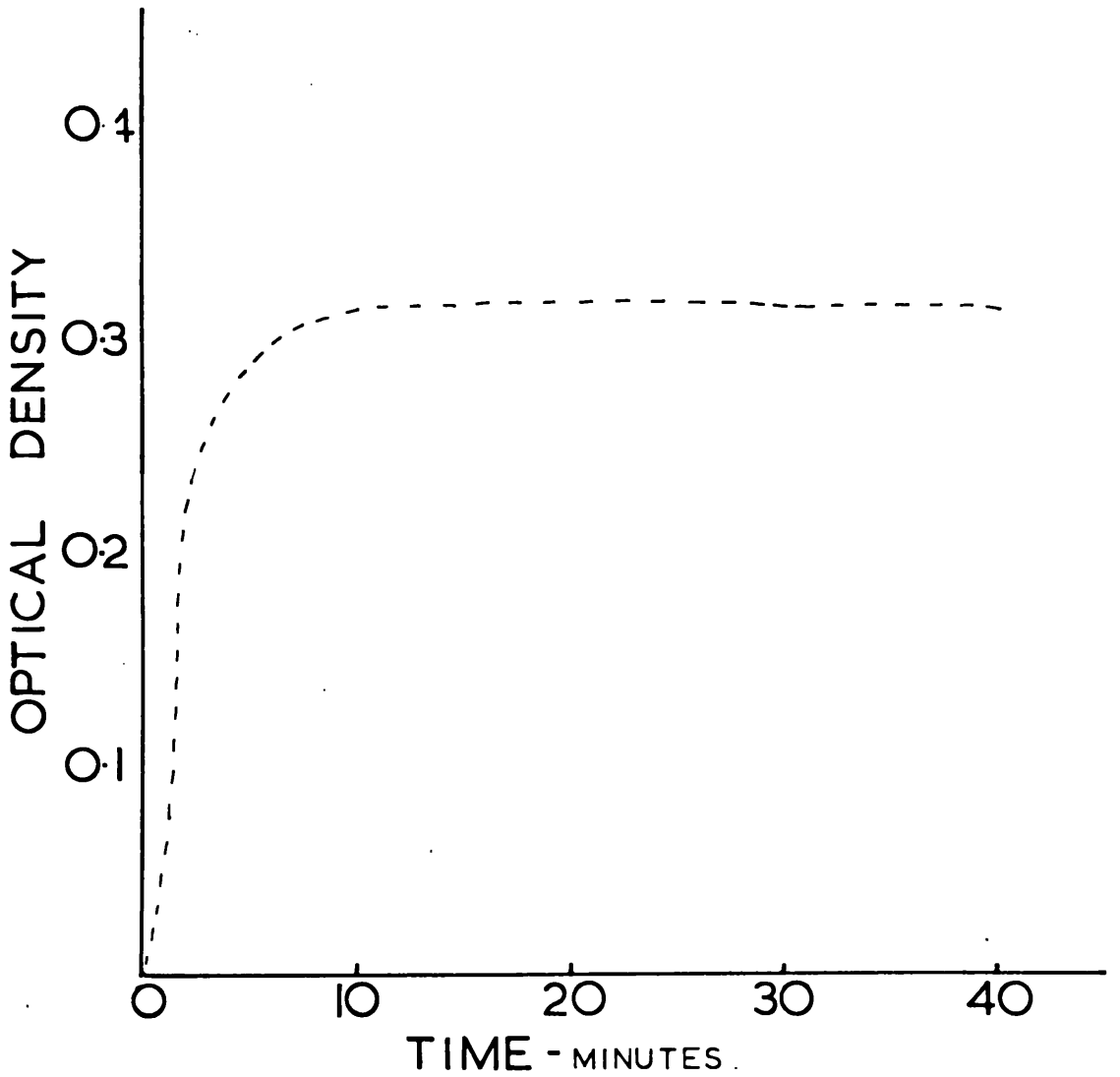


Figure 12. Rate of colour development after completion of coupling reaction at 0°C, in the estimation of sodium para-amino hippurate. Optical density was measured at 540 mμ.

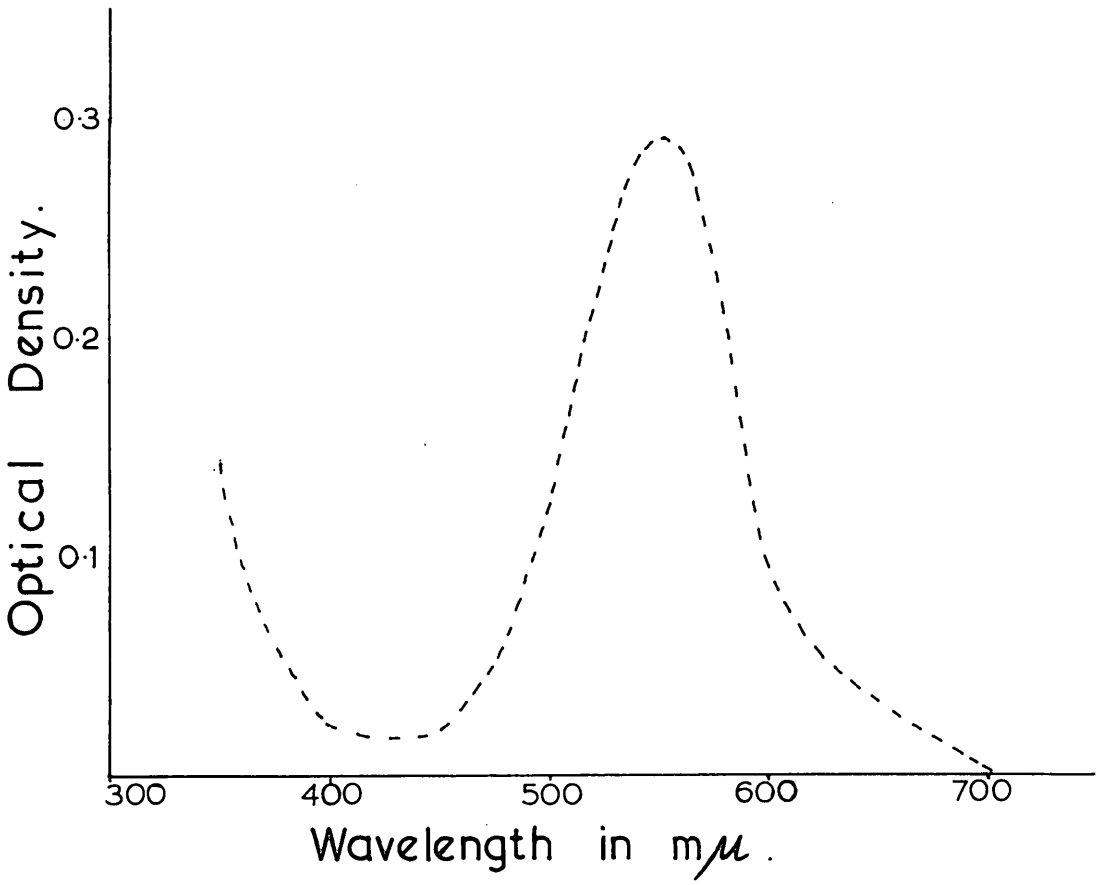


Figure 13. Absorption curve for PAI estimation.

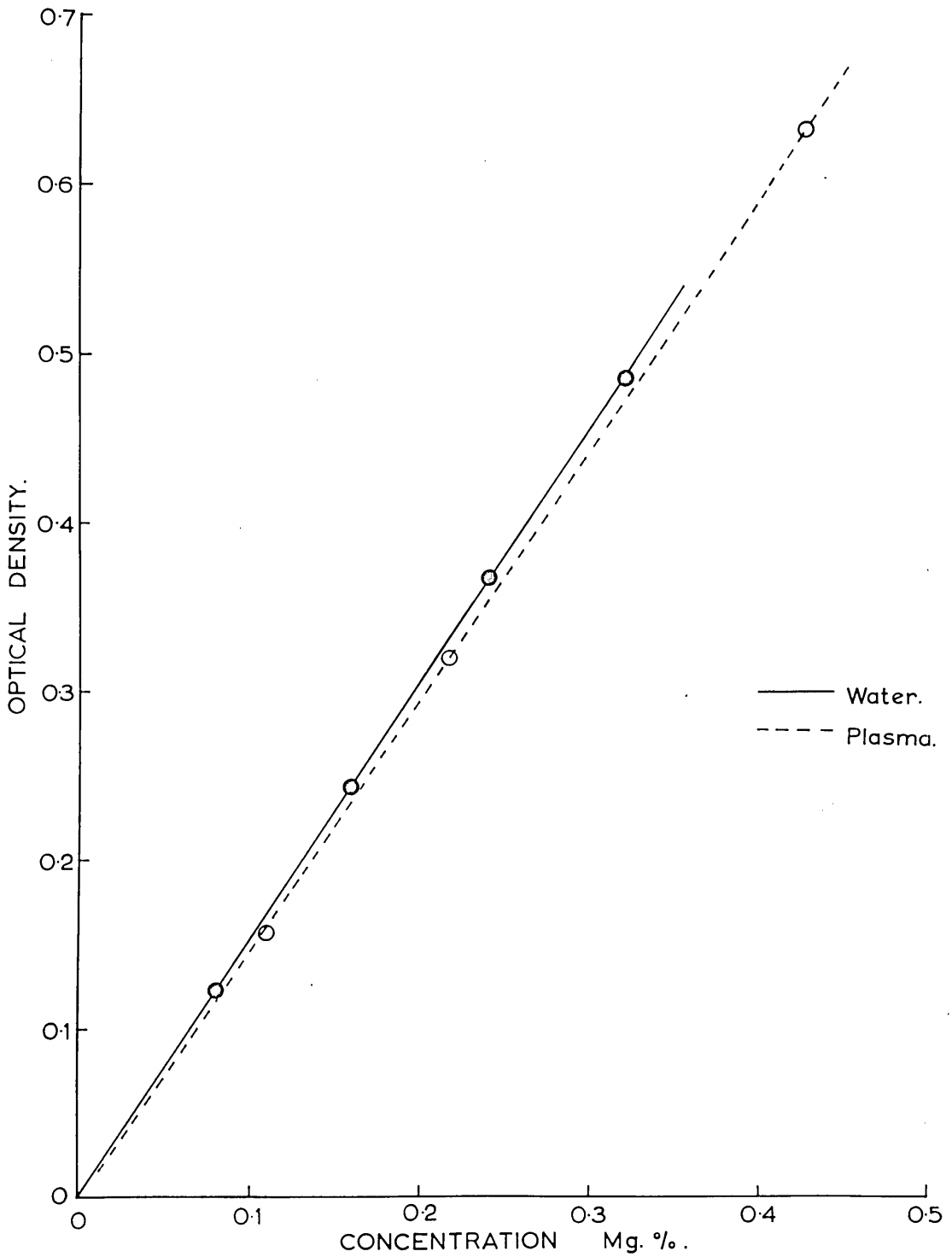


Figure 14. Calibration curves for the estimation of PAH in water and in plasma. PAH concentration in plasma is expressed as mg per 100 ml of protein-free filtrate. Optical density was measured at 540 mμ.

and 2 ml of each diluted urine sample, water standard and water blank, was pipetted into centrifuge tubes.

Colorimetric reaction.

(1) Add 4 ml of reagent to each sample and mix. Leave to stand for 20 minutes.

(2) Centrifuge plasma samples for 20 minutes at 3000 rev/min. Remove supernatant using a Pasteur pipette. This step removes any slight opacity in the solution caused by precipitation of excess cadmium.

Optical density readings were made on the Beckman spectrophotometer. The absorption curve for creatinine in water is shown in figure 15. Readings were made at a wavelength of 520 mμ on the descending slope of the curve, so as to minimize the reading of the water blank; adequate sensivity was obtained at this wavelength. The calibration curves for creatinine in water and plasma are shown in figure 16. Plasma recoveries were rather more variable than in the case of the PAH reaction and ranged from 92-100%.

Determination of Inulin.

The method of Roe, Epstein and Goldstein modified by Schreiner (165), was used.

Reagents:- (a) 200 mg resorcinol dissolved in 200 ml of 95% alcohol. Can be kept up to 3 weeks in a dark bottle in the cold.

(b) Add 112 ml distilled water to 500 ml of concentrated HCl (10 N) It is essential that the alcohol

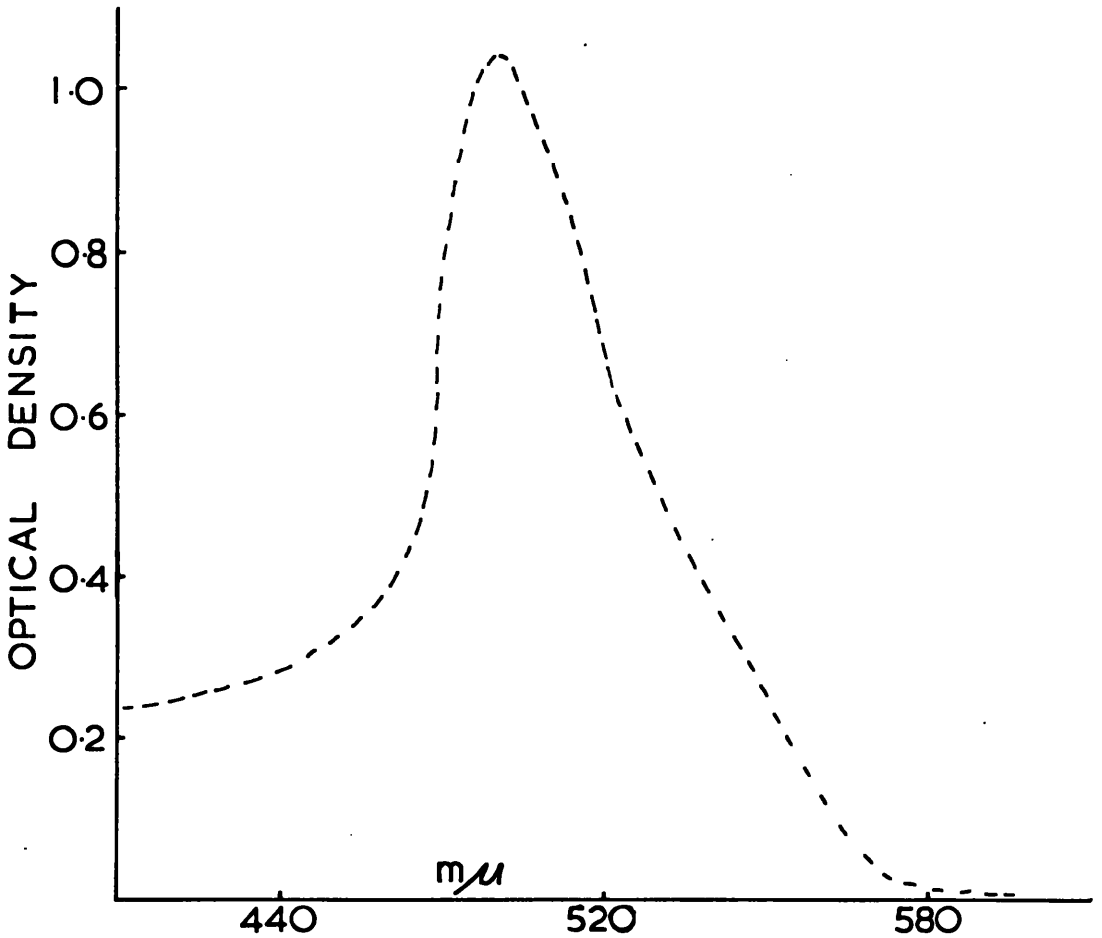


Figure 15. Absorption curve for creatinine estimation.

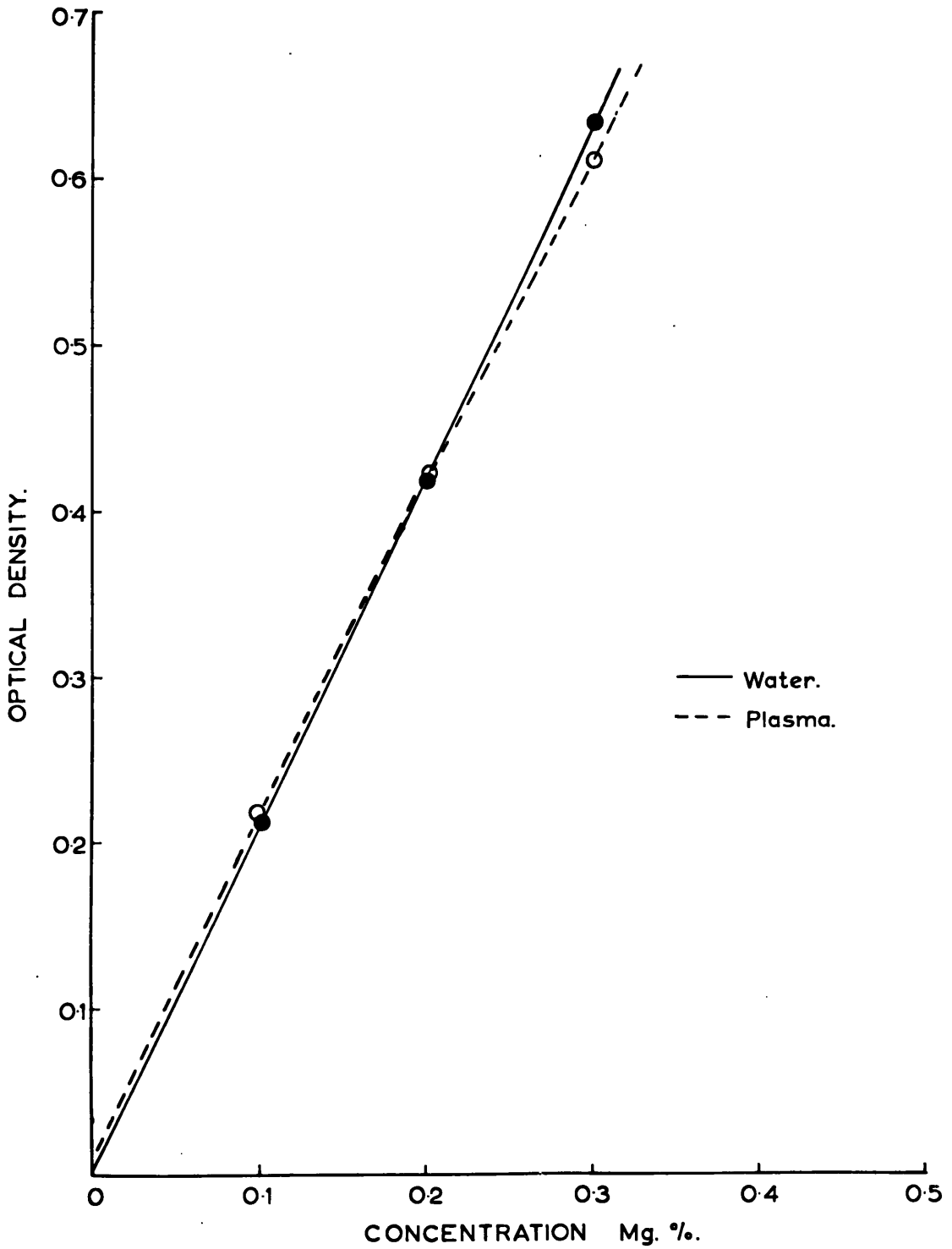


Figure 16. Calibration curves for the estimation of creatinine in water and plasma. Concentration of creatinine in plasma is expressed as mg per 100 ml of protein-free filtrate. Optical density measured at 520 mμ.

used in preparing alcoholic resorcinol is free from impurities, as these may cause opacity in the water blank. Absolute alcohol (C.S.R.) was stored for 3 weeks with 4 gm per litre of M-phenylene diamine dihydrochloride. It was then double - distilled, refluxed for 4 hours with KOH 1 gm %, and distilled again. During the distillation, impurities were given off at 78° C, and pure alcohol at 80° C.

Preliminary steps. Urine specimens were diluted as for the PAH reaction. The inulin stock solution was prepared by dissolving 1 gm of inulin (B.D.H.) in 1 litre of water at 90° C. The water standards were 1/100, 2/100, 3/100 and 4/100 dilutions of the stock solution. 1 ml of each plasma filtrate plus 1 ml water, and 2 ml of each diluted urine sample, water standard and water blank, was pipetted into "Quickfit" tubes calibrated at 9 ml. The tubes were equipped with tight-fitting plastic stoppers.

Colorimetric reaction.

(1) Add 2 ml of alcohol resorcinol (a) to each tube.

(2) Add 5 ml of concentrated HCl (b), using an automatic pipette-filler (Griffin) or suction pump to fill the pipette. Stopper tube tightly and invert several times.

(3) Incubate in thermostatically-controlled water bath at 80° C for 25 minutes.

(4) Cool in tap water for 3 minutes, check contents of each tube and make volume up to 9 ml with distilled water

as necessary.

Optical density readings were made within one hour at 490 mμ on the Beckman spectrophotometer. The calibration curves for inulin in water and plasma are shown in figure 17.

THE EXPERIMENTAL PRODUCTION OF ANAEMIA.

Three types of post-haemorrhegic anaemia were studied, and for the purposes of differentiation these have been called (1) acute normovolaemic anaemia, (2) acute hypovolaemic anaemia, (3) "chronic" anaemia.

(1) Acute Normovolaemic Anaemia.

In order to produce an acute reduction of haematocrit ratio with minimal blood volume changes, anaemia was produced by bleeding and simultaneous replacement of plasma. The plasma used was freshly collected from a donor animal. In preliminary experiments it was observed that the animals tolerated these procedures better if the haematocrit was reduced in a given experiment by not more than half of its initial value. Accordingly two groups of animals were used in these experiments.

Group I

In 6 animals initial measurements of renal blood flow, renal PAH extraction ratio, glomerular filtration rate and arterial pressure were made at their normal haematocrit (range 38-30%). Following these measurements the sustaining infusion was stopped. The animal was then allowed to bleed

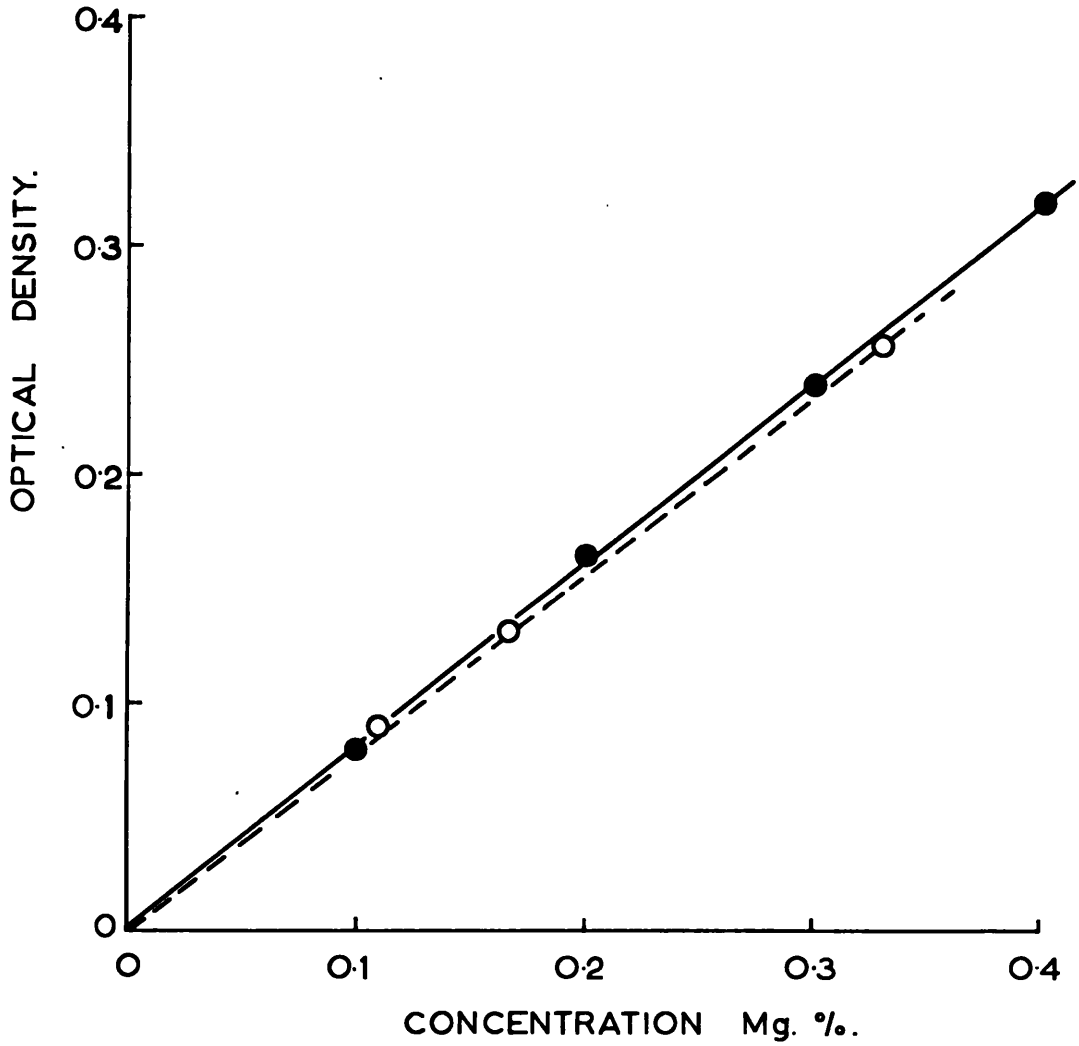


Figure 17. Calibration curves for the estimation of inulin in water and plasma. Concentration of inulin in plasma is expressed as mg per 100 ml of protein-free filtrate. Optical density was measured at 490 mμ (Smith, 1956).

(20-25 ml/Kg) slowly from the ear artery catheter while 80-100% of the blood lost was replaced by infusion of plasma. This process lasted 30-45 minutes, after which the sustaining infusion was recommenced, and the second set of observations of RBF, etc. carried out 1 hour later. The range of haematocrits during these observations was 24-18%. In 3 of these animals measurements of renal venous O_2 content were also made before and after production of anaemia. The effect of the bleeding and plasma replacement procedure on blood volume was determined in 2 additional animals. They were given the same infusion solutions and made anaemic in the same way as animals used for renal circulatory studies, but renal vein catheterization and renal clearance estimations were not carried out.

Group II

In 23 animals an initial state of moderate anaemia was induced by two preliminary bleeds of 15 ml/Kg/day from the marginal ear vein. Two days after the second bleed, renal vein catheterization was carried out, and on the following day initial measurements of renal blood flow, E_{PAH}, glomerular filtration rate and arterial pressure were made. The range of haematocrit values was 29-15%. The procedure of acute bleeding and plasma replacement was carried out as described for Group I, and a second set of measurements of RBF, etc. was made with haematocrit values ranging from 15-8%. In 2 of these animals the process of bleeding and

plasma replacement was repeated and the animals were thus studied at three levels of haematocrit. In 10 animals a third set of measurements was carried out after breathing 100% O_2 for 30 minutes. Measurements of renal venous O_2 content were made before and after acute production of anaemia in these 10 animals and also in 3 others of the group. Blood volume changes were determined in 3 additional unoperated animals as described for Group I.

(2) Acute Hypovolaemic Anaemia.

During these experiments no measures were taken to prevent blood volume changes, so that haemorrhage resulted in both a reduction in haematocrit and a fall in total blood volume.

In 3 animals, measurements of RBF, E_{PAH}, GFR and arterial pressure were made at their normal haematocrit (38%). The animals were then bled (15-20 ml/Kg) from the ear artery catheter for 15-20 minutes while the sustaining infusion was continued. Serial measurements of RBF, etc. were recommenced immediately and continued for a period of one hour after bleeding. The range of haematocrits during these observations was 31-24%.

In 12 animals with ureter catheters, measurements of RBF, GFR, arterial pressure and ratios of left to right renal clearances were also made before haemorrhage and during the subsequent hour. Their haematocrits were initially 44-36%, and 34-17% after bleeding. Time trends

in L/R clearance ratios were studied by analysis of variance.

In 4 additional ureter animals, the period of observation after haemorrhage was extended to 2½ hours, three clearance determinations being carried out with 30 minutes between each determination. Blood volume changes were measured in 3 of these animals.

(3) "Chronic" Anaemia.

In 6 animals, measurements of RBF, renal PAH extraction ratio (E_{PAH}), GFR, arterial pressure and total blood volume were made at their normal haematocrit (range 38-30%). The animals were then bled 15 ml/Kg daily for 3 days. 24 hours after the last bleed, measurements were repeated at haematocrits ranging from 18-8%. This procedure was also carried out in 8 animals in which tubular maximum for PAH (Tm_{PAH}) was measured, and in a separate series of 5 animals in which no infusions were given, blood volume alone being determined.

BLOOD VOLUME MEASUREMENTS.

Total blood volume was measured as the sum of separately determined plasma and red cell volumes before and after bleeding in 5 animals of the acute normovolaemic anaemia series, 3 animals of the acute hypovolaemic anaemia series, and 11 animals of the "chronic" anaemia series. Measurements were based on the estimation of the dilution of a known amount of an injected substance which remained firmly attached to plasma proteins or red cells.

Plasma volume was determined using the dye T-1824; in 5 animals simultaneous measurements with T-1824 and I^{131}-labelled human serum albumin were made. Red cell volume was determined using Cr^{51}- labelled red cells.

Determination of Plasma Volume using T-1824.

When T-1824 is introduced into the circulation it forms a dye-protein complex with plasma albumin, and becomes uniformly distributed by intravascular mixing within about 10 minutes (134). Subsequently there is a constant slow decline of dye concentration in the plasma due to leakage of albumin into extravascular spaces. If several plasma samples are taken at different times starting from 10 minutes after injection, the dye concentration at the time of injection can be obtained by semilogarithmic plotting of their concentrations and extrapolation back to zero time. Colorimetric readings of dye concentration may be affected by the presence of haemolysis or lipaemia in the plasma. Thus it is preferable to extract the dye chemically from the plasma constituents before determination.

In the present study attempts to apply Allen's cellulose-acetone method of dye extraction (2) were unsuccessful, the percentage of dye extracted being too low for accuracy. As an alternative to extraction, the plasma samples were diluted 1:100 in normal saline before reading at a wavelength of 620 mμ on the Beckman

spectrophotometer. At this wavelength, haemolysis adds relatively little to the optical density (75) and the reading of the plasma blank averaged 0.002, indicating that lipaemia did not cause significant turbidity after dilution of the plasma. It was shown that the absorption of T-1824 in saline is altered by the addition of plasma (figs 18,19), as has been reported elsewhere (3). Thus dye standards containing 1 part plasma in 100 parts normal saline were prepared from the plasma blank of each animal.

Injections of dye were made with a syringe calibrated for delivery by repeated weighings of distilled water. For each determination of plasma volume, 1 ml of a 5% solution of T-1824 in normal saline was injected into the ear vein catheter and flushed through with 1 ml of Ringer solution. Samples were taken at 10, 20 and 30 minutes following injection, and plasma concentration at zero time obtained by semilogarithmic extrapolation.

Determination of Plasma Volume using I^{131}- labelled Human Serum Albumin.

In man the distribution volumes of T-1824 and I^{131} - labelled albumin appear identical (5), and an identical dilution of both indicators is found during their first circulation through the heart and lungs or through the lower extremity (69). This suggests that the union of T-1824 and plasma protein is complete and prompt in man. In the rabbit Zizza and Reeve (187) have demonstrated a

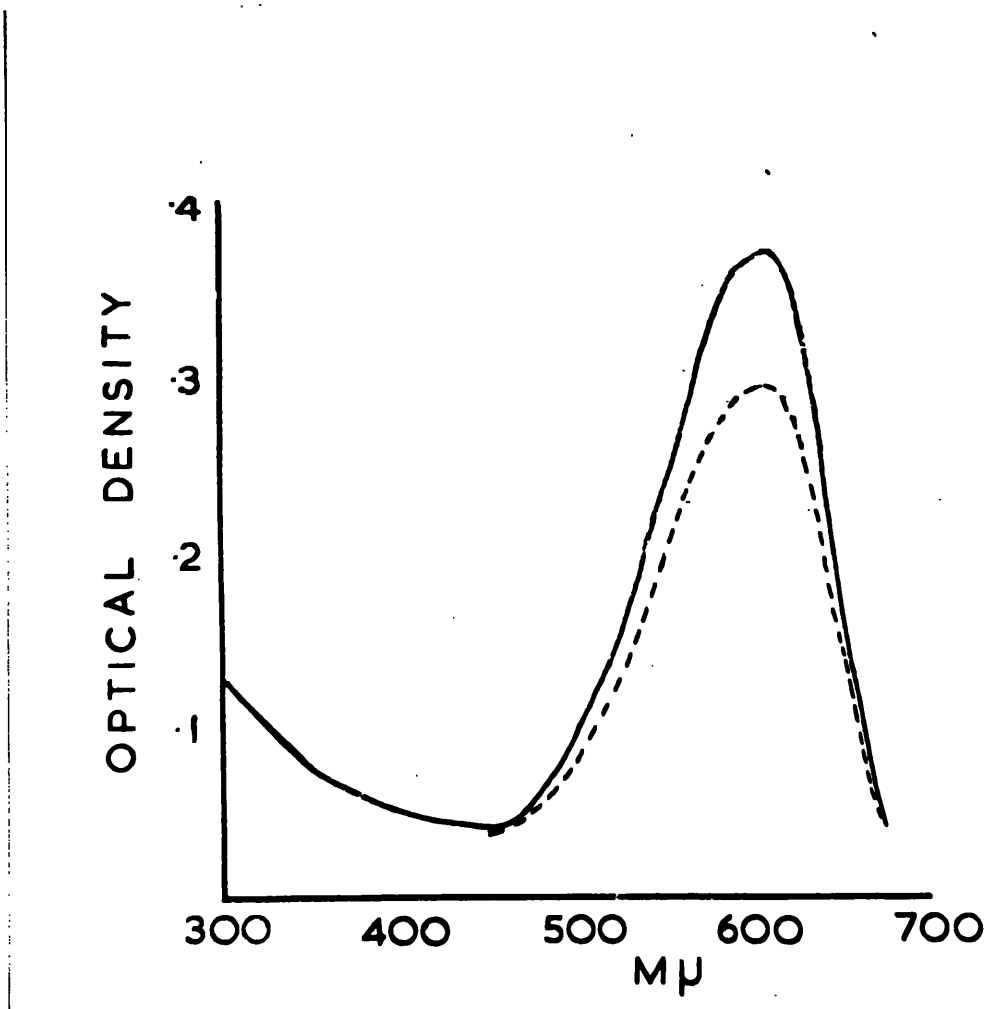


Figure 19. Calibration curves for the dye T-1824 in 0.9% saline (open circles) and in plasma (black circles).

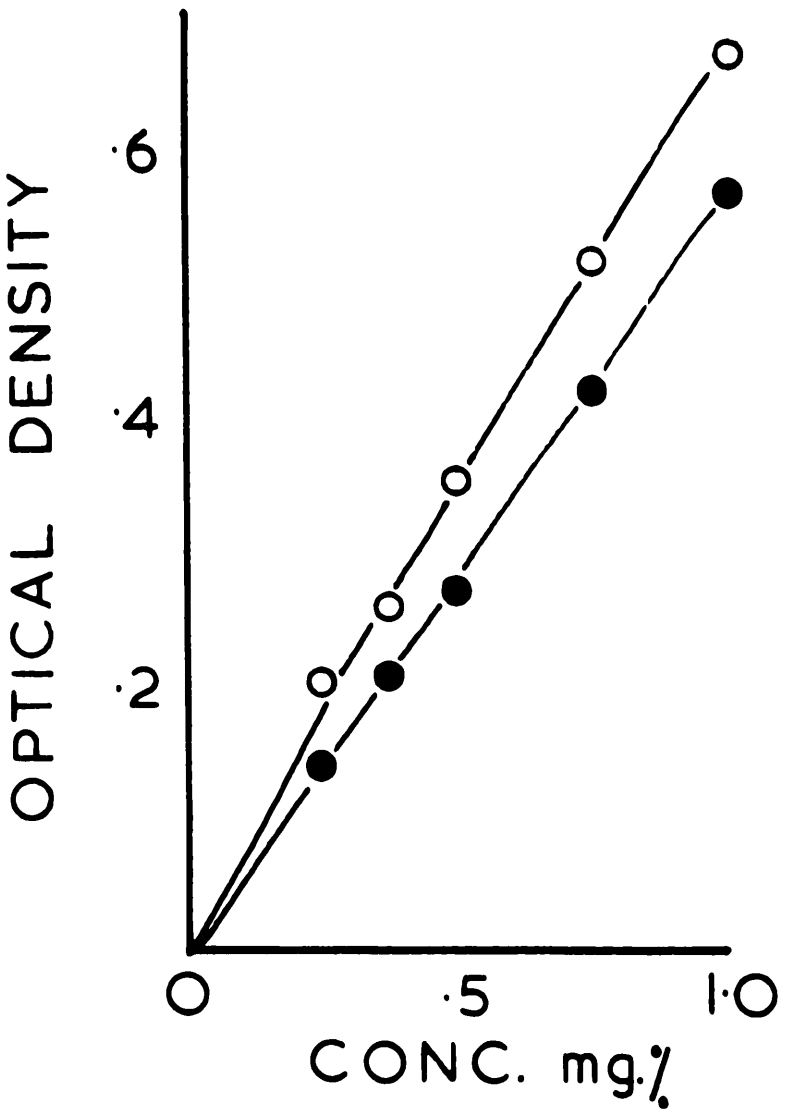


Figure 19. Calibration curves for the dye T-1824 in 0.9% saline (open circles) and in plasma (black circles).

discrepancy between measurements using T-1824 and I^{131}-labelled albumin, which they attribute to a loss of unbound T-1824 from the plasma. They suggest that in the rabbit, T-1824 may provide falsely high estimates of plasma volume.

The decay rates of T-1824 and I^{131}-albumin concentrations were studied simultaneously in 3 rabbits. 50 mg T-1824 and 3 μc I^{131}-labelled human serum albumin (Radiochemical Centre, Amersham) in 1 ml saline were injected intravenously and plasma indicator concentrations determined at 10,20,30,40 and 50 minutes after injection. Figure 20 shows the results expressed as percentages of the concentration at zero time; there was no evidence that T-1824 was lost from the circulation at a more rapid rate than I^{131}-labelled albumin. Seven estimations of plasma volume in 5 animals were carried out using both T-1824 and I^{131}-labelled albumin. T-1824 gave results for plasma volume averaging 1.6±1.8(SE)% greater than I^{131}-albumin, a difference which was not significant (table 6). Thus in the present study, T-1824 appeared to provide accurate estimates of plasma volume.

Determination of Red Cell Volume.

Red cell volume was determined from the 10 minute sample, after injecting 1 ml of the animal's own cells labelled with CR^{51} at the same time as the dye injection. The method used for labelling erythrocytes has been described by Lajtha (104).

ADMINISTRATION OF GASES.

Carbon Monoxide Mixtures.

The effects of breathing 0.2% and/or 0.3% carbon

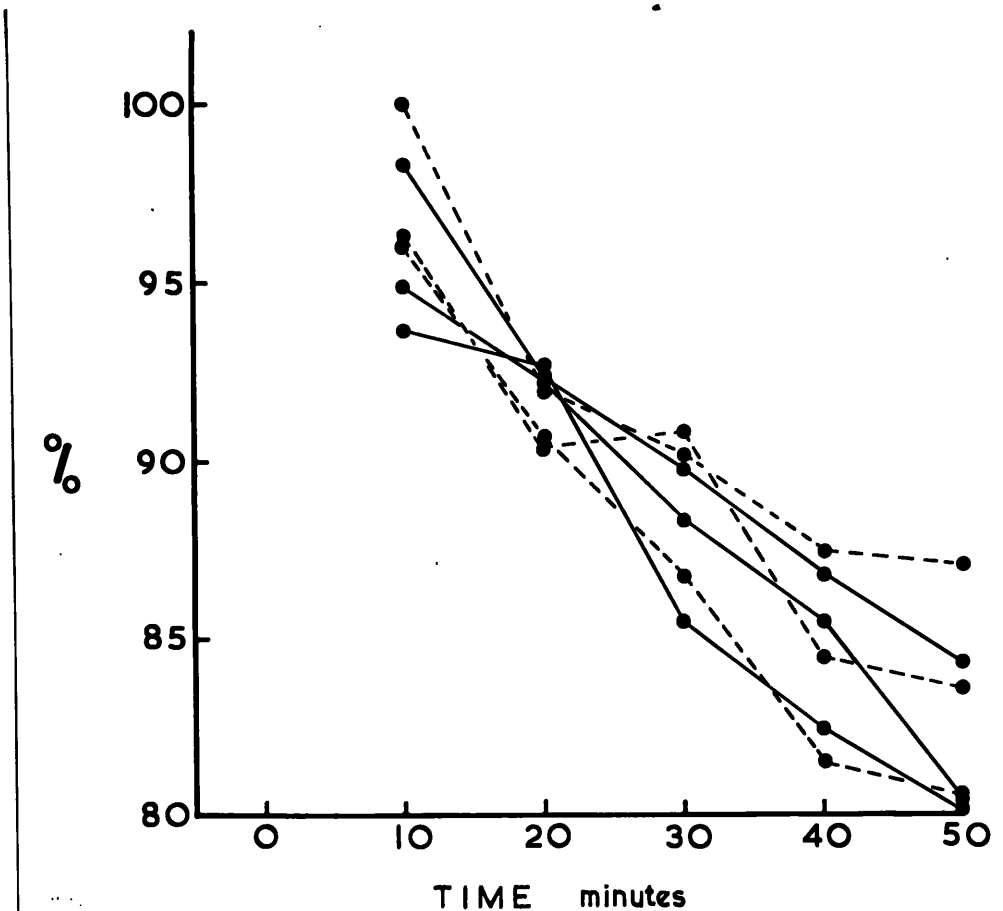


Figure 20. Rates of disappearance of I^{131} - albumin (dashed lines) and T-1824 (unbroken lines) measured simultaneously during 50 minutes after injection in 3 animals. Plasma concentration of each indicator at zero time was obtained by semilogarithmic extrapolation, and the plasma concentrations at 10-50 minutes expressed as percentages of this figure.

TABLE 6.

COMPARISON OF METHODS OF DETERMINING PLASMA VOLUME.

		Haematocrit (%)	^{131}I - albumin Volume (ml)	T-1824 Volume (ml)	Difference (%)
Animal	1	34.0	120	121	+0.8
	2	37.2	98	101	+3.1
	3	39.2	97	101	+4.1
	4	34.9	124	117	-5.6
	5	44.0	85	92	+8.2
Animal	3	12.4	107	111	+3.7
	4	12.6	148	143	-3.4

MEAN DIFFERENCE = +1.6%

S.E. = $\pm$ 1.8%

monoxide in air were studied in 11 animals. The animals were placed in a gas-tight perspex box, and the catheters were brought to the exterior through rubber seals (figure 21). Gas was drawn through this box from a 100 litre Douglas bag at the rate of 4-6 litres per minute, using a water suction pump. The flow rate was monitored with a rotameter (Gap Meter). During the control period room air was drawn through the box, and subsequently the animals breathed 0.2% CO or 3% CO for 30 minutes before commencing clearance measurements.

The gas mixtures were prepared freshly for each experiment from a stock mixture of 0.45-0.55% carbon monoxide in 21% O_2 which was kept under pressure in a cylinder, and was of accurately determined composition (this was a special mixture supplied by Commonwealth Industrial Gases, Sydney). The stock CO mixture, and compressed air from another cylinder, were passed through rotameters into a Douglas bag at appropriate flow rates to obtain the diluted CO mixture required.

Oxygen Mixtures.

During experiments in which low oxygen mixtures or 100% oxygen were administered, the animals breathed through a tracheotomy tube and repiratory valve assembly (Fig 23) as described by Edwards et al (52). It was estimated that this system increased the normal respiratory dead space by no more than 1 ml. The inlet of the respiratory valve was

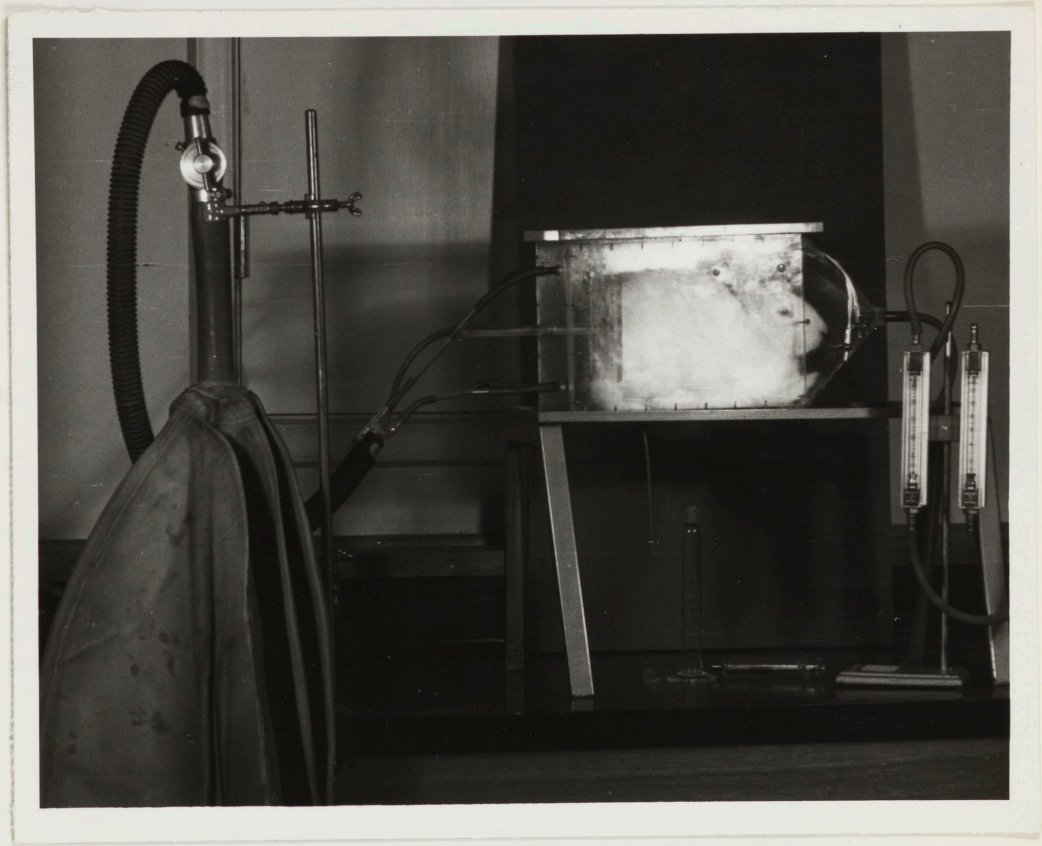


Figure 21. Apparatus for administration of carbon monoxide mixtures.

connected to a light polythene bag, which was replenished periodically from a Douglas bag containing the gas mixture (figure 22).

Low oxygen mixtures.

20 animals breathed low oxygen mixtures, ranging from 7.9-8.7% O_2 in N_2 in different experiments. The gas was given as a test procedure at the end of experiments involving bilateral ureteric catheterization, and was designed to show up differances in renal innervation on the two sides. It was administered for 10 minutes before renal clearance measurements were recommenced. In all animals in which the left kidney had been chronically denervated, there was a marked increase in the ratio of left/right clearances due apparently to chemoreceptor stimulation and resultant vasoconstriction on the innervated side (98).

The mixtures were made up freshly in a Douglas bag by passing air and nitrogen each from a cylinder through a rotameter at flow rates such that $O_2:N_2 \simeq 10:14$. The O_2 concentration of the resultant mixture was checked using a Beckman model "C" O_2 analyser, and adjusted as necessary.

100% oxygen.

In 10 experiments in severely anaemic animals (Group II animals - see p. 72), 100% O_2 was administered as a means of increasing the transport of O_2 in blood at low red cell concentrations. After renal measurements

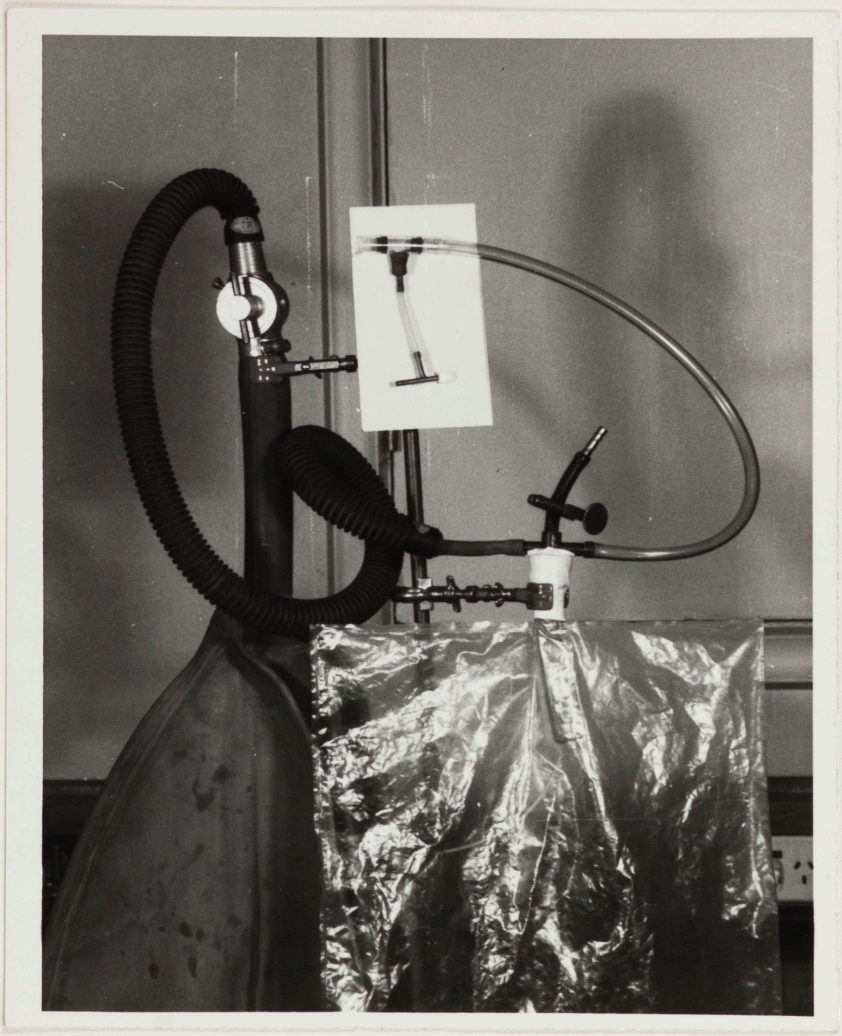


Figure 22. Apparatus for administration of oxygen mixtures.

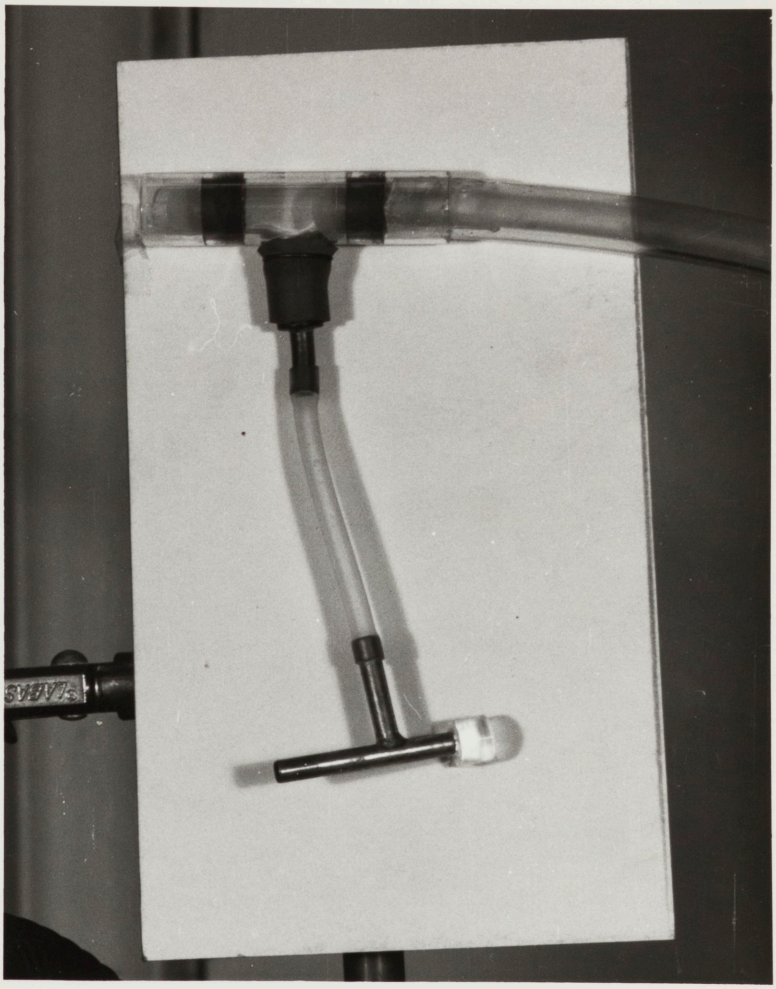


Figure 23. Details of respiratory valves and tracheotomy tube. The flap of the inspiratory valve is in the open position.

while breathing room air, the animals breathed 100% O_2 for 30 minutes and then renal measurements were repeated.

MEASUREMENT OF HAEMOGLOBIN, BLOOD O_2 SATURATION AND RENAL O_2 CONSUMPTION.

Haemoglobin.

The cyanmethaemoglobin method of Drabkin and Austin (50) was used.

Reagents (a) 3% potassium ferricyanide.

(b) 2% potassium cyanide.

Using an opsonic pipette transfer 0.2 ml blood to a 50 ml volumetric flask containing 1 ml of (a) and 30 ml water. Wash out pipette into this solution. Stand for 20 minutes. Add 0.5 ml of (b), and make up to 50 ml with water. Read on Beckman spectrophotometer at 540 mμ.

In this method, oxygenated and reduced forms of haemoglobin are first converted to methaemoglobin by the addition of ferricyanide. This is an unsatisfactory pigment for spectrophotometric determination because the shape of its absorption curve is greatly affected by minor changes in the pH and temperature of the solution, but the addition of KCN results in the formation of cyanmethaemoglobin, a very stable compound.

The cyanmethaemoglobin concentration in optical density units was converted to haemoglobin concentration in gm per 100 ml blood, using a factor of 38.43. The data for estimating this factor was obtained by measuring the O_2

capacity of a range of blood samples, and then determining these samples by the cyanmethaemoglobin method. (Data in the rabbit was kindly supplied by Dr. P.I. Korner and was obtained using the same Van Slyke apparatus and spectrophotometer as used in the present study). The haemoglobin concentration of the samples was estimated from their O_2 capacity, and the conversion factor derived from a calibration curve.

Determination of Blood O_2 Content.

Blood was sampled and stored using Scholander's technique (148). The tuberculin syringes used for sampling had a relatively wide bore, with the calibration mark at about two-thirds the length of the barrel, so that the plunger did not have to be withdrawn to its limits when filling the syringe. Each syringe was lubricated with paraffin grease, and a little concentrated heparin was drawn into the lower part of the barrel and then expelled, leaving heparin free of air bubbles in the nozzle. When sampling blood from an animal, the ear artery or renal vein catheter was primed with blood, the syringe connected and 1.2 ml of blood withdrawn slowly. The syringe was disconnected, a little mercury was drawn into its nozzle to act as a seal, and it was stored in ice. Figure 24 shows the perspex bath used to hold the ice and syringes. A clip was placed on the plunger of each syringe to prevent it sliding. Immediate storage of blood samples in ice has been

Figure 24. Perspex bath for storing blood samples in ice.

shown to prevent glycolysis for over 4 hours (99,112). In the present study, analyses were carried out within 3 hours of collecting the samples.

Measurements of O_2 content were made in a Van Slyke manometric apparatus (Fig 25) by the method of Van Slyke and Neill (177), using 0.5 ml samples of blood. The syringe containing the sample was inverted several times, using the drop of mercury inside to disperse sedimented red cells evenly throughout the sample. It was then mounted nozzle upwards in the transfer apparatus shown in figure 26. This consisted of a 19 gauge luer intramuscular needle through a rubber stopper clamped vertically in a retort stand. A Van Slyke stopcock pipette was placed over the point of the needle and pressed gently down onto the rubber so that the needle lay within its tip. It was then filled above the calibration mark by ejecting blood from the syringe. The volume was adjusted to 0.5 ml, and the contents of the pipette transferred to the extraction chamber of the Van Slyke manometric apparatus. The residue of blood in the syringe was used for a haemoglobin determination.

In the method of Van Slyke and Neill, oxygen in blood is released from oxyhemoglobin by shaking in a vacuum with potassium ferricyanide, and then measured in a manometer. The technical details have been fully described by Consolazio et al. (35); their procedure was followed exactly except for the use of a 0.5 ml blood sample, and 1 ml of

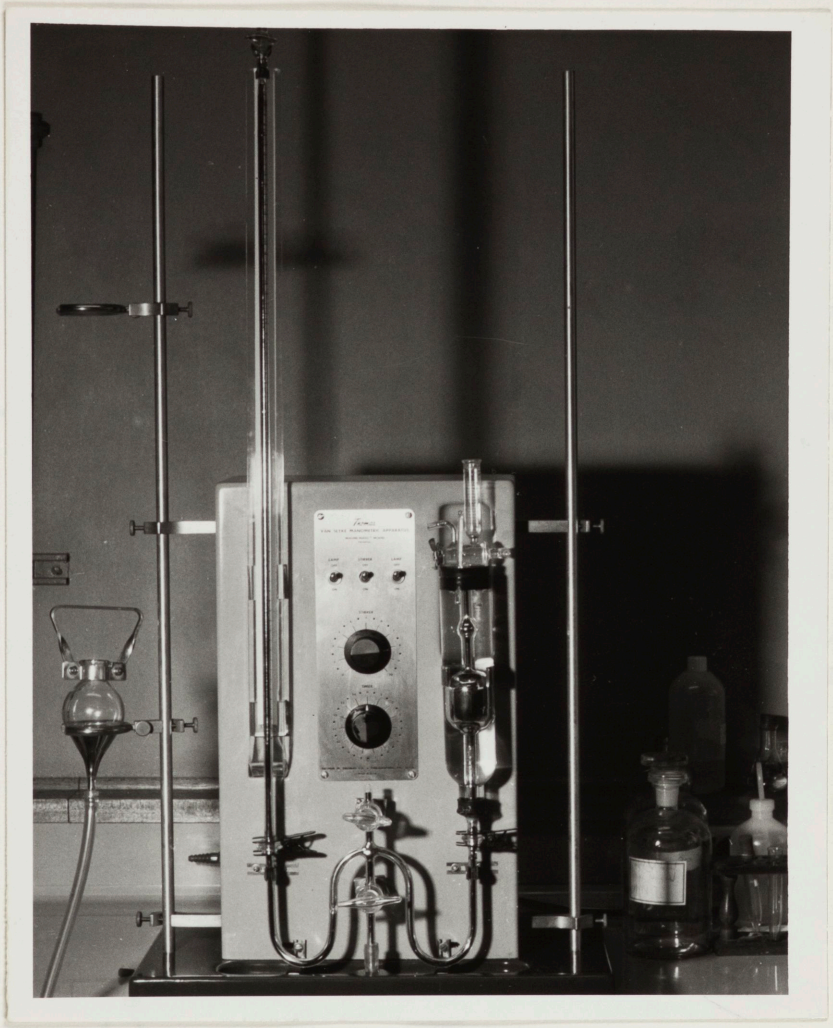


Figure 25. Van Slyke manometric apparatus.

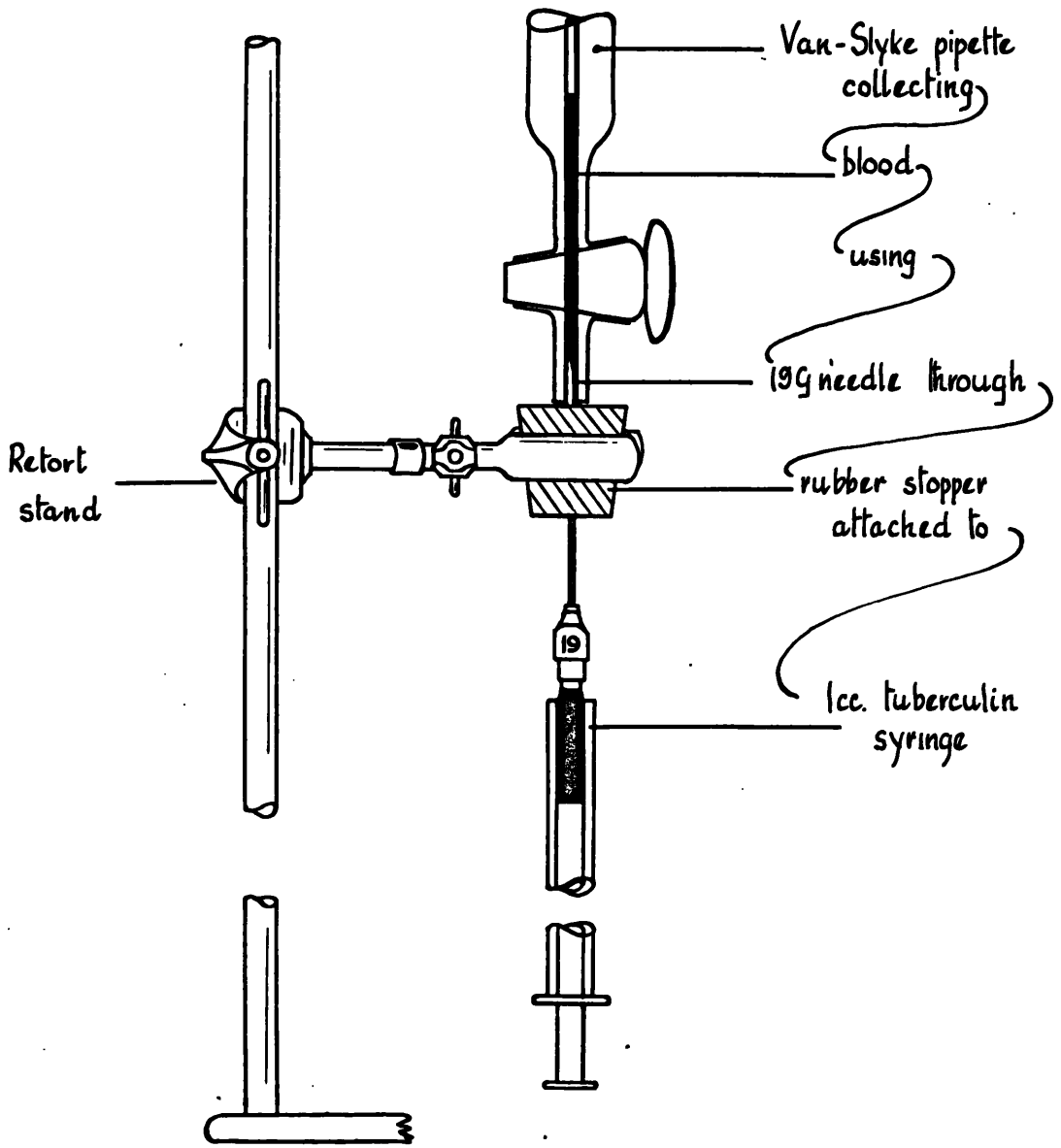


Figure 26. Transferring blood sample to Van Slyke pipette.

oxygen reagent instead of 2 ml. If P_1 is the manometer reading after reduction of oxyhaemoglobin, and P_2 the reading after absorption of the released O_2 by sodium hydrosulphite:-

Total O_2 content (ml/100ml blood)= $(P_1-P_2-C) \times F$, where C is the machine correction (which corrects for pressure changes due to addition of CO_2 and O_2 absorption reagents, and is constant for a given machine), and F is the Van Slyke oxygen factor for 0.5ml blood samples (which is constant for a given temperature).

Calculation of O_2 Saturation.

The calculation of O_2 saturation differed according to whether the animal was breathing air, 100% O_2 or carbon monoxide in air, during observations of blood O_2 content. In general the relationships used were:-

O_2 bound by Hb/100 ml blood = total O_2 content − dissolved O_2

O_2 capacity/100 ml blood = Hb X 1.34

O_2 saturation = $\frac{O_2 \text{ bound by Hb}}{O_2 \text{ capacity}}$.

Calculation of dissolved O_2

Since the solubility of O_2 in blood at normal body temperature in the rabbit (40°C - range 39.5-41.0°C (unpublished observations of Dr. P.I. Korner)) is approximately 2.3 ml/100 ml (77), then at a partial pressure of oxygen, p,

$$\text{dissolved } O_2 = 2.3 \times \frac{p}{760} \text{ (ml/100 ml).}$$

Arterial blood. When breathing air or carbon monoxide in air p = 100 mm, and dissolved O_2 was equal to 0.28 ml/100 ml blood (expressed at STPD). When breathing 100% O_2, p= 680 mm (assuming water vapour pressure of 43 mm, and pCO_2 of 37 mm), and dissolved O_2 was approximately 2.0 ml/100 ml (expressed at STPD).

Renal venous blood. Dividing the total O_2 content of renal venous blood by its O_2 capacity gave an approximation of the renal venous O_2 saturation. When breathing air or 100% O_2, p could then be obtained approximately from the O_2 dissociation curve for rabbit's blood. When breathing carbon monoxide, p was obtained indirectly from this curve, applying corrections for the presence of carboxyhaemoglobin as explained below.

Calculation of % Saturation of Haemoglobin with CO.

The percentage of total haemoglobin bound as carboxyhaemoglobin (COHb %) was calculated from the haemoglobin and O_2 content of arterial blood. This is best demonstrated by working through a sample calculation, using data from a typical experiment in which an animal breathed 0.3% CO in air.

Experimental data: Hb = 9.03 gm per 100 ml, total O_2 content of arterial blood = 5.53 ml per 100 ml.

O_2 bound by Hb = total O_2 content - dissolved O_2

= 5.53 - 0.28 = 5.25.

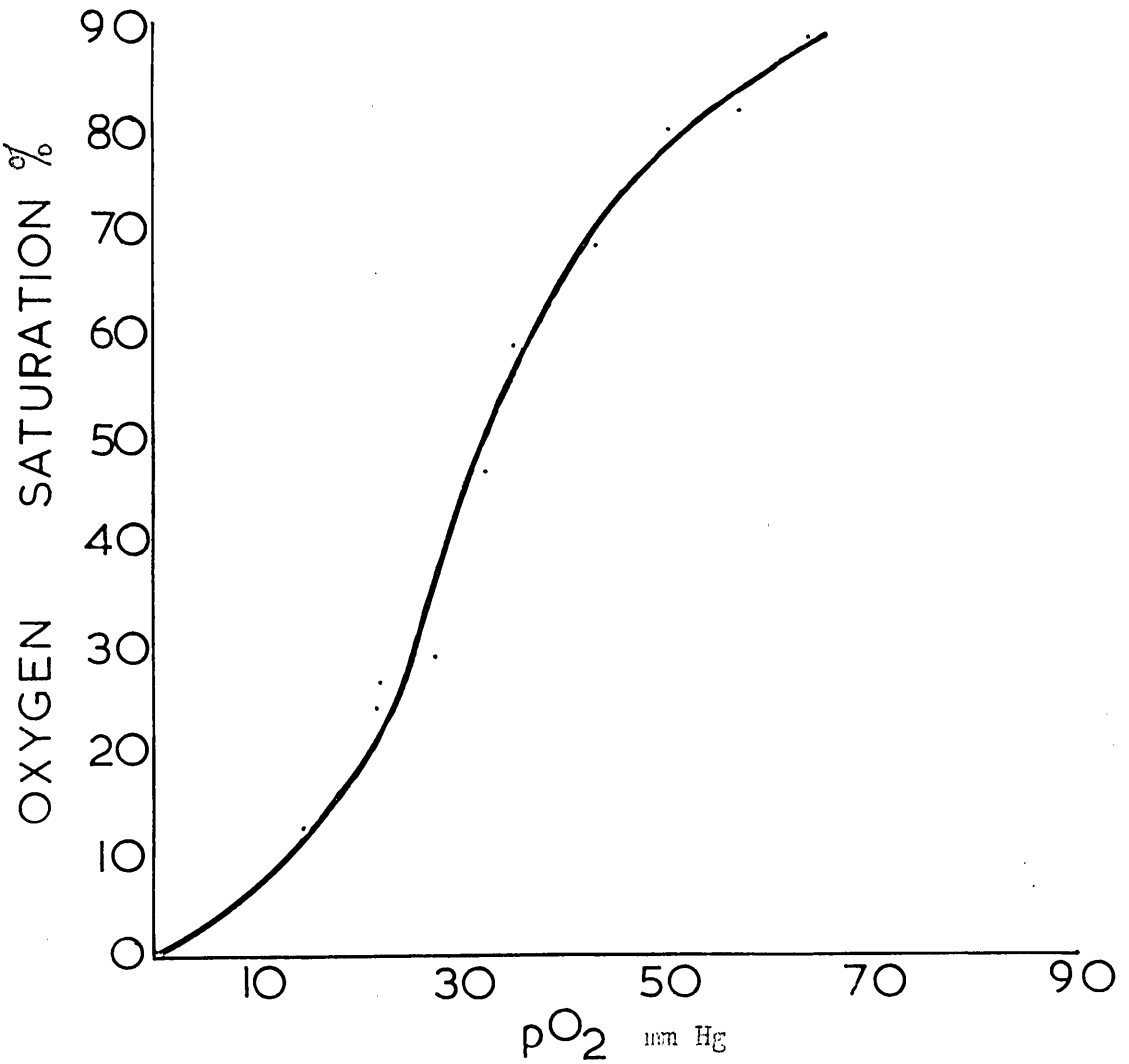


Figure 27. O_2 dissociation curve for rabbit blood at 40 mm Hg CO_2 tension and 40°C (derived from data of Korner and Darian Smith, 1954).

Assuming that the haemoglobin available for binding O_2 (i.e. O_2 Hb + reduced Hb) is 97% saturated with O_2 (99), then O_2 bound at 100% saturation = $\frac{5.25}{0.97} = 5.40$

but total O_2 capacity = 9.03 x 1.34 = 12.10

$$\therefore \text{COHb\%} = \frac{12.1 - 5.4}{12.1}$$

$$= 55\%$$

Calculation of Renal Venous O_2 Tension.

Figure 27 shows the O_2 dissociation curve for rabbit's blood at a CO_2 tension of 40 mm Hg and 40°C (body temperature of the normal rabbit). This curve was obtained by applying a temperature correction to the data of Korner and Darian Smith (99). Knowing the O_2 saturation of renal venous blood, the O_2 tension could be read off directly from this curve, if the animal was breathing air or 100% O_2.

When the animal was breathing carbon monoxide, the method described by Roughton and Darling (147) was applied. By this method, the O_2 tension may be calculated from the O_2 dissociation curve in absence of CO. It is based on two assumptions, which were originally proposed by Haldane (76).

(i) The amount of reduced haemoglobin present in a mixture of O_2 at partial pressure p O_2 and CO at partial pressure pCO, is the same as it would be in absence of CO if the partial pressure of O_2 was equal to $pO_2 + M.pCO$.

(ii) Even when appreciable amounts of reduced haemoglobin are present, haemoglobin combined with gas is still assumed

to be partitioned between COHb and O_2Hb according to the equation

$$\frac{COHb}{O_2Hb} = \frac{MpCO}{pO_2}$$, where M is a constant (called the relative affinity constant).

The calculation of pO_2 by this method is demonstrated by the following example, using data from the same experiment as before.

COHb = 55%, renal venous O_2Hb = 24%

Total saturation = COHb% + O_2Hb%

= 79%

The total gas tension is then read off the O_2 dissociation curve (figure 27), in which 79% saturation is found to correspond to a tension of 44 mm Hg. From assumption (i),

$$pO_2 + MpCO = 44 \text{ mm} = pO_2 \left(1 + \frac{MpCO}{pO_2}\right)$$

From (ii) $$\frac{MpCO}{pO_2} = \frac{COHb}{O_2Hb}$$

$$\therefore pO_2 - MpCO = pO_2 \left(1 + \frac{COHb}{O_2Hb}\right) = pO_2 (1 + 55/24) = 44 \text{ mm}$$

$$\therefore pO_2 = 44/(1 + 55/24)$$

$$= 13.4 \text{ mm Hg.}$$

Thus the O_2 tension of renal venous blood in animals breathing carbon monoxide could be calculated from the saturation data without recourse to measurements of pCO or of the relative affinity constant.

Calculation of Renal O_2 Consumption.

The renal O_2 consumption in ml per minute per total renal mass is the product of the renal arterio-venous O_2 difference (ml/100 ml blood) and the renal blood flow (ml/min). In experiments using carbon monoxide mixtures, in which the total O_2 content of both arterial blood and renal venous blood were measured, the renal A-V difference was obtained simply by subtraction. Where the animals breathed room air or 100% O_2, arterial O_2 content was not measured but was derived from O_2 capacity.

(a) Breathing room air. For normal rabbits breathing room air it has been shown (99) that arterial O_2 saturation averaged 97 ± 1.4 (SD) %; during anaemia no reduction in arterial O_2 saturation was demonstrated (43).

Assuming that arterial blood is 97% saturated with O_2, arterial O_2 content/100 ml of blood=O_2 capacity x 0.97 + 0.28.

(b) Breathing 100% O_2. Assuming that arterial blood is 100% saturated with O_2.

arterial O_2 content/100ml of blood = O_2 capacity +2.0.

MEASUREMENTS OF BLOOD PRESSURE, HEART RATE AND RENAL RESISTANCE.

Arterial blood pressure and pulse rate were recorded from the ear artery using a Statham P23AC pressure transducer and a Grass polygraph. Pressures were referred to a level 7 cm. above the floor of the experimental cage. Edwards et al (52) have shown that mean pressure in the ear artery is on the average 10 mm Hg below that in the contralateral

carotid artery, the difference being approximately constant over a range of mean ear artery pressures from 62 to 92mm Hg in 6 rabbits.

Analysis of renal vascular resistance into various components has been attempted by Lamport (105) and Gomez (72,164), who have formulated equations for calculating afferent and efferent arteriolar resistances. However, no attempt has been made in the present study to derive detailed information about changes in renal resistance in anaemia, as no data was obtained regarding renal venous pressure, renal interstitial pressure or intrarenal blood distribution and viscosity. Total renal vascular resistance was calculated simply as ear artery blood pressure divided by total renal blood flow and was expressed in "peripheral resistance units" (mm Hg/ml/sec); this index was used merely as a means of representing the relationship between renal blood flow and arterial pressure, rather than as a meaningful expression of the degree of renal vasoconstriction or renal vasodilatation. Changes in filtration fraction, i.e. in the ratio of glomerular filtration rate to renal plasma flow, have been frequently interpreted in the literature simply as indicating alterations in the ratio of afferent and efferent arteriolar resistance. Such an interpretation is questionable, particularly in post-haemorrhagic anaemia, as during this condition various vascular shunting mechanisms

have been postulated (136,176). However in this study, filtration fraction was used as an approximate indication of the resistance of the total post-glomerular vasculature relative to that of the preglomerular vessels.

ADMINISTRATION OF PHARMACOLOGICAL AGENTS.

Arfonad

In 2 animals measurements of arterial pressure, renal blood flow, etc. were carried out before, during and after an infusion of trimethylene thiophanium d-camphorsulphonate ("Arfonad" - Roche), which was given intravenously at a constant rate of 2 mg per minute after a priming dose of 1.5 mg. This is a short-acting but potent ganglion-blocking agent, which in addition causes direct vasodilator effects (110). The onset of hypotension after starting the infusion was rapid, and within one minute the blood pressure had fallen 15-20 mm Hg. While the infusion continued, the arterial pressure remained constant at this lower level, but it returned to control levels promptly after stopping the infusion.

Adrenaline and Angiotensin.

Renal blood flow, GFR, arterial pressure and L/R renal clearance ratios were determined in 4 animals with ureter catheters before, during and after intravenous infusion of adrenaline bitartrate (BDH) 0.6-1.2 µg/Kg/min (in normal saline 0.35 ml/min). The effects of angiotensin

II ("Hypertensin", Ciba) 0.1 µg/Kg/min were similarly determined in 3 ureter animals. The effects of adrenaline and angiotensin on the renal PAH extraction ratio were tested in 2 animals with renal vein catheters, one of which had a preliminary left renal denervation.

The rate of infusion of each of these agents was the lowest with which a sustained pressor response could be obtained. In the case of adrenaline this was established in preliminary experiments. Data about angiotensin dosage in the rabbit was obtained from a report by Brown (22).

HISTOLOGICAL TECHNIQUES.

Kidneys from 3 normal rabbits were examined microscopically with particular reference to the distribution of vasa recta in the outer medulla. In 2 animals, using pentobarbital anaesthesia, an abdominal incision was made, and the renal vessels of both sides were clamped simultaneously. The kidneys were removed and placed in neutral formalin. In another animal, the left kidney was perfused with neutral formalin followed by 10% saccharated iron oxide, before fixation. After 12 hours in formalin, the whole kidneys were cut into sections 3-5 mm thick, which were returned to formalin for a further 4-6 hours. The sections were then processed using the rapid paraffin processing technique described by Culling (39):-

70% alcohol for ½ hour

95% alcohol for 1 hour (one change)

Absolute alcohol for 1 hour (one change)

Xylol for 1 hour (one change)

Paraffin 1½-2 hours

Block in fresh wax.

6-8 μ sections were cut from the blocked tissue using a rotary microtome, and attached to glass microscope slides using egg-albumin solution.

The saccharated iron oxide in perfused tissue was stained using 2% potassium ferrocyanide (Prussian blue reaction). Other sections were stained using Masson's trichrome technique (39) with slight modifications, as follows:-

(1) Bring to water. Mordant in Zenker's fluid for 30 minutes.

(2) Stain in Weigert's iron haemotoxylin for 20 minutes, wash in water.

(3) Differentiate in 1% acid-alcohol until only nuclei are stained. Wash in water until sections are blue.

(4) Stain for 4 minutes in 1% ponceau-fuchsin in 1% acetic acid. Rinse rapidly in distilled water.

(5) Mordant in 1% aqueous phosphomolybdic acid for 5 minutes or until collagen around large blood vessels is de-stained.

(6) Drain slide, and pour on 2% light green in 2% acetic acid for 10 minutes.

(7) Differentiate in 1% acetic acid for 2 minutes to

remove excess green.

The cell nuclei were stained blue-black, cytoplasm blue red, collagen green and red cells brilliant red.

STATISTICAL PROCEDURES.

The statistical procedures and terminology used are described in "Introductory Statistics" and "Associated Measurements" by M.H. Quenouille (139, 140) and in "Statistical Analysis in Biology" by K. Mather (118). The statistical tables used were those of R.A. Fisher and F. Yates (62). The abbreviations used were-

n = the number of observations

V = variance

SD = standard deviation

SE = standard error

D/F = degrees of freedom

MS = mean square

SS = sum of quares

p = probability of an event occurring by chance.

The following techniques have been applied:

Calculation of the mean. The mean is the sum of individual observations divided by the total number of observations.

Calculation of variance. The variance of a distribution is the sum of the squares of the differences of individual observations from the mean, divided by the number of degrees of freedom; the number of D/F is n-1.

To facilitate the calculation of variance, the following function was used:-

Where a,b, ...y,z are individual observations in a series of n observations,

$$V = \frac{a^2 + b^2 + \ldots\ldots y^2 + z^2 - \frac{(a + b + \ldots\ldots + y + z)^2}{n}}{n - 1}$$

Calculation of standard deviation. The standard deviation is the square root of the variance.

Calculation of standard error. In a normal distribution, the standard error equals the standard deviation of the distribution divided by the square root of the number of observations.

In the present study the standard error was often based on comparisons within animals, each animal of a series acting as its own control. The SE was used to test the significance of the mean effect produced in the animals by a given treatment. The mean effect divided by the SE gave an estimate of the deviate, "t", and the probability of this deviate being exceeded by change was then obtained by reference to the table of the distribution of "t".

Analysis of Variance. In the analysis of variance technique, the total variance is split into its several components and the the variance of effects of interest then compared to the residual or error variance. An example of the application if this technique is described in detail on p133.

Regression Equations were used to express the relationship between a dependent and an independent variable (e.g.Table 8).

CHAPTER 3

NORMAL RENAL FUNCTION IN THE UNANAESTHETIZED RABBIT.

Introduction.

Before analysing the effects of various treatment procedures on the renal circulation, it was necessary first to examine the "baseline" values obtained for indices of renal function under experimental conditions, to establish that they were reproducible and to compare them with results obtained by other workers. This chapter deals with the findings during control observations in 36 "normal" rabbits and 21 rabbits with bilateral ureteric catheters. Measurements were carried out during moderate water and mannitol diuresis, following administration of a sustaining infusion (see Table 3) for one hour in "normal" animals or for 2-3 hours in "ureter" animals. Experiments lasted for a period of between one and four hours, and the animals were infused at a constant rate throughout.

Evidence regarding the apparent relationships between urine flow, glomerular filtration rate (91) and renal blood flow (47) during extreme degrees of water loading in the rabbit, has already been discussed. In the present study, no definitive water loads were given to the "normal" animals, although they were permitted to drink ad libitum during experiments; the "ureter" animals received 40 ml water initially and 10 ml per hour thereafter. In both groups 5% mannitol in Ringer-Locke solution was infused at a rate of 40 ml per hour. The measures taken to promote diuresis thus resembled those employed by Forster (65), Brod and Sirota (21) and Korner (97), and were moderate in comparison to those

used by the earlier investigators. It has been shown under these conditions that no relationship exists between changes in urine flow and changes in GFR or RBF (21), and also that both GFR and RBF remain relatively constant for periods of up to 4 or 5 hours (65, 97). In nine experiments in which the infusion and clearance techniques were the same as for the present series, the standard error of replicate determinations of RBF and GFR during a period of 3 hours averaged 8% of their mean values (97). Because of this previous evidence of stability in the type of preparation used for the "normal" animals, no further testing was considered necessary. Evidence that the renal circulation in animals with ureter catheters could also maintain a steady basal state was obtained in 4 experiments which showed no significant changes between control observations of RBF and GFR one hour apart (e.g. Fig 44), and in 3 experiments in which RBF and GFR returned consistently to their control levels despite repeated vasoconstrictor stimuli during a 3 hour period (Fig 45).

Renal Blood Flow and Glomerular Filtration Rate.

Table 7 shows results obtained in 26 "normal" animals and 8 animals with bilateral ureter catheters. The values and standard deviations shown for ear artery blood pressure and heart rate are closely similar to results reported by Edwards et al (52) from rabbits which had received no anaesthetic agents or infusions. The haematocrit ratios obtained were probably slightly lower than the normal values, due to haemodilution caused by the sustaining infusion. In 10 "normal" animals the haematocrit ratio averaged 39.1% just before starting the infusion; after the infusion had been administered for 80 minutes, this value was decreased

TABLE 7

Mean values and standard deviations obtained in 27 "normal" rabbits (each with a chronic renal vein catheter) and 8 rabbits with bilateral ureteric catheters.

Measurements	NORMAL ANIMALS Mean	SD	n	URETER ANIMALS Mean	SD	n
Body weight (Kg)	2.69	0.26	(27)	2.42	0.41	(8)
Kidney weight (g)	20.1	3.06	(22)	17.0	3.2	(8)
Ear artery pressure (mm Hg)	84.0	8.04	(27)	89	8.9	(8)
Heart rate per min	282	32.2	(26)	239	19.6	(8)
Haematocrit (%)	33.9	7.60	(27)	40.7	3.71	(8)
Renal plasma flow* (ml/min)	87.4	16.9	(26)	54.3	16.4	(8)
Renal blood flow (ml/min)	134.6	25.0	(26)	91.8	28.0	(8)
RBF/BW (ml/min/Kg)	50.6	10.3	(26)	38.0	10.6	(8)
RBF/KW (ml/min/g)	6.77	1.48	(21)	5.42	1.54	(8)
Glomerular filtration rate (ml/min)	18.7	5.03	(26)	14.8	4.92	(8)
GFR/BW	7.10	2.15	(26)	6.06	1.59	(8)
GFR/KW	0.957	0.324	(22)	0.881	0.303	(8)
Filtration fraction	0.214	0.049	(25)	0.272	0.040	(8)

*Renal plasma flow was estimated from the ratio of PAH clearance to E_{PAH}: in "normal" animals E_{PAH} was measured in each animal, in "ureter" animals a value of 95% was assumed for E_{PAH}.

significantly ($p = 0.01$) to 34.8%. The expansion of blood volume caused by the infusion appeared to be of smaller degree in the "ureter" animals.

The values shown for renal plasma flow in normal animals were determined from PAH clearance and renal PAH extraction ratio; E_{PAH} was measured in each experiment by means of a chronic renal vein catheter. Relationships between renal blood flow, glomerular filtration rate, body weight and kidney weight are illustrated in figures 28-32. Linear regression equations for these interrelationships are given in table 8. Renal blood flow was significantly related to both body weight and kidney weight, but was more closely associated with kidney weight. There was no significant relationship between glomerular filtration rate and body weight or kidney weight in this series, but results are expressed in terms of body and kidney weight for the purpose of comparison with other series. The relationship between GFR and renal plasma flow (Fig 32) was highly significant, thus there was a relatively small variation in filtration fraction between animals. The average value for filtration fraction was 0.214, which is similar to values reported in man but lower than in the dog (164).

Whether values for renal blood flow and glomerular filtration rate were expressed in absolute units or in terms of body or kidney weight, the results obtained appeared to be considerably higher than those reported by most other workers. This point is demonstrated in table 9, which is based on previous reports of renal clearance values observed during resting conditions in unanaesthetized rabbits.

In the series of Korner (97), clearance measurements were carried out in the same way as in the present investigation, and results obtained

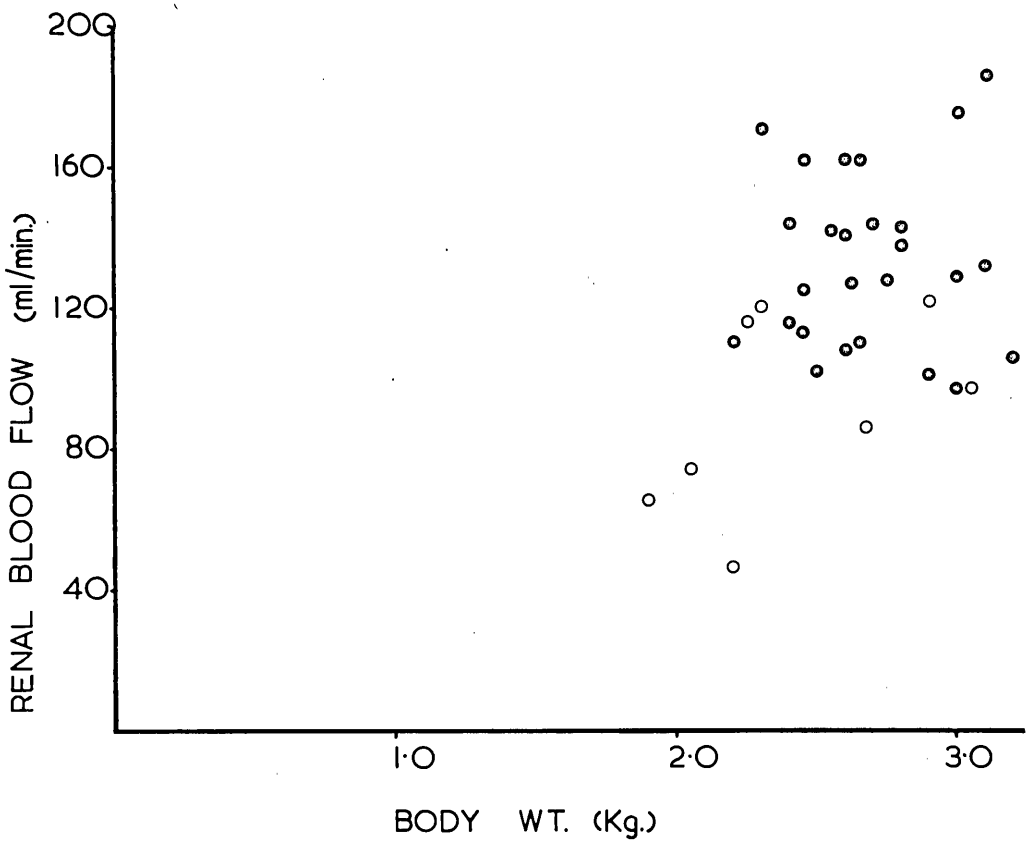


Figure 28. Relationship between body weight and renal blood flow in normal rabbits (black circles) and rabbits with ureter catheters (open circles). The equation of the linear regression is given in Table 8.

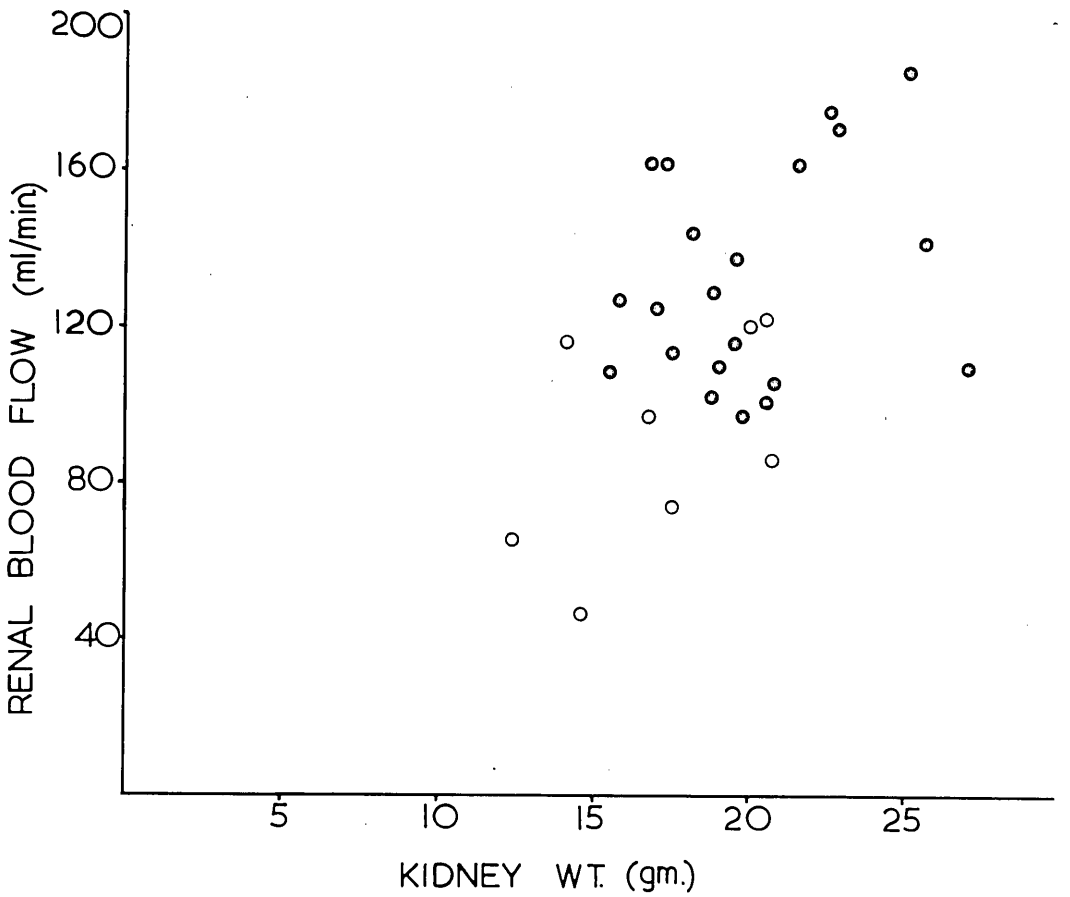


Figure 29. Relationship between kidney weight and renal blood flow in normal rabbits (black circles) and rabbits with ureter catheters (open circles). The equation of the linear regression is given in Table 8.

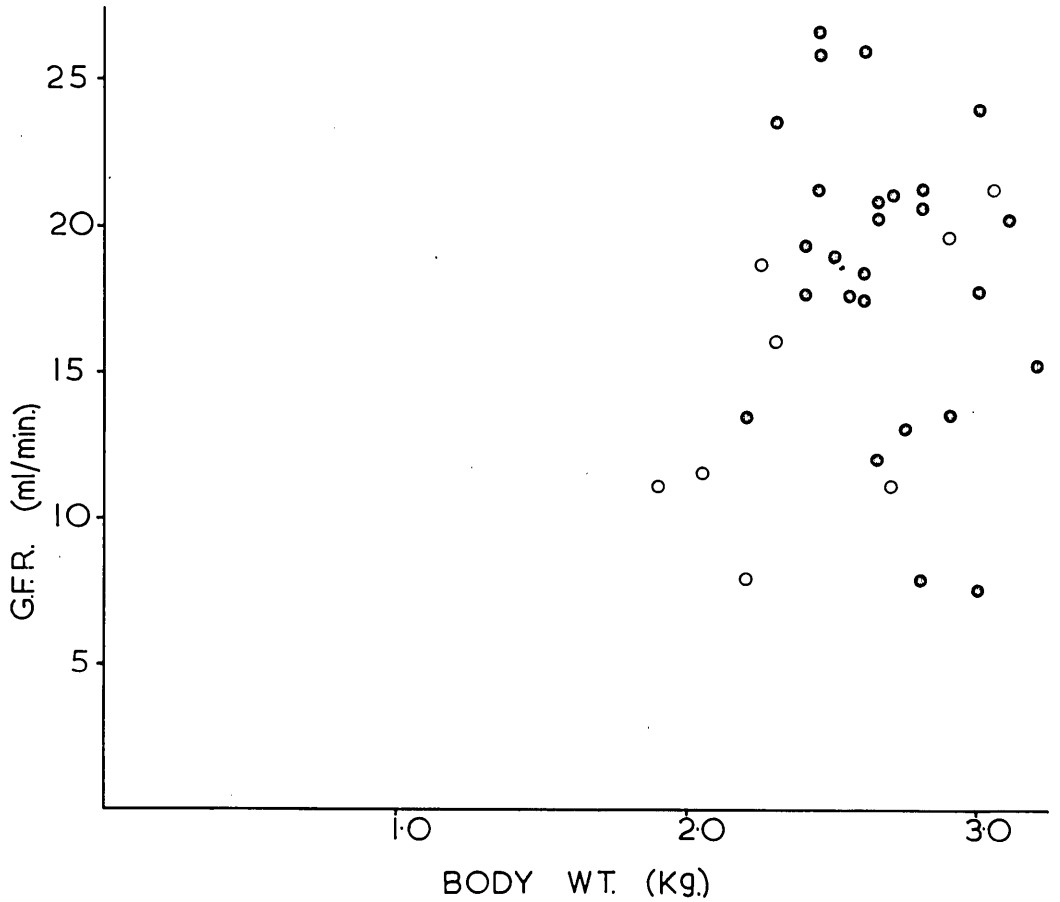


Figure 30. Relationship between body weight and glomerular filtration rate in normal rabbits (black circles) and rabbits with ureter catheters (open circles). This was not statistically significant (Table 8).

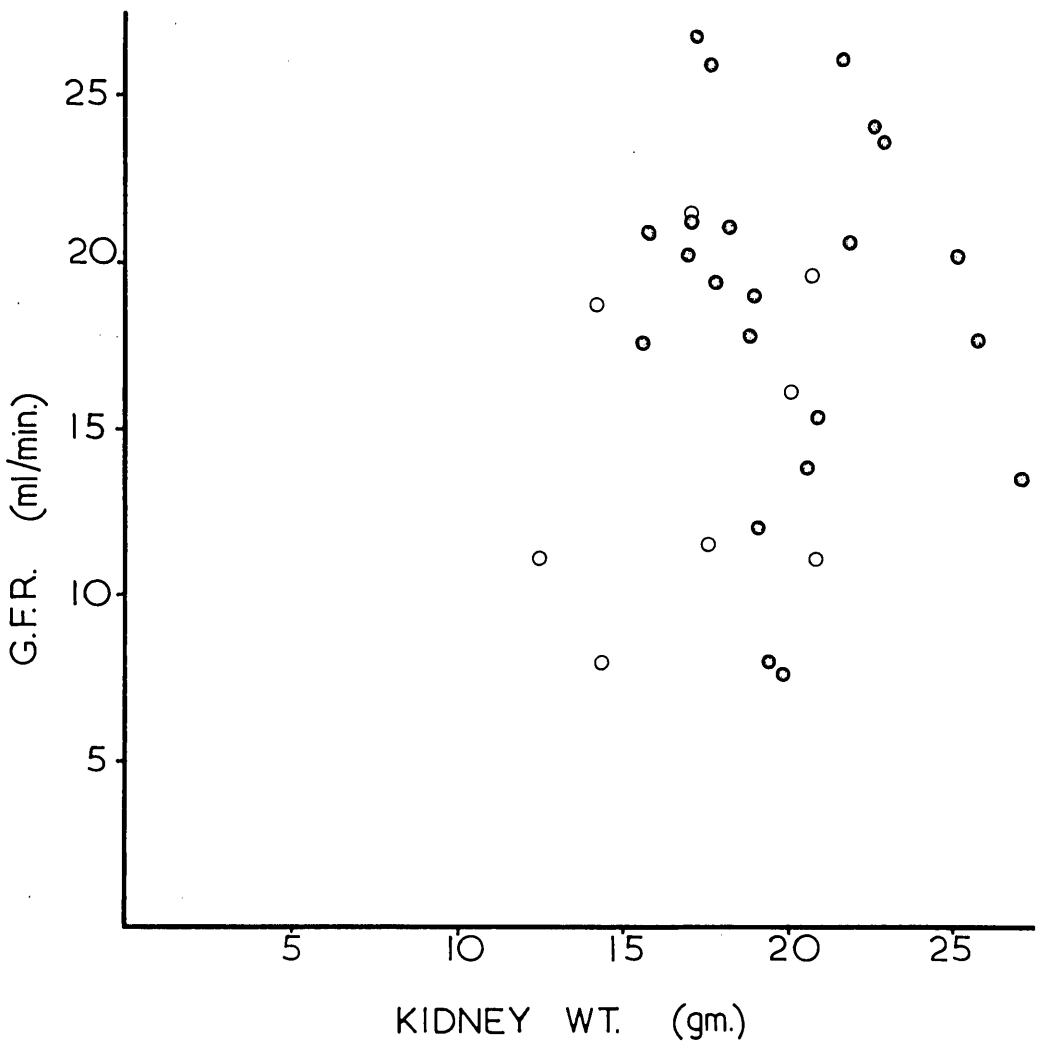


Figure 31. Relationship between kidney weight and glomerular filtration rate in normal rabbits (black circles) and rabbits with ureter catheters (open circles). This was not statistically significant (Table 8).

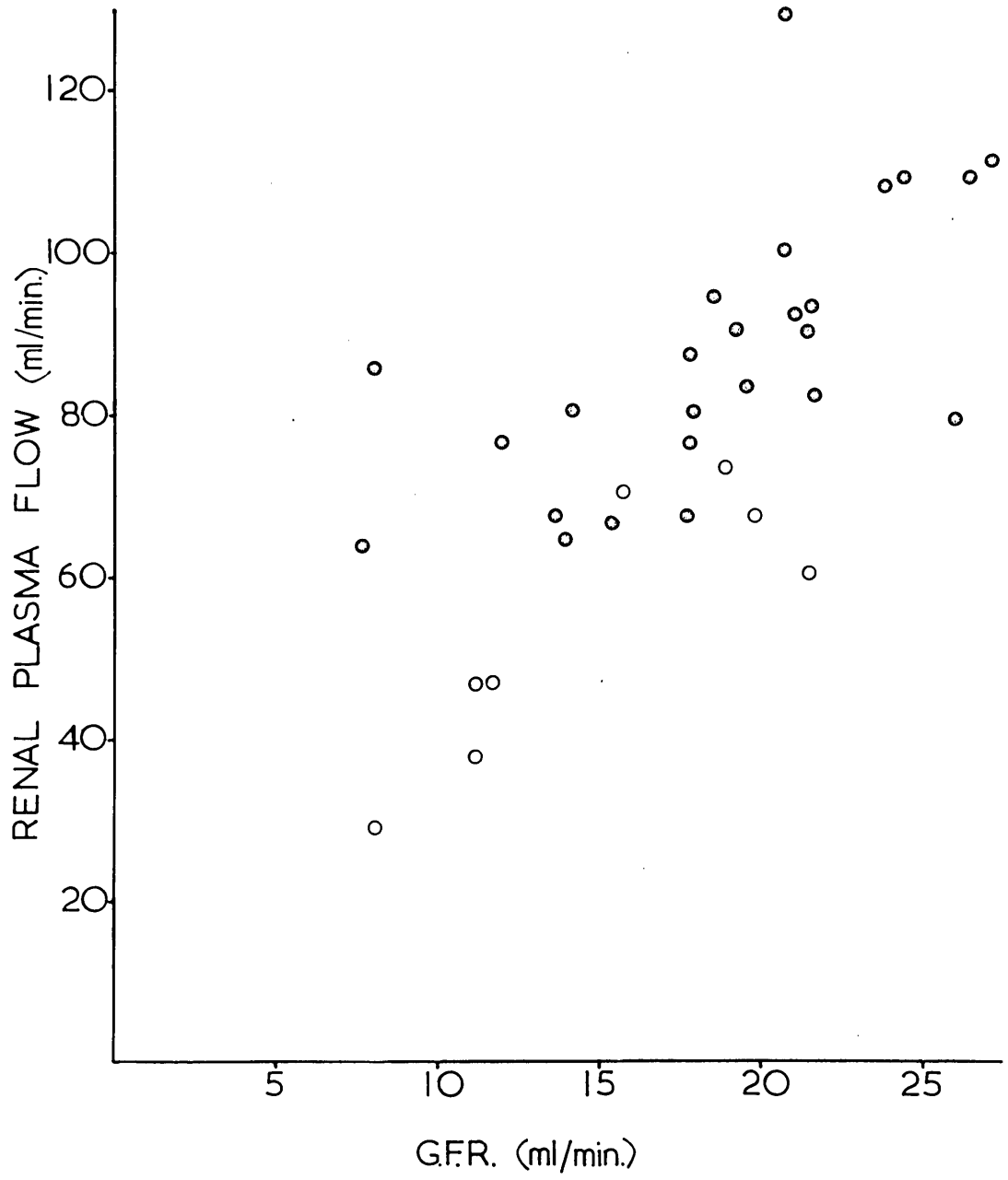


Figure 32. Relationship between renal plasma flow and glomerular filtration rate in normal rabbits (black circles) and rabbits with ureter catheters (open circles). The equation of the linear regression is given in Table 8.

TABLE 8

RELATIONSHIPS OF RBF AND GFR TO BODY WEIGHT (BW) AND KIDNEY WEIGHT (KW). ALSO RELATIONSHIP OF GFR TO RPF

Number of Animals	Regression equation	Standard error of regression coefficient	"t"	"p"
34	RBF = 35.1 + 34 BW	16.3	2.09	0.05
29	RBF = 30.8 + 4.76 KW	4.76	2.85	0.01
34	GFR = 12.64 + 1.96 BW	3.25	0.60	0.6 N.S.
30	GFR = 14.02 + 0.19 KW	0.185	1.03	0.4 N.S.
33	GFR = 4.47 + 0.167 RPF	0.031	5.39	0.001

TABLE 9

UNANAESTHETIZED RABBIT: COMPARISON OF RENAL HAEMODYNAMIC DATA REPORTED IN DIFFERENT SERIES

Source	Present Series		Kerner 1963 (97)		Brod & Sirota 1949 (21,164)		Forster 1947 (65)	Smith.W.1941 (169)	Josephson et al. 1953. (87)
	Mean	SE	Mean	SE	Mean	SE	Mean	Mean	Mean
RPF++ (ml/min)	87.4	± 3.31 (26)+	64.8	± 2.55 (42)	-	-	52.6 (6)	-	-
RPF/BW (ml/min/Kg)	32.9	± 1.37 (26)	28.2 (42)		19.2	± 3.6 (21)	19.5 (3)	-	-
RPF/KW (ml/min/g)	4.39	± 0.22 (21)	3.88 (20)		-	-	-	2.61 (8)	
GFR (ml/min)	18.7	± 0.99 (26)	13.6	± 0.83 (42)	-	-	13.2 (6)	-	14.6 (8)
GFR/BW (ml/min/Kg)	7.10	± 0.42 (26)	5.91 (42)		3.12	± 0.06 (21)	5.21 (3)	-	3.35 (8)
GFR/KW (ml/min/g)	0.96	± 0.07 (22)	0.81 (20)		-	-	-	0.66 (8)	-
FILTRATION FRACTION	0.214	± 0.010 (25)	0.210 (42)		0.16 (21)		0.26 (6)	0.26 (8)	-

++ Values for "effective" renal plasma flow (PAH clearance) from all the quoted series have been divided by 0.95 (average value for E_{PAH} in the present series) to allow comparison with RPF in the present series.

+ The numbers in brackets indicate the number of animals in each series.

for renal plasma flow (assuming E_{PAH} = 95%) were only slightly lower if expressed in terms of body weight or kidney weight; the same was true about results for GFR. These differences could be attributed in part to the characteristics of the animals used. Uncastrated New Zealand Giant rabbits (which are particularly lean and muscular) were used in the present study whereas castrated rabbits of mixed breed were used by Korner.

Recently Korner has reported that New Zealand Giant rabbits appear to have a significantly higher cardiac output than rabbits of other strains and of comparable body weight (98B). He found that cardiac output in New Zealand Giant crossbreds (average body weight 2.6 Kg) was 556 $\pm$ 22.6 (SE) ml/min using a thermodilution technique in one group, and 513 $\pm$ 18.7 (SE) ml/min using the Fick method in another group. The mean value for renal blood flow in the present series of animals (average body weight 2.7 Kg) was 134.6 ml/min (Table 7). Using the average of the above results for cardiac output as an approximation of cardiac output in the present series, the renal fraction of the cardiac output is estimated as 25.1%. In the renal study of Korner under consideration (97), renal blood flow averaged 98.6 ml/min and cardiac output averaged 405 ml/min, giving a result of 24.4% for the renal fraction of the cardiac output. Thus the differences between the present results for RBF and GFR, and those of Korner are attributable in large part to dissimilarities in body weight and breed of rabbits used. However the apparent differences in renal haemodynamics between the present series and the remaining four series cited are too great to be explicable on this basis.

It has been clearly demonstrated by Brod and Sirota (21) that "emotional stress" of various types (produced by tube feeding, faradic shocks, etc) can produce a marked reduction in GFR and RPF values in the

rabbit, as has also been observed in man (164). It is possible that stress was a factor tending to lower RPF and GFR in the series of Forster (65) and Smith (169), because the animals in their experiments were tied supine to a board and blood samples were obtained by needling the ear vessels or heart. During control observations in the experiments of Brod and Sirota, the animals were allowed to rest in a box, but blood samples were collected by cardiac puncture. This suggests the possibility that reflex renal vasoconstriction may have been excited in their experiments at an earlier stage than was intended. No RPF values were reported by Josephson and Kallas (87), but the values they obtained for GFR in giant rabbits with minimal restraint and handling, are included in table 9 to make the record complete; these values again appeared lower than in the present series.

Results for renal blood flow could not be derived for the four earlier series cited, as haematocrit ratios were not reported. It should not be assumed that differences in RPF values between series necessarily indicate that similar disparities would be found for RBF values. Evidence is presented in chapter 4 which indicates that RPF can increase without a change in RBF when haematocrit is reduced and total plasma volume expanded. Thus the higher values for renal plasma flow in the present series may have been due in part to a lower average haematocrit ratio or to greater haemodilution by the sustaining infusion.

In animals with ureter catheters, values obtained for total renal blood flow and total glomerular filtration rate (table 7) were significantly lower than in normal animals ($p = 0.01$), even when differences in body and kidney weight were taken into consideration.

Reflex nervous renal vasoconstriction has been shown to occur after catheterization of the ureters in dogs (123). The possible role of increased vasomotor tone in causing renal vasoconstriction in rabbits with ureter catheters was investigated in 13 animals whose left kidneys had been chronically denervated (Table 10). Clearance values were 7% lower on the right (innervated) side, the difference between sides being statistically significant for RBF values ($p = 0.001$) and GFR values ($p = 0.05$). In 8 animals with both kidneys innervated (table 10) no significant differences were demonstrated between the right and left sides. Thus there was evidence of nervous vasoconstriction in the animals with one kidney denervated. Since it appears that vasomotor pathways to the kidney are normally at rest (163), the apparent depression of renal clearances after catheterization of the ureters was probably due in part to nervous factors, although humoral and other factors may have also been operative. Values for filtration fraction in "ureter" animals were significantly higher than in "normal" animals.

Renal PAH Extraction Ratio

Table 11 shows the mean values obtained for renal PAH extraction ratio during control observations in 43 rabbits. Since in 36 of these animals renal clearance measurements were also carried out, they were receiving an infusion of 5% mannitol in Ringer-Locke solution (40 ml per hour) at the time of E_{PAH} determinations, and probably had a slightly expanded plasma volume. It has been observed in other species that marked reduction of renal PAH extraction ratio can occur following infusion of 20% mannitol (20) or concentrated albumin solution (11, 29, 54, 122). To investigate the possibility that mannitol infusion or blood volume expansion in the present experiments might have resulted

TABLE 10

COMPARISON OF RBF AND GFR IN LEFT AND RIGHT KIDNEY
(Each value shown is mean of 4 clearance periods)

Animal		RENAL BLOOD FLOW			GLOMERULAR FILTRATION RATE		
		Left	Right	L/R	Left	Right	L/R
1		22.3	22.8	0.89	3.8	3.8	1.00
2	Both	31.0	33.2	0.93	5.7	6.1	0.92
3		39.4	36.2	1.09	6.0	5.6	1.08
4	Kidneys	58.6	58.2	1.01	9.8	9.1	1.07
5		51.6	52.5	0.98	10.2	10.6	0.97
6	Innervated	43.6	43.7	0.99	5.5	5.6	0.99
7		61.4	60.9	1.01	10.0	10.4	0.96
8		60.4	60.6	0.99	7.6	8.6	0.91
	MEAN	46.0	46.0	1.00	7.3	7.5	0.99
9		57.4	50.8	1.13	7.0	6.0	1.16
10		48.9	44.5	1.10	8.5	7.3	1.17
11	Left	48.5	48.4	1.00	5.1	4.7	1.09
12		55.6	50.6	1.10	7.6	7.3	1.04
13		50.5	49.2	1.03	7.8	7.5	1.04
14	Kidney	44.2	39.8	1.11	7.7	7.1	1.07
15		53.8	47.6	1.13	8.0	7.8	1.03
16		81.6	77.4	1.05	11.4	10.9	1.05
17	Denervated	52.4	51.9	1.01	9.5	9.7	0.98
18		43.3	44.3	0.98	7.0	7.5	0.93
+ 19		38.4	39.9	0.96	4.3	4.5	0.96
+ 20		66.8	60.0	1.11	7.9	7.3	1.08
+ 21		57.4	47.6	1.20	7.8	5.9	1.32
	MEAN	53.8	50.2	1.07	7.7	7.2	1.07

+ Mean of 2 clearance periods

TABLE 11

Mean values and standard deviations for renal PAH extraction ratio (E_{PAH}) and Tm_{PAH} in animals with chronic renal vein catheters.

Measurement	Mean	SD	n	Arterial PAH (mg %)	Haematocrit (%)
E_{PAH} (%)	95.9	± 2.71	(7)	1.96	37.4
E_{PAH} (%) - mannitol diuresis	94.8	± 3.00	(36)	1.70	35.6
Tm_{PAH} (mg/min)	19.08	± 3.01	(10)	49.3	33.1
Tm_{PAH}/BW (mg/min/Kg)	7.09	± 1.18	(10)		
Tm_{PAH}/KW (mg/min/g)	0.94	± 0.196	(8)		
E_{PAH} (%) IN EWE SHEEP	97.2	-	(2)	1.59	31.5

TABLE 12

REPRODUCIBILITY OF E_{PAH} VALUES IN ONE RABBIT

Time (min)	E_{PAH}+ (%)	Arterial PAH concn. (mg %)
0	93.6	2.6
10	93.0	2.7
75	93.6	2.4
90	93.5	2.3

+ Each value based on one arterial sample and one renal venous sample.

in depression of E_{PAH}, determinations of E_{PAH} were also made in rabbits which received no mannitol and were infused only with PAH in Ringer-Locke solution at rates of 10-20 ml per hour. In these 7 animals E_{PAH} averaged 95.9 $\pm$ 1.02 (SE)%, while in the mannitol-infused group the average was 94.8 $\pm$ 0.5 (SE)%. The difference between the two groups was not statistically significant ($p = 0.4$), and together with the high level of extraction observed in the mannitol-infused animals, indicate that there was no significant alteration in tubular function due to the conditions necessary for determining renal clearances.

Using a previously-implanted renal vein catheter to sample renal venous blood, the reproducibility within animals of values determined for E_{PAH} was very high. Table 12 shows a series of E_{PAH} values obtained consecutively in a typical experiment. The difference between replicate control measurements did not exceed 5% in any one of the animals tested, and was usually less than 2%.

Renal PAH extraction ratio was measured at arterial PAH levels of 1-3 mg per 100 ml plasma (mean = 1.77 mg %) during most experiments in these studies. Figure 33 shows the results of 5 experiments during which the arterial PAH concentration was increased stepwise to much higher levels, by changing the rate of PAH infusion. In these animals it was demonstrated that tubular saturation occurred at an arterial PAH concentration of about 25 mg %; between 5 mg % and 20 mg %, E_{PAH} was independent of the arterial PAH concentration, and between 0.5 mg % and 5 mg %, there were small decrements in E_{PAH} as the arterial PAH concentration was increased. Since PAH in high plasma concentrations was observed to alter renal haemodynamics (Table 2), during clearance determinations its arterial concentration was kept as low as was compatible with accurate analysis of renal venous

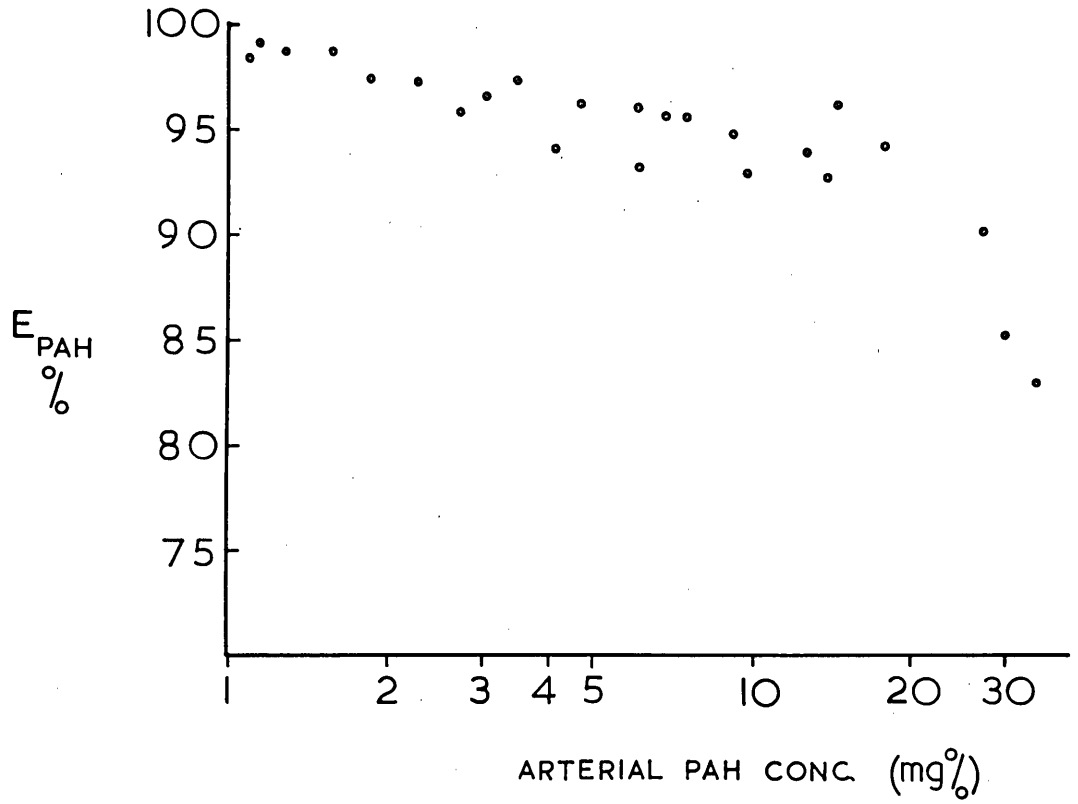


Figure 33. Renal PAH extraction ratio (E_{PAH}) in relation to arterial PAH concentration in 5 normal rabbits. Arterial PAH concentration is shown on a logarithmic scale.

samples, i.e. 1-3 mg %. It is apparent that in this range, changes in arterial PAH concentration such as were observed during the course of most experiments (e.g.) Table 12), were an unimportant factor in accounting for variation in E_{PAH}.

The average value obtained for renal PAH extraction ratio in the rabbit was slightly higher than that reported in man (cited in 164). However in studies of E_{PAH} in man, the kidney and catheterized renal vein have not been directly visualised as in the present study, and the average value quoted (92%) was probably weighted by low values due to renal abnormalities or due to contamination of the renal vein stream with blood from small tributaries arising in other tissues. In view of the contrast between values for E_{PAH} obtained in these two species and the lower and more variable figures reported from other animals (164), it is of interest to speculate if E_{PAH} varies from species to species, or whether perhaps the discrepancies stem from the use of anaesthetized preparations. Certainly in one other mammalian species in which a limited number of measurements have been made after recovery from anaesthesia, very high renal extraction of PAH was observed. In 2 conscious merino sheep, E_{PAH} was determined by a similar technique to that used in the rabbit (details were described in appendix 3) and an average value of 97% was obtained (Table 11 and Appendix 3).

Several suggestions have been put forward to explain the fact that in all species tested, the renal extraction of PAH falls short of 100% (136, 164, 176). Homer Smith maintained that this is due to that portion of the renal blood flow which passes through non-excretory tissue (capsule, pelvis, calyces, etc.). Accepting the figure of 95% as the normal value of E_{PAH} in the rabbit and 7.8 ml/min as the normal blood flow per gram of

kidney, and using Pappenheimer's assumption that the inert tissue of the kidney does not exceed 20% of the total renal mass, then the flow through this tissue would be 0.05 x 7.8 ÷ 0.2, or 1.9 ml/min/gm tissue. This rate of flow seems too high for an inert tissue (136), and thus it has been suggested that a portion of the blood flow through renal parenchyma is shunted along channels in which its PAH content is not exposed to uptake by the proximal tubular cells.

A possible anatomical route for such a distribution of blood flow in the rabbit kidney has been indicated by the histological studies of Trueta et al. (176). These workers demonstrated that the efferent flow from juxtamedullary glomeruli does not enter a cortical peritubular capillary network as does the flow from more peripheral glomeruli, but passes directly into the medulla down long straight capillaries ("vasa recta vera") which loop back at various levels to join the interlobular veins in the cortex. Trueta and his colleagues believed that blood flowing in the vasa recta does not come into proximity with parenchyma other than the thin segments of loops of Henle and the collecting tubules, and thus can escape the excretory operations of the proximal tubular cells. It was pointed out by Smith et al (119, 164) that this hypothesis neglected the presence in the outer medullary zone, of the straight descending limb of the proximal tubule, which is capable of PAH transport. However Trueta's concept is validated to some extent, when the anatomical relationships between the vasa recta and the proximal tubules in this outer third of the medulla are considered in detail.

Figure 34 shows a typical low power microscopic field of an outer

medullary cross-section in the rabbit's kidney. The vasa recta are in bundles, which are arranged in a fairly uniform fashion, as described by Trueta et al (176). Figure 35 is a high power view of one of these bundles showing that it contains no proximal or distal tubules; thus in the axial part of the bundle some vessels are isolated from the surrounding secretory tissue. It is suggested that diffusion of PAH from the plasma in these axial vessles to the proximal tubular cells may be limited at normal rates of medullary plasma flow. If the rate of plasma flow in the vasa recta is increased, then a fall in E_{PAH} would be expected; the possibility that this situation arises in anaemia is discussed in chapter 4.

Thorburn and Stacy have found that in the sheep, vasa recta bundles consist of many more vessels than in the rabbit (175). Figure 36 illustrates typical bundles in cross-section; they contain an average of 160 vessels (175) compared to about 30 vessels in the rabbit. Arguing from the concept that vasa recta bundles may limit diffusion, a lower PAH extraction ratio might be expected in the sheep because of the greater remoteness of the axial vessels of its bundles from secretory tissue. This does not appear to be the case, as shown in table 11. However it is conceivable that the rate of plasma flow through the vasa recta of the sheep may be slower than in the rabbit, allowing more time for diffusion of PAH to tubular cells.

In the anaesthetised dog, it has been shown that outer medullary blood flow represents approximately 5% of the total renal blood flow (174). If this result is accepted as an approximate indication of the intrarenal distribution of blood flow in the rabbit, and if the dynamic haematocrit of vasa recta blood is assumed to be the same as in other regions of the kidney, then to account for a PAH extraction ratio of 95% it would be necessary to postulate that all the plasma flowing through the vasa recta bundles

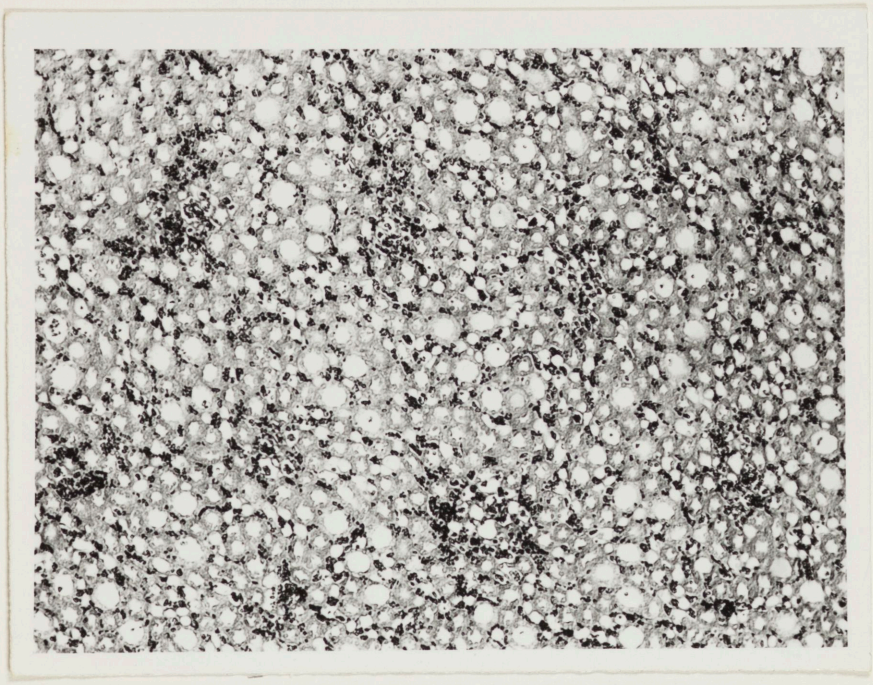


Figure 34. Cross-section of outer third of renal medulla in the rabbit, showing the distribution of vasa recta bundles Stain - Masson's trichrome. X75.

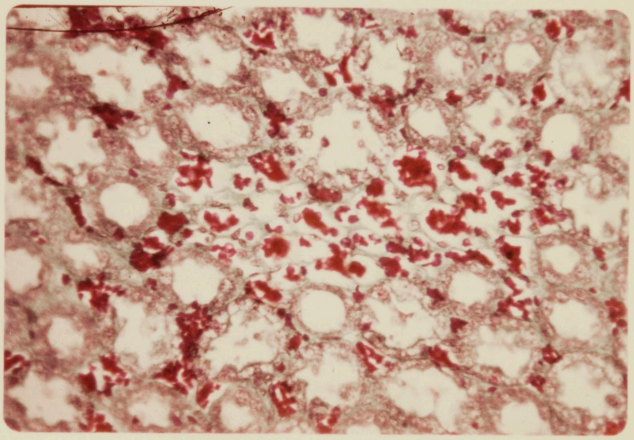


Figure 35. High power view of the section shown in fig.34. Vasa recta bundle in cross-section.
Stain - Masson's trichrome. X300.

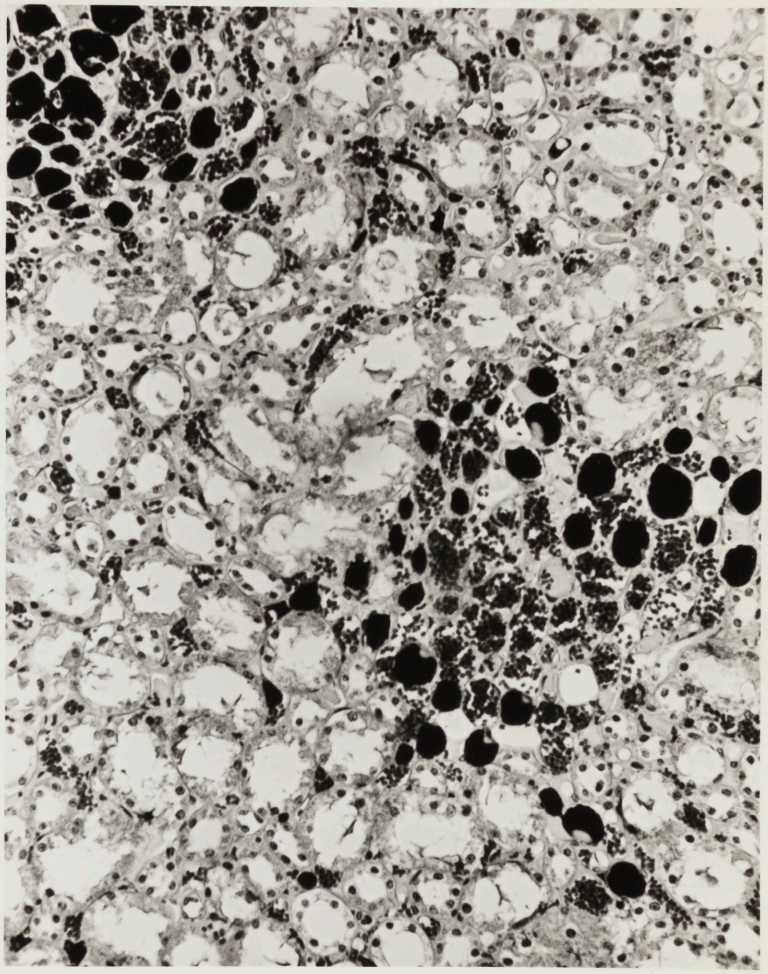


Figure 36. Cross-section of the outer third of the renal medulla in the sheep, showing one vasa recta bundle and portion of another. Carbon black was injected intra-arterially. Stain - Masson's trichrome. X120.

escapes extraction of its PAH content. On morphological grounds this is most unlikely, as the vessels at the periphery of bundles are juxtaposed to tubules (see Figure 35). However several lines of evidence indicate that the haematocrit of blood perfusing the renal medulla is lower than that perfusing the cortex (57, 58, 111, 136), possibly due to a plasma-skimming effect in the interlobular arteries and afferent arterioles (136). Thus perhaps 7-8% of the total renal plasma flow would pass through the vasa recta bundles, and if limitation of PAH diffusion were the whole explanation of E_{PAH} averaging 95% instead of 100%, then the extraction of PAH from the vasa recta bundles would approximate 40%.

In summary, a very high level of renal PAH extraction was found in the unanaesthetized rabbit, which was little affected by small variations in arterial PAH concentration in the range of 1-3 mg % or by the presence of mannitol in the sustaining infusion. The level of extraction was less than 100%; it is concluded that a portion of the renal plasma flow may pass through vasa recta bundles and escape complete extraction, while some supplies only the inert tissue of the renal capsule, calyces and pelvis.

Tm_{PAH}

In 10 normal rabbits, Tm_{PAH} averaged 19.1 mg/min (Table 11). The values obtained ranged from 12.4-22.5 mg/min (Appendix 3), and were derived from clearance measurements while the arterial PAH concentration was high, with the load/T ratio exceeding 1.25.

It has been mentioned earlier that in another series of animals in which the arterial PAH concentration was increased stepwise, renal PAH extraction ratio remained at a level of approximately 95% until the arterial concentration reached 25 mg %, when E_{PAH} was decreased, apparently due to

saturation of the tubular transport mechanism (Figure 33). From a knowledge of the arterial PAH concentration at which tubular saturation is attained, and using the average value for renal plasma flow in normal rabbits, a rough estimate of Tm_{PAH} can be made without recourse to clearance data. Thus $Tm_{PAH} = 0.87 \times 25 = 21.7$ mg/min. This estimate is within the range of values determined by the clearance method.

The reproducibility of Tm values using the clearance method was tested in one animal by carrying out 4 separate determinations on two consecutive days (Table 13). Due to the repeated blood sampling which was necessary, the haematocrit was reduced progressively with each determination. It is demonstrated later (Figure 50) that a reduction in haematocrit results in significant depression of Tm_{PAH}. Thus in this experiment the variation in Tm values may not have been as great if the fall in haematocrit had not occurred.

In table 14 the mean values obtained for Tm_{PAH} in the present study and in the rabbits investigated by Josephson et al (86), are compared with results obtained by other workers in the rat, the dog and man. While there is no important difference between the present findings and those of Josephson et al, the values reported from other species appear to indicate that they have less than 50% of the PAH excretory capacity of the rabbit when a comparison is made on the basis of body weight or kidney weight. However some of this apparent dissimilarity can be explained in terms of differences in body temperature between species. For instance, in man Tm_{PAH} has a temperature coefficient (Q_{10}) of about 2.0; i.e. a rise of 1.0°F in rectal temperature will increase Tm by 10% (165). Thus as the body temperature of the rabbit (approximately 103.5°F), Tm_{PAH} in man would

TABLE 13

REPRODUCIBILITY OF Tm VALUES IN ONE ANIMAL

	HAEMATOCRIT %	Tm_{PAH} (mg/min)	LOAD/T	E_{PAH} %	ARTERIAL PAH mg %
DAY 1	33.8	19.9	1.57	70.4	47.7
	32.8	20.5	1.55	71.1	57.4
DAY 2	25.3	18.8	2.86	44.4	60.4
	25.1	18.9	2.9	40.6	65.7

Mean 19.5

S.D. = $\pm$ 0.82 mg/min.

SE = 1.1% of mean.

TABLE 14

COMPARISON OF RENAL FUNCTION IN SEVERAL SPECIES

Species	Source	Per Kg. Body Weight			Per Gm. Kidney Weight		
		GFR	*RPF	Tm_{PAH}	GFR	*RPF	Tm_{PAH}
		ml/min	ml/min	mg/min	ml/min	ml/min	mg/min
Rabbit	Present series. Josephson et al, 1953 (86).	7.10	32.9	7.09 6.01	0.96	4.39	0.94
Rat	Cited by Smith, 1951 (164)	3.12	23.2	3.0	0.75	2.90	0.38
Dog		4.29	15.5	0.97	0.62	2.20	0.14
Man		1.97	12.5	1.28	0.46	2.56	0.30

* Values for RPF in quoted series were calculated from C_{PAH}/E_{PAH}, using a value for E_{PAH} of 0.87 for the dog and 0.91 for man (164); E_{PAH} for the rat was assumed to be 0.95.

rise to a value of (0.3 mg/min/g ÷ 50/100) = 0.45 mg/min per gram kidney weight. This correction makes the differences in Tm_{PAH}/KW between the rabbit and man comparable to the differences in glomerular filtration rate and renal plasma flow between these 2 species (Table 14).

Renal venous O_2 saturation, renal venous O_2 tension and renal O_2 consumption.

Mean values and standard deviations for measurements in 10 normal rabbits are shown in table 15. The O_2 saturation of renal venous blood averaged 74 ± 1.8 (SE) %, which is higher than the O_2 saturation of venous blood from most other organs (50-70% (136)), and significantly greater than the average value obtained for mixed venous O_2 saturation in unanaesthetized rabbits (61 ± 1.1 (SE) % (99); 58 ± 1.1 (SE) % (97)). However it was somewhat lower than values obtained for renal venous O_2 saturation by other workers (49, 97, 181). Renal venous O_2 tension was derived from the saturation data using the O_2 dissociation curve for rabbit's blood at 40°C (Figure 27), and averaged 46 mm Hg.

The average value shown for renal O_2 consumption per gram kidney weight (Table 15), is about 50% higher than obtained by Dole et al (49) in the dog and Bucht et al (25) in man. Since the rabbit's body temperature is higher than in the dog and in man, this finding is not unexpected. However renal O_2 consumption was also apparently higher in animals in the present study then it was during similar conditions of diuresis, etc. in rabbits used by Korner (97). The reason for this difference is not clear.

SUMMARY

Values obtained for various measurements of renal function in the

TABLE 15

Mean values and standard deviations for renal venous O_2 saturation ($S_{RV_{O_2}}$), renal venous O_2 tension ($P_{RV_{O_2}}$) and renal O_2 consumption ($\dot{V}_{R_{O_2}}$) in 10 animals with chronic renal vein catheters.

Observation	Mean	SD	n
$S_{RV_{O_2}}$ (%)	74	5.8	(10)
$P_{RV_{O_2}}$ (mm Hg)	46	-	(10)
$\dot{V}_{R_{O_2}}$ (ml/min STPD)	4.15	1.22	(10)
$\dot{V}_{R_{O_2}}$/KW (ml/min/g. STPD)	0.21	0.06	(9)

resting unanaesthetized rabbit are presented. Evidence is presented which indicates that the renal circulation was stable under the conditions of diuresis, etc. needed for measuring renal clearances, and renal tubular function was not depressed; the reproducibility of estimates of E_{PAH} and Tm_{PAH} appeared high. Renal blood flow was significantly related to body weight and to kidney weight. Both RBF and GFR were decreased after catheterization of the ureters. Reasons for the differences found between results in this study and other studies of the renal circulation in the rabbit are discussed; it is concluded that some of these differences may arise because of the presence of renal vasoconstriction in animals which are handled or forcibly restrained. Comparison is also made with results in other species, and the effects of higher body temperature in the rabbit examined. The level of renal PAH extraction in the rabbit and its possible significance in relation to intrarenal blood flow distribution, is discussed in detail.

CHAPTER 4.

THE RENAL CIRCULATION IN ACUTE NORMOVOLAEMIC ANAEMIA.

In this initial study, acute anaemia was produced by a method that was designed to minimize changes in total blood volume. As each animal was bled, plasma was infused at a rate approximately equal to the rate of blood loss. The endogenous replacement of plasma after haemorrhage is rapid in the rabbit (43); thus in experiments in which the rate of bleeding was slow, allowance was made for this factor by keeping the amount of plasma replaced slightly lower than the amount of blood lost.

A similar technique for producing acute anaemia was used by Share (160), Kinter and Pappenheimer (94,95) and Thompson et al (173) in studies of the renal circulation in anaesthetized animals. While these studies were not strictly comparable in regard to species, degree of replacement of blood by plasma, etc., there is a surprising dissimilarity between some of their findings. Thus Kinter et al showed that renal blood flow increased more than twofold when the arterial haematocrit was reduced from 42% to about 7% with arterial pressure maintained constant, whereas Thompson et al found no significant change in RBF after a similar reduction in haematocrit; arterial pressure declined slightly during the latter experiments, but this difference could account for little of the apparent disparity between the two series. In both series a fall in renal PAH extraction ratio occurred with reduction in haematocrit ratio, but this effect was greater in the experiments of Kinter et al.

In the present experiments, 2 groups of animals were investigated. Measurements in the first group were made at their normal haematocrit

values and then repeated when the haematocrit had been reduced to approximately 20%. The second group were rendered chronically anaemic by preliminary bleeding over several days; measurements in these animals were made initially at a haematocrit of approximately 20%, and again after the bleeding procedure outlined above when the haematocrit was about 10%.

RESULTS.

Changes in the Renal Circulation in Acute Normovolaemic Anaemia.

(Data from individual experiments is listed in appendix 4).

Haemodynamic effects.

Figure 37 shows results obtained in 4 animals from Group I and 17 animals from Group II, studied in each case at 2 levels of haematocrit (at 3 levels in 2 animals). There was no significant change in RBF as a result of anaemia, but there was significant reduction in GFR ($p = 0.02$) and filtration fraction ($p = 0.001$) with the more severe grades of anaemia (Group II). Other changes noted were a significant fall in arterial pressure in Group II animals, and a slight but significant tachycardia (mean 109.3± 2.7 (SE) % of control value) in this group.

There was a reduction in resistance to blood flow in severe anaemia (i.e. maintenance of RBF despite a fall in arterial pressure). This may have been in part due to the reduction in blood viscosity. The fall in filtration fraction suggests that changes may also have occurred in the ratio of pre - to post-glomerular resistance.

Renal venous O_2 saturation and renal O_2 consumption.

Figure 38 shows that there was no significant reduction in renal venous O_2 saturation and renal O_2 consumption in anaemia. These findings

Figure 37. Haematocrit ratio in relation to renal blood flow (RBF) glomerular filtration rate (GFR), ear artery blood pressure (BP) and filtration fraction. Black circles - determinations in Group 1 animals before and after bleeding with plasma replacement. Open circles - determinations in Group 11 animals before and after bleeding with plasma replacement. Results from one animal are joined by a straight line.

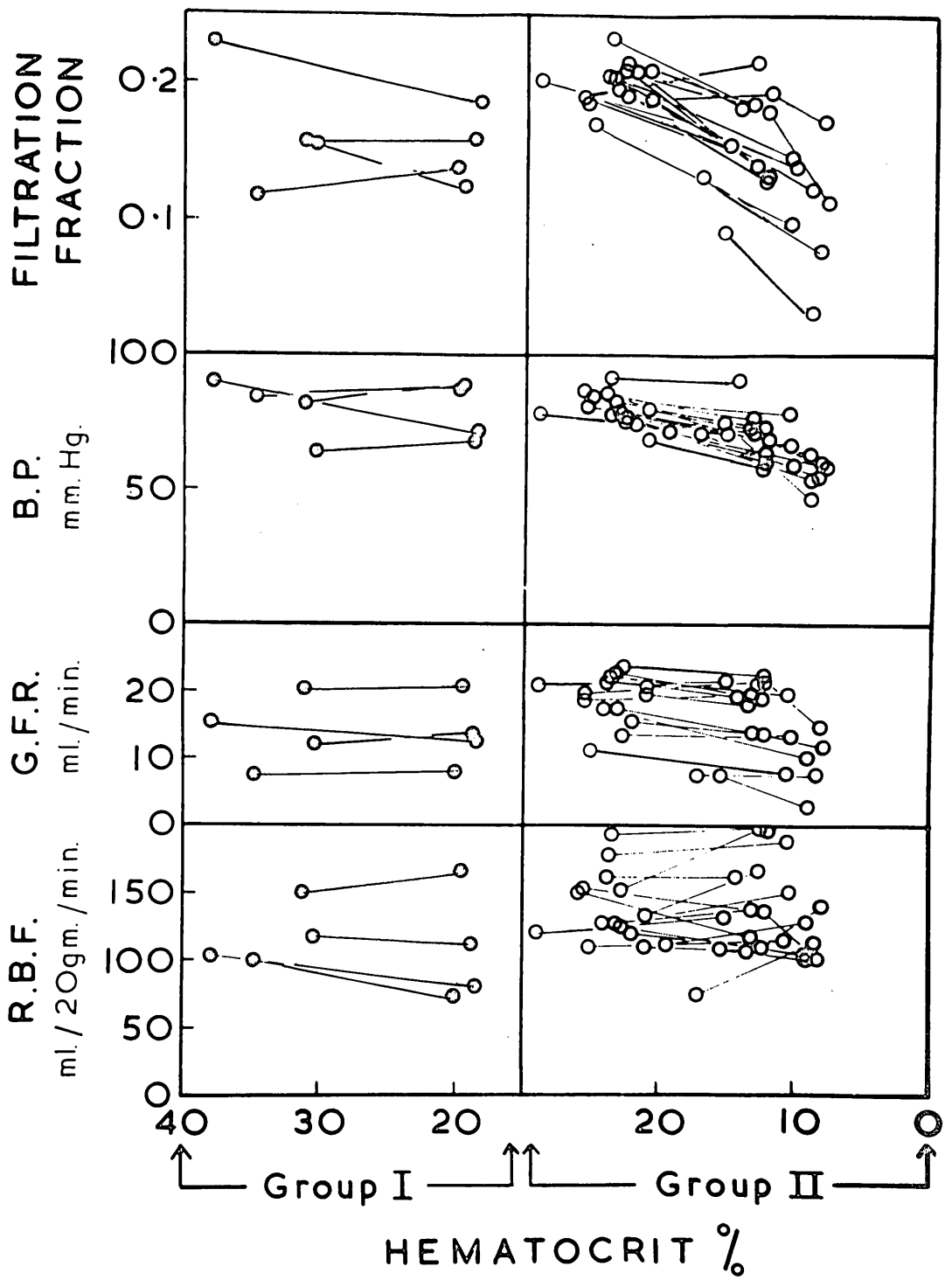


Figure 37. Legend on opposite page.

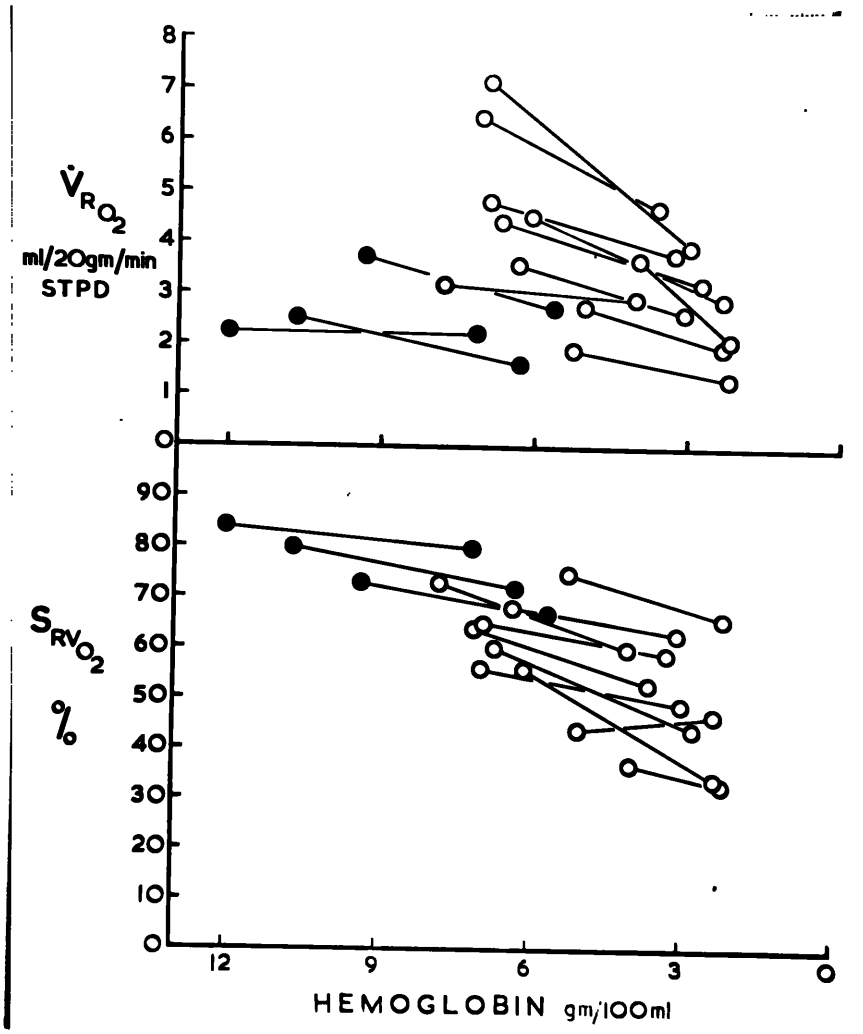


Figure 38. Haemoglobin in relation to renal vein blood oxygen saturation ($S_{RV_{O_2}}$) and renal oxygen consumption ($\dot{V}_{R_{O_2}}$) Black circles - determinations in Group 1 animals. Open circles - Group 11 animals. Results from one animal are joined by a straight line.

suggest an increasing degree of tissue hypoxia with increasing severity of anaemia.

Renal PAH extraction ratio.

Figure 39 illustrates the relationship of renal PAH extraction ratio to the haematocrit in 85 tests obtained from 56 animals. In tests carried out with the haematocrit above 30%, the renal PAH extraction ratios averaged 96.2$\pm$ 0.4 (SE)%. There was little change in extraction ratio down to haematocrit values of about 20%, but below this there was progressive reduction in extraction ratio which reached an average of 84.3$\pm$ 2.3 (SE) % for haematocrit values below 10%.

In 26 animals the extraction ratio was determined at 2 levels of haematocrit in the same animal, thus permitting a better quantitative assessment of the effects of anaemia on renal PAH extraction. In 6 experiments on Group I animals the renal PAH extraction was 96.9% and 95.2% (0.1 p 0.2) when the haematocrit was reduced from 34.5% to 20.4%. In 20 animals from Group II there was a significant reduction in extraction ratio from 95.0% to 91.3% ($p = 0.001$) when the haematocrit was reduced from 22.5% to 11.5%. The results of these comparisons within animals are in agreement with those of the whole series (Fig.39), and it is evident that the effect of anaemia on renal PAH extraction was relatively slight except where the degree of anaemia was severe (i.e. haematocrit below 10%).

Evaluation of Some Factors Influencing the Renal Circulation in Anaemia.

An attempt has been made to assess the part played by renal tissue hypoxia, fall in red cell concentration, fall in arterial pressure and variation

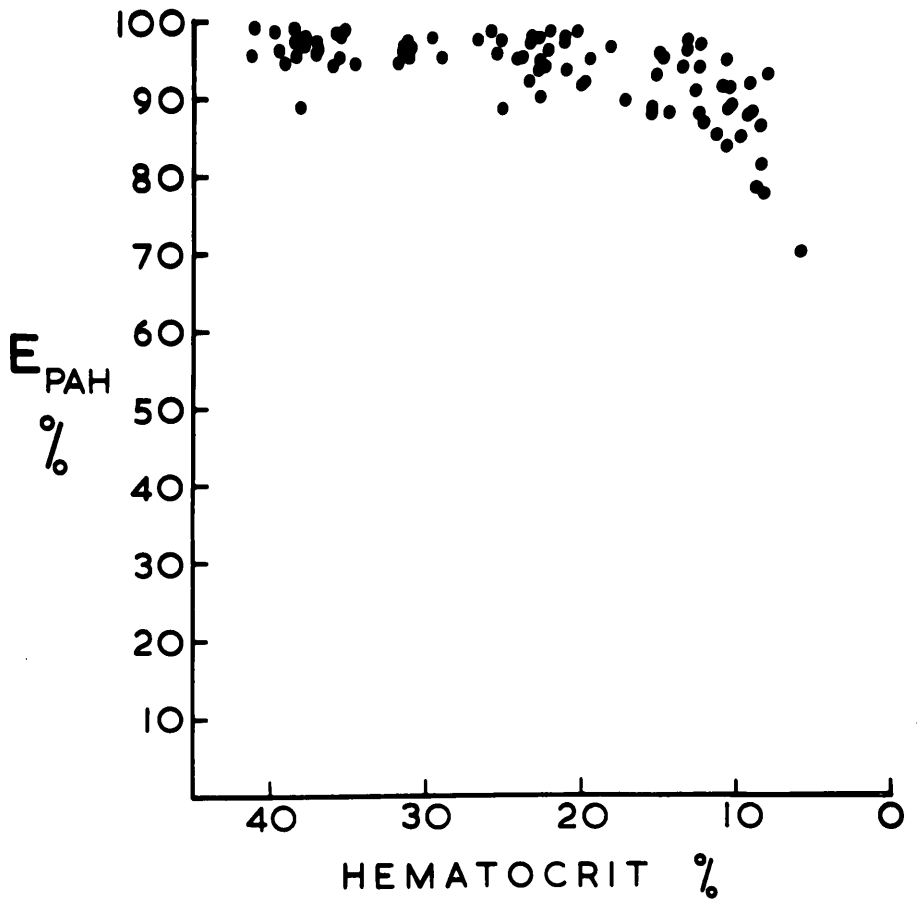


Figure 10. Renal [illegible] (E_{PAH}) [illegible]
[illegible]

in arterial PAH concentration on the various renal circulatory findings in anaemia in this series. In addition the effect of bleeding with plasma replacement on total blood volume was investigated.

Contributions of renal hypoxia and change in red cell concentration.

In anaemia there is a reduction both in the blood O_2 carrying capacity and in the number of circulating red cells, leading to a decrease in O_2 available to the tissues on the one hand, and a decreased blood viscosity and possibly an alteration in the distribution of red cells and plasma in certain vascular beds (136), on the other. The part played by these two factors was investigated (i) by administration of 100% O_2 to severely anaemic animals (ii) by administration of graded low concentrations of carbon monoxide to animals with a normal haematocrit.

Breathing 100% O_2 increases the amount of dissolved oxygen and thus the O_2 content of the arterial blood. At normal haematocrits the proportionate increase in oxygen available to the tissues is slight, but in severe anaemia with low haematocrit ratios this effect is greater, and presents a means of relieving tissue hypoxia with little or no change in haematocrit or blood viscosity.

Conversely, breathing low concentrations of carbon monoxide affords a method of studying increasing degrees of tissue hypoxia in the presence of a normal haematocrit and normal blood viscosity. Carboxyhaemoglobin is formed and there is consequently a reduction in the haemoglobin available for O_2 transport without change in arterial O_2 pressure.

Effects of breathing 100% O_2. Measurements of RBF etc., were obtained in 10 animals with the haematocrit approximately 20%, whilst the animals were breathing room air (column A, Table 16). The haematocrit was reduced to

TABLE 16.

Mean values obtained in 10 animals from Group II: (A) during moderate chronic anaemia while breathing room air (21% O_2); (B) after bleeding with plasma replacement while breathing room air; (C) after bleeding with plasma replacement while breathing 100% O_2. The treatments were carried out consecutively in each animal and the standard errors shown are based on comparisons within animals.

Measurement	(A) Breathing 21% O_2	(B) After bleeding -breathing 21% O_2	(C) After bleed- -breathing 100% O_2	SE of difference (B - C)
Haematocrit %	20.7	10.2	9.6	
E_{PAH} %	93.2	88.3^{++}	89.6^{+++}	± 0.7
Blood pressure (mmHg)	76.0	57.2^{++}	61.3^{++}	± 1.5
Heart rate per min.	286	313^{++}	305	± 7.7
RBF (ml/20gm/min)$^{+}$	135	148	136	± 8.8
Renal vascular resistance^{+} (mmHg/ml/sec)	45.3	29.3^{++}	34.5^{+++}	± 2.5
GFR (ml/min)$^{+}$	15.7	12.4^{++}	13.1	± 0.4
RPF (ml/min)$^{+}$	86.5	120^{++}	112	± 7.8
Filtration fraction^{+}	0.177	0.108^{++}	0.130^{++}	± 0.007
Renal venous S_{O_2} %	53.8	43.3^{++}	76.3^{++}	± 3.15
Renal $\dot{V}_{O_2}$ (ml/min STPD)$^{+}$	3.93	2.56^{++}	3.11^{++}	± 0.14

+ Based on 6 measurements (within animals)

++ Treatment effect (A - B or B - C) significant ($p < 0.05$)

+++ $0.05 < p \leq 0.1$

approximately 10% by the method of bleeding with plasma replacement and this resulted in the usual significant reduction in arterial pressure, GFR, filtration fraction, renal vascular resistance, renal PAH extraction ratio, renal venous O_2 saturation and renal O_2 consumption (column B, Table 16).

In these animals the O_2 capacity of the arterial blood averaged 3.8 ml/100 ml of blood with the haematocrit ratio 10%. Breathing 100% O_2 increased the amount of O_2 in physical solution in the arterial blood by approximately 2 vol/100 ml of blood, and thus increased the amount of O_2 available to the tissues by approximately 50% in these severely anaemic animals. The effects observed are shown in column C of table 16. In these animals the haematocrit ratio was 10.2% while breathing 21% O_2, and 9.6% while breathing 100% O_2. There was thus a negligible change of blood viscosity in these two treatment periods.

Breathing 100% O_2 produced no significant changes in RBF and GFR. The filtration fraction increased significantly, and the renal vascular resistance increased in 5 out of 6 animals; the overall effect being small but probably significant. These results observed in the presence of constant blood viscosity suggest that part of the reduction in filtration fraction and renal vascular resistance was due to hypoxic post-glomerular vasodilatation.

There was a large increase in the renal venous O_2 saturation and the value reached was greater than before bleeding. There was a significant increase in renal O_2 consumption, though this did not return to its initial value (cf column A and C, Table 16).

The renal PAH extraction ratio increased slightly in 7 animals, remained unchanged in one, and decreased in 2 animals. The mean effect was small and not statistically significant ($0.05>p>0.1$).

In summary, there was an increase in renal venous O_2 saturation and renal oxygen consumption suggesting relief of renal hypoxia. The main haemodynamic effect in the severely anaemic animal of breathing 100% O_2 was an increase in filtration fraction.

Effects of breathing carbon monoxide Fig. 40 shows the changes produced in the renal circulation in rabbits breathing 0.1% - 0.3% carbon monoxide in air. The data comprises results from an earlier series (98), and in addition results from 11 animals breathing 0.2% or 0.3% carbon monoxide in the author's series (see appendix 4). Despite minor technical differences in the procedures employed, the response in the two series was similar and the results have accordingly been pooled.

There were significant reductions in RBF, GFR, and arterial pressure in animals breathing carbon monoxide (Fig 40). Reference to Figure 37 shows that the haemodynamic effects of breathing carbon monoxide differed somewhat from these produced by severe anaemia. In animals breathing carbon monoxide, RBF and GFR were reduced in proportion to the blood pressure and there was little or no change in filtration fraction. In severe anaemia, the fall in arterial pressure was approximately similar to that observed with carbon monoxide, and the GFR was also reduced. However in anaemia there was no reduction in RBF and a marked fall in filtration fraction.

Table 17 shows the effects observed with carbon monoxide on renal venous O_2 saturation, renal O_2 consumption and renal PAH extraction ratio

Figure 40. Arterial oxyhaemoglobin/total haemoglobin % (i.e. Art O_2 Hb/Art O_2 Hb + CO Hb + Hb%) in relation to renal blood flow (RBF), glomerular filtration rate (GFR), ear artery blood pressure (BP) and filtration fraction (FF) in animals breathing 21% O_2 during treatment period (open circles), in animals breathing 0.1% and 0.2% CO in the previous series (half-black circles) and in animals breathing 0.2% and 0.3% CO in the present series (black circles). Changes in above measurements are expressed as percentage of the animal's own control value.

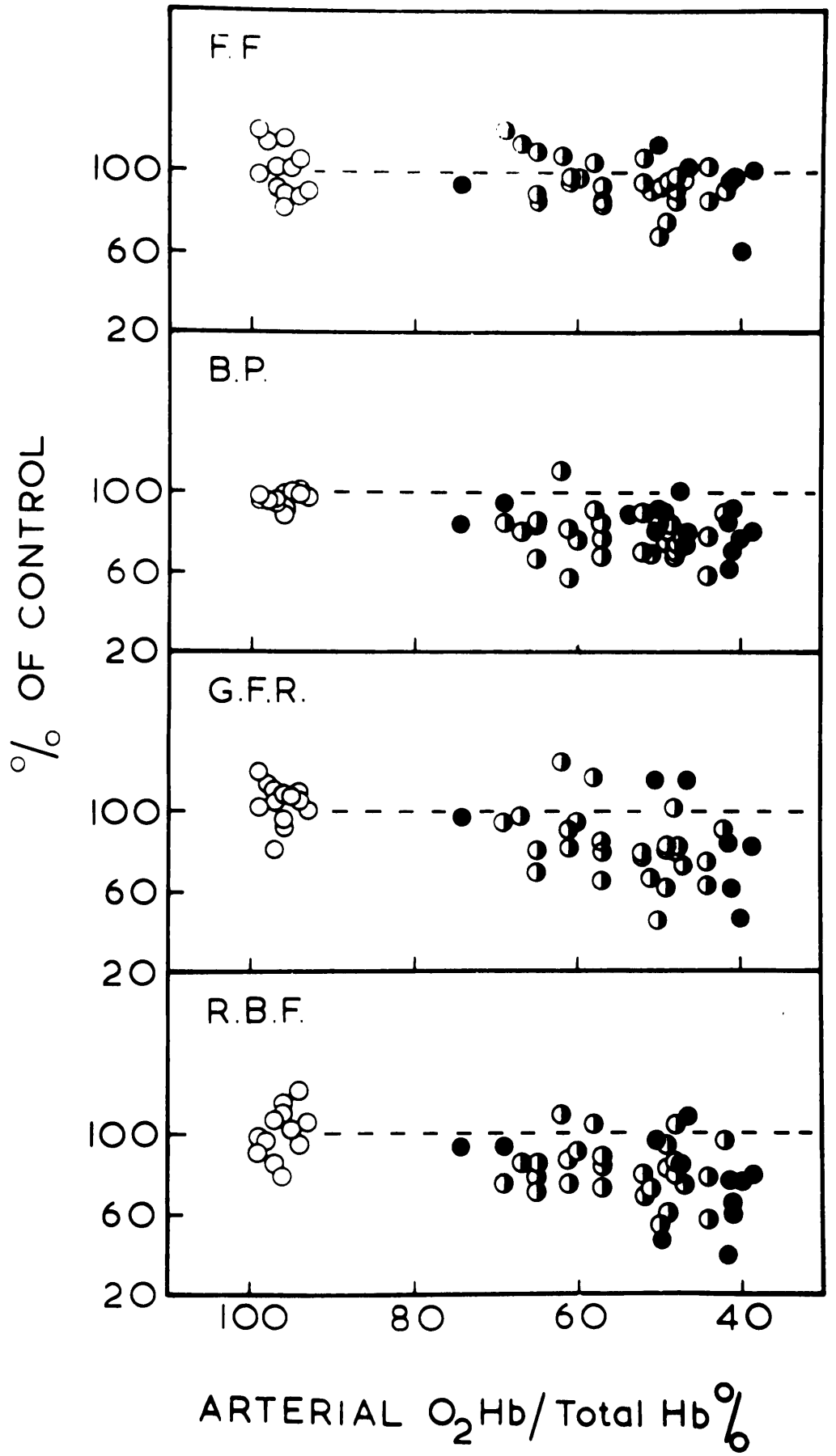

F.F
B.P.
G.F.R.
R.B.F.
100
60
20
100
80
60
40
% OF CONTROL
ARTERIAL O_2Hb/ Total Hb%

TABLE 17

Results for renal PAH extraction ratio, arterial oxygen saturation, renal venous oxygen saturation and renal oxygen consumption in 16 animals. Each group breathed room air during the control period (C), but breathed 0.2% CO in air and or 0.3% CO in air during the treatment period (T).

Treatment	0.2% CO+			0.3% CO		
No. of animals	12			7		
	C	T	SE'	C	T	SE'
$E_{PAH\%}$	95.3	93.2*	± 0.39	94.8	88.4*	± 0.98
++Arterial O_2 Hb/Total Hb-%	96.0**	56.1*	± 3.51	96.0**	45.0*	± 0.52
++Renal venous S_{O_2} - %	75.1	36.0*	± 4.73	71.7	26.5*	± 2.48
+++Renal $\dot{V}_{O_2}$ (ml/min STPD)	3.32	2.92	± 0.31	4.56	2.25*	± 0.26

' Standard error based on comparisons within animals, each animal acting on its own control.

\+ 6 animals from this group taken from previous series (98)

++ Data from 10/12 animals in 0.2% CO group and 6/7 animals in 0.3% CO group.

+++ Data from 7/12 animals in 0.2% CO group and 5/7 animals in 0.3% CO group.

* Treatment effect significant ($p<0.05$).

** Mean value from 4 animals only.

in animals breathing 0.2% and 0.3% carbon monoxide. There was a significant reduction in renal O_2 saturation with both mixtures, and in renal O_2 consumption with 0.3% carbon monoxide. Though the renal venous O_2 pressure was not measured directly, it may be estimated approximately from the saturation data. Using the rabbit's oxyhaemoglobin dissociation curve (see figure 27), and assuming a pCO_2 of 40 mm Hg (150), and a body temperature of 40°C, the estimated renal venous pO_2 averaged 23 $\pm$ 2.3 (SE) mm Hg in the experiments with 0.2% carbon monoxide and 17 $\pm$ 1.5 (SE) mm Hg in the experiments with 0.3% carbon monoxide. In severe anaemia (Group II), the estimated renal venous pO_2 (corresponding to a haematocrit of 10.8%) was 33 $\pm$ 1.1 (SE) mm Hg. Thus in animals breathing 0.2% and 0.3% carbon monoxide, there was probably a greater degree of renal hypoxia present than in the most severe degree of anaemia observed in the present study.

Despite these differences in the estimated degree of renal hypoxia, there was a smaller effect on the renal PAH extraction ratio in the carbon monoxide experiments than in severe anaemia (Table 17). With 0.2% carbon monoxide the mean reduction in renal PAH extraction ratio was only 2%, and only in animals breathing 0.3% carbon monoxide was a change in PAH extraction ratio observed comparable to that found in severe anaemia. This point was investigated in more detail in 4 animals, where the responses to breathing 0.3% carbon monoxide and the responses to severe anaemia were compared in the same animal. Following the initial set of observations with 0.3% carbon monoxide in air the animals were allowed to recover and were then bled over a period of 2-3 days to make them moderately anaemic. Severe anaemia was then produced by the usual technique of bleeding and plasma replacement. The results in figure 41

demonstrate that in the carbon monoxide experiments the estimated renal venous O_2 pressure was lower than in severe anaemia, but there was an approximately similar reduction in extraction ratio.

Effect of bleeding with plasma replacement on blood volume.

Figure 42 shows that the technique of bleeding with plasma replacement used to produce anaemia, caused minimal changes in total blood volume in 4 out of 5 animals where the blood volume was measured; in one animal there was an increase in blood volume of 26%. It seems likely that the technique of bleeding with plasma replacement minimized changes in total blood volume.

Effect of reduction of arterial pressure.

Two animals with normal haematocrit ratios were given the ganglion blocking drug "Arfonad" by constant intravenous infusion. These experiments were carried out to examine the possibility that the fall in arterial pressure observed in anaemia might contribute to the reduction in renal PAH extraction ratio. The arterial pressure was lowered from 80 to 64 mm Hg in one animal, and from 70 to 50 mm Hg in the second animal. Details of other measurements are listed in the appendix. In neither animal was there any significant change in RBF, GFR, renal vein O_2 saturation or renal PAH extraction ratio. It is thus unlikely that reduction in arterial pressure alone accounts for any of the findings in anaemia.

Effect of changes in arterial PAH concentration on renal PAH extraction ratio.

In all the above experiments the arterial plasma PAH concentration varied from 1-3 mg/100 ml. In further experiments the relationship of

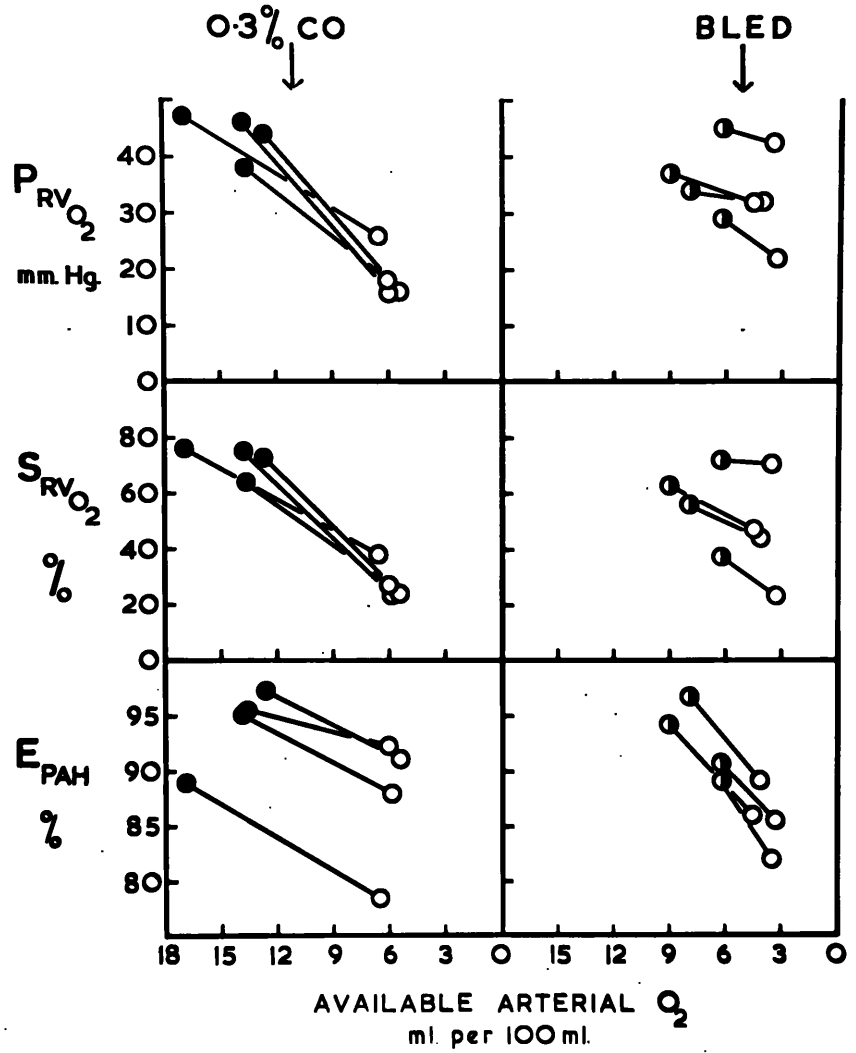


Figure 41. Comparison of the effects of 0.3% CO inhalation and acute anaemia studied consecutively in each of 4 animals. Available arterial oxygen is shown in relation to renal PAH extraction ratio (E_{PAH}), renal venous oxygen saturation (S_{RVO_2}) and estimated renal venous oxygen tension (P_{RVO_2}). Left half of figures - Black circles - breathing room air; open circles - breathing 0.3% CO. Right half of figure:- Half-black circles - moderate anaemia (produced by Group 11 bleeding procedure); open circles - acute severe anaemia following bleeding with plasma replacement.

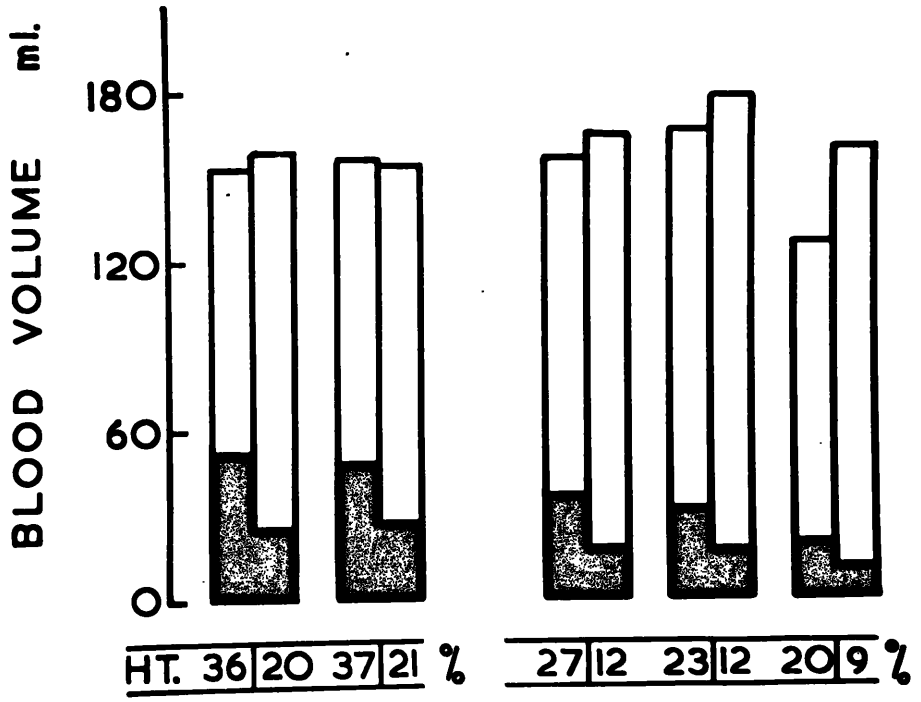


Figure 42. Blood volume estimations in 2 animals of Group 1, and 3 of Group II before bleeding (left side of each column), and after bleeding with plasma replacement (right side of each column). The haematocrit ratio before and after bleeding is shown beneath each column.

Black area - Cr^{51} red blood cell volume.
White area - T-1824 plasma volume.

of renal PAH extraction ratio to haematocrit was examined over a wider range of arterial PAH concentrations. This was done in order to investigate the possibility that the fall in extraction ratio observed in anaemia might be due to a minor change in arterial PAH concentration between the taking of control measurements and measurements in anaemia. In addition the experiments were designed to test whether reducing the renal tubular PAH load might diminish the effect of severe anameia on the PAH extraction of the kidney.

In 3 animals the plasma PAH concentration was increased stepwise, and simultaneous arterial and renal vein specimens were taken when the PAH level had become stable at each infusion rate. The results for renal PAH extraction ratios at three different haematocrit values in each animal (i.e. approximately 35%, 20%, and 10%) are shown in figure 43. At haematocrit values of about 20%, the renal PAH extraction ratio differed from normal only at higher arterial PAH concentrations, but at low haematocrit values (mean 9.8%) it was reduced at all arterial PAH levels in all 3 animals. At any level of haematocrit the extraction ratio was only slightly affected by variation of arterial PAH concentration in the range 1-3 mg/100 ml.

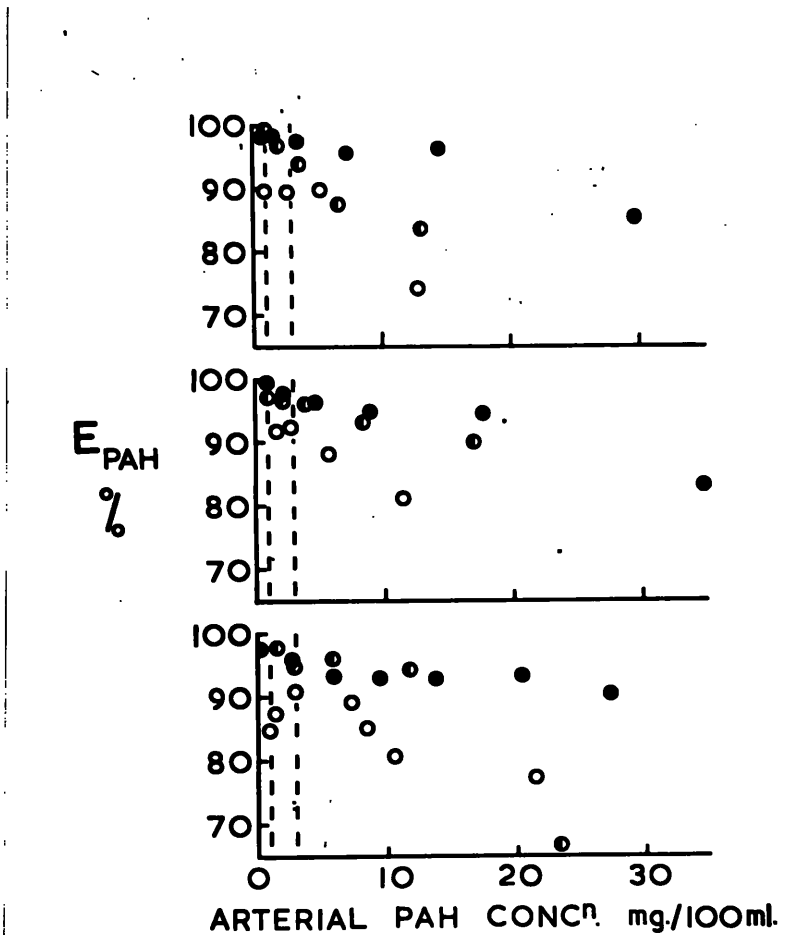


Figure 43. Renal PAH extraction ratio (E_{PAH}) in relation to arterial PAH concentration in 3 animals (one to each panel). Black circles - normal haematocrit ratios. Half-black circles - moderate anaemia (haematocrit ratios 20, 17 and 24 respectively). Open circles - more severe acute anaemia (haematocrit ratios 9, 10 and 10) produced by bleeding with plasma replacement. The two broken straight lines mark the limits of the range of arterial PAH concentrations used in all other experiments in the series.

DISCUSSION.

In the renal circulation in anaemia there was no change in RBF, and a decrease in GFR, filtration fraction, renal PAH extraction ratio, renal venous O_2 saturation and renal O_2 consumption.

The changes observed in anaemia differed from those reported previously in unanaesthetized rabbits with normal haematocrits in other types of hypoxia (98). In contrast to the fall in filtration fraction in anaemia there was no change in filtration fraction with carbon monoxide. This may represent differences in the effects of these treatments on the pre- and post-glomerular circulation. It is unlikely that the different effects observed in the carbon monoxide experiments and in anaemia depend on differences in renal pCO_2, since the arterial pCO_2 is essentially normal in anaemia (150), and is only slightly altered when breathing carbon monoxide in man (7) and in the rabbit (unpublished observations of Dr. P.I. Korner). The arterial pO_2 is normal both in anaemia and with carbon monoxide (7), and the O_2 carrying capacity of the blood is reduced in both types of experiment. The differences in haemodynamic findings in anaemia and in the carbon monoxide experiments thus probably depend on the differences in the red cell concentrations in the two types of experiment.

The contribution of changes in blood viscosity to the decreased renal vascular resistance in anaemia is difficult to assess quantitatively in the absence of general agreement regarding the distribution of intra-renal blood flow in anaemia. Whittaker and Winton (186) have shown that the relative blood viscosity in the isolated perfused hindlimb is reduced by less than 10% with a fall of haematocrit from 20% to 10% (the range of Group II animals). This would account only for a part of the observed fall in renal

vascular resistance.

The experiments with 100% O_2 indicate that tissue hypoxia also plays a part in some of the circulatory findings in anaemia. Thus when animals with severe anaemia breathed 100% O_2, the renal venous O_2 saturation increased above its control level, and this was accompanied by a significant increase of filtration fraction and renal vascular resistance, although the latter measurements did not return to their control values. Since these effects were produced without alteration of blood viscosity, the results suggest that hypoxic post-glomerular vasodilatation accounts for part of the fall in the renal filtration fraction.

The renal O_2 consumption was reduced in anaemia. The changes observed in anaemia appeared to be greater for a given reduction in renal pO_2 than those observed in the rabbit when breathing low oxygen mixtures or low concentrations of carbon monoxide in air (98). The latter experiments suggested that renal O_2 consumption fell only in severe hypoxia (renal vein pO_2 20 - 25 mm Hg), or where the RBF fell by about 30%, in agreement with the behaviour reported for other species (cited in 136). The present experiments throw no light on the mechanism of this apparently greater reduction in renal O_2 consumption in anaemia. It is possible that reduction in O_2 consumption may have been associated with a decrease in GFR and a fall in sodium load available for reabsorption (101) or with redistribution of intra-renal blood flow (102B, 176) from areas of high oxygen consumption to areas of low oxygen consumption.

The observations concerning the renal PAH extraction ratio are of interest in relation to the findings of Kinter and Pappanheimer (94) and Thompson et al (173), obtained from anaesthetized cats and dogs. In both the latter series the initial control extraction ratios at normal haematocrit

values were much lower than in the present series. The present experiments demonstrate that there was no change in renal PAH extraction ratio in moderate anaemia (haematocrit 20%), whereas there was a small but definite reduction in PAH extraction ratio in severe anaemia (haematocrit 10%). In these experiments the fall in extraction ratio was approximately similar to that observed by Thompson et al, and was smaller than that observed by Kinter and Pappenheimer. At the lowest haematocrit values (3-10%) studied by Kinter and Pappenheimer, the PAH or diodrast extraction ratio was about 60% of the control value. In the present series the PAH extraction ratio was about 95% of the control value with a haematocrit of 11.5%, and was about 88% of the control value at haematocrits between 6-10%. Minor fluctuations in plasma PAH level did not contribute to the change in PAH extraction ratio in anaemia, and the effect occurred over a wide range of tubular loads of PAH.

The effect of breathing 100% O_2 on PAH extraction ratio was slight and inconclusive. However, comparison of the results in anaemia and in carbon monoxide experiments demonstrated that at any given level of renal hypoxia there was a smaller reduction in renal PAH extraction ration when the haematocrit was normal than when it was low. It was shown that only with very severe degrees of renal tissue hypoxia produced by breathing carbon monoxide, was there a marked reduction in renal PAH extraction ratio. At the levels of renal vein pO_2 observed in anaemia it seems that the reduction in red cell concentration was a factor in bringing about the reduction in renal PAH extraction ratio.

The present results are thus consistent with the possibility that in anaemia some plasma (and PAH) is diverted away from the tubules through

hypothetical vascular shunts in the renal cortex, as proposed by Kinter and Pappenheimer (94, 136). However in contrast to the findings of these workers, the present experiments indicate that any shunting of PAH away from the tubules is small even in the most severe grades of anaemia studied here and is negligible in moderate anaemia.

That portion of the reduction in PAH extraction ratio in severe anaemia which cannot be attributed to renal hypoxia, is also explicable in terms of a possible redistribution of blood flow between renal cortex and medulla. Kramer, Thurau and Deetjen (102B) have demonstrated that the flow of blood in medullary vessels may not be regulated by the same mechanisms as flow through the cortex. If partition of blood flow between cortex and medulla is altered in anaemia, due to extrinsic nervous or humoral factors (176) or due due to plasma-skimming within the kidney (136), the medullary vasa recta could receive a greater proportion of the total renal plasma flow than normally. Since in the present experiments total renal plasma flow was increased approximately in inverse proportion to the fall in haematocrit ratio, then a redistribution in severe anaemia favouring the medulla would also involve an increase in the absolute rate of plasma flow through the vasa recta bundles. The time available for PAH to diffuse from the axial vessels of these bundles would be decreased and thus more may have escaped excretion than at normal arterial haematocrit values.

A similar interpretation of decreased renal PAH extraction during conditions of increased total renal plasma flow has been advanced in studies in which the total plasma volume of human subjects or dogs was expanded by infusion of serum albumin (11, 29, 54, 122). It has also been shown that the depression in E_{PAH} can occur without a change in Tm_{PAH} (54, 122).

Kinter et al (94) and Reubi et al (144) report that reduction of E_{PAH} in anaemia is reversible by a red cell infusion. This finding is consistent with the possibility of redistribution of intrarenal blood flow in anaemia, but does not necessarily rule out the possibility that the direct effect of hypoxia on the renal tubular cells contributes to the depression of E_{PAH} in severe anaemia.

Redistribution of intrarenal blood flow may also have contributed to the marked decrease in filtration fraction which was observed in the more anaemic rabbits. The juxtamedullary glomeruli which supply the vasa recta of the medulla have efferent arterioles of much wider bore than do the glomeruli in the outer cortex (176), and it is probable that a greater proportion of the plasma with which they are perfused escapes filtration. Therefore if flow through the juxtamedullary glomeruli and vasa recta increased relative to the flow through the remainder of the kidney, the overall filtration fraction would be expected to decrease.

The present observations suggest that renal tissue hypoxia and reduction in red cell concentration contribute in varying degrees to the changes observed in the renal vascular bed and the PAH extraction ratio.

SUMMARY

In the unanaesthetized rabbit made acutely anaemia by bleeding with plasma replacement, there was no change in renal blood flow, a reduction in glomerular filtration rate, and a reduction in renal vascular resistance with a fall in the filtration fraction. There was evidence of renal tissue hypoxia and reduction in renal PAH extraction ratio. The extraction ratio was 97% at a haematocrit ratio of 34.5%, 91% at a haematocrit of 11.5%, and 84% with haematocrits between 6-10%. The effects of carboxyhaemoglobinaemia

at normal blood viscosity were compared with the effects of anaemia; a smaller reduction in renal PAH extraction ratio was found and there was no reduction in filtration fraction. The reduction in renal vascular resistance and filtration fraction in anaemia were partly reversed by breathing 100% O_2, viscosity changes again being minimized. It was concluded that renal tissue hypoxia and reduction in red cell concentration contribute in varying degrees to the changes in the renal vascular bed and the PAH extraction ratio.

CHAPTER 5

THE RENAL CIRCULATION IN ACUTE HYPOVOLAEMIC ANAEMIA.

It is well known that there is considerable reduction in renal blood flow in the hypovolaemic phase following haemorrhage. The maintenance of circulation through the kidneys is often said to be subordinate to the needs of the body as a whole (153), so that in haemorrhagic shock there is a renal "shut down" to enable the large fraction of the cardiac output normally perfusing the kidney to be shunted to other regions, such as the cerebral and coronary circulations. This concept is well supported by evidence in acute renal failure in man (130) and in prolonged hypotension in the anaesthetized dog (152). Also after moderate, recoverable degrees of blood loss, it has been demonstrated in numerous clearance studies (36, 74, 80, 107, 138) that there is a reduction in estimated renal blood flow which exceeds the fall in arterial pressure; from these findings renal vasoconstriction has been inferred. However Balint et al (8, 10) have questioned the validity of this inference on the grounds that oliguria after haemorrhage prevents accurate clearance measurements and leads to falsely low estimates of renal blood flow.

Using unanaesthetized rabbits with bilateral ureteric catheters and under conditions of moderate mannitol diuresis, it is initially shown that valid clearance measurements can be obtained during the hypovolaemic phase after haemorrhage, and that these measurements

indicate marked renal vasoconstriction. The nature of the renal vasoconstriction is then analysed in animals with one kidney chronically denervated and the other kidney intact.

RESULTS.

The results of this study fell into three sections:-

I Effects of acute hypovolaemic anaemia on the renal circulation.

II Demonstration of nervous and humoral effects on the circulations of the innervated and denervated kidney.

III Role of nervous and humoral factors in the renal vascular response to haemorrhage.

I Effects of Acute Hypovolaemic Anaemia on the Renal Circulation

The changes observed in 8 animals following a bleed of 15-20 ml/Kg are shown in figure 44. The renal vascular response during the first 60 minutes after haemorrhage was studied in 6 animals, including 4 animals with both ureters catheterized and 2 animals with bladder catheters (i.e. without preliminary anaesthesia on the day of the test). The responses in these two types of animal were similar and accordingly the results are pooled and considered together. There was a significant reduction from control levels ($p < 0.05$) in renal blood flow, GFR, arterial pressure, and a significant increase ($p < 0.001$) in filtration fraction and renal vascular resistance. Values calculated for renal vascular resistance averaged 141 ± 5 (SE) % of control.

Although the rate of urine flow decreased after haemorrhage, it averaged 0.4 ml/min or more during subsequent clearance periods (see figure 46). Thus in "ureter" animals the urine flow from each ureteric catheter during a typical 10 minute clearance period after

Figure 44. Time before and after haemorrhage (15-20 ml/Kg) in relation to mean values for renal blood flow (RBF), glomerular filtration rate (GFR), blood pressure (BP), filtration fraction (FF), haematocrit (HT), and total blood volume (TBV) in 2 animals with bladder catheters (squares), in 4 "ureter" animals observed for 60 minutes after bleeding (circles) and in 2 "ureter" animals observed for 150 minutes after bleeding (circles).
Black symbols - control values.
White symbols - values during hypovolaemic anaemia.
Half-black symbols - values obtained after infusion of packed red cells.

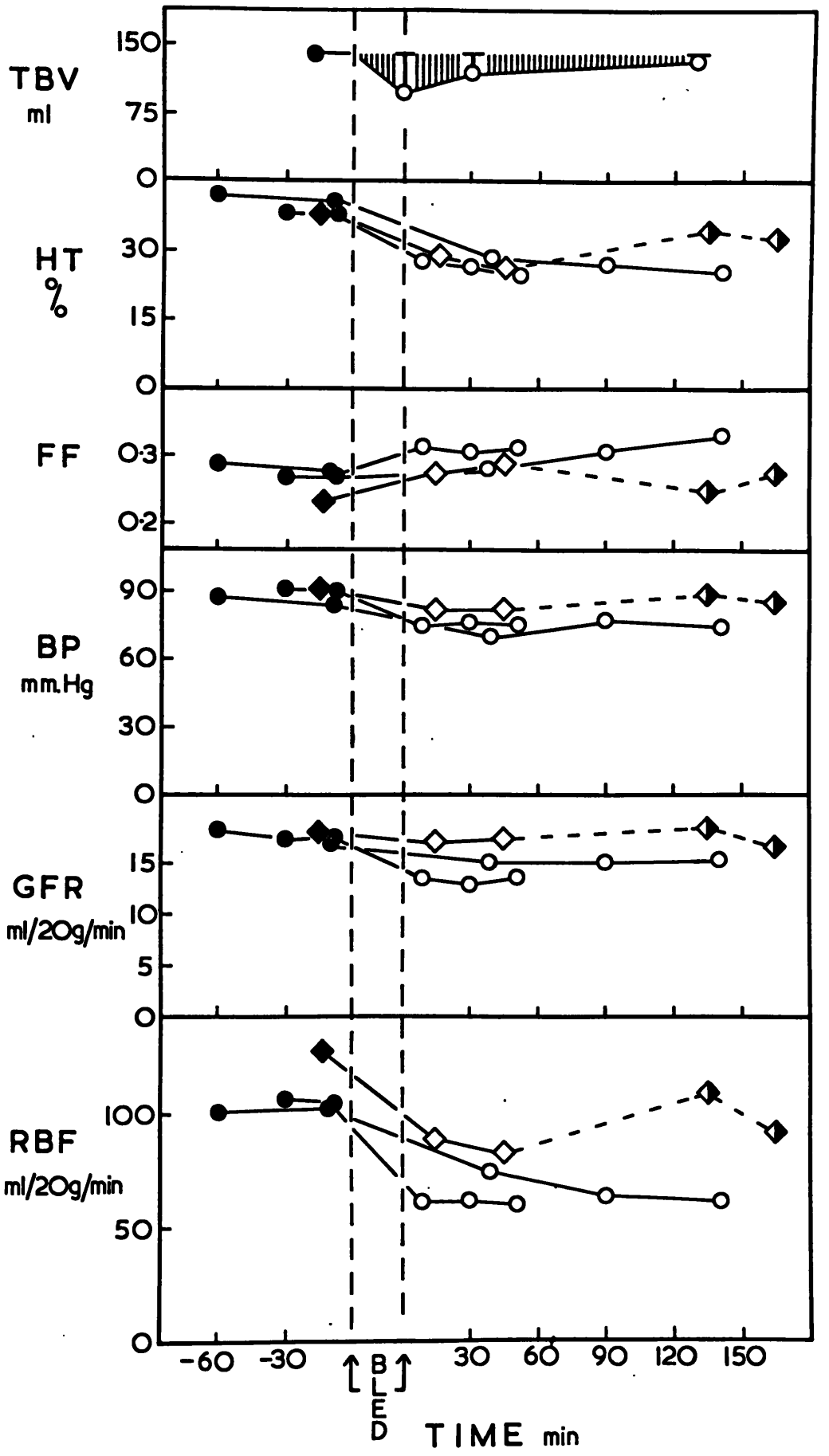

TBV
ml
150
75
0
HT
%
30
15
0
FF
0·3
0·2
BP
mm.Hg
90
60
30
0
GFR
ml/20g/min
15
10
5
0
RBF
ml/20g/min
100
50
0
-60
-30
BLED
30
60
90
120
150
TIME min

Figure 44.

bleeding was 2.0 ml or more; this is approximately 3-4 times as great as the dead space represented by the renal calyces, pelvis, upper ureter and catheter. Table 18 shows the values obtained for renal PAH extraction ratio before and after haemorrhage in the 2 animals with bladder catheters, and in two other animals in which clearance measurements were not carried out. No significant change in E_{PAH} was observed immediately after haemorrhage, or during the subsequent hour. The results for urine flow and renal PAH extraction indicate that there was no appreciable accumulation of PAH in the urinary passages or in the interstitial tissue of the kidney.

In 2 animals studied during the period between 30 and 150 minutes after bleeding (figure 44), the changes observed in the renal circulation were similar to those observed during the first 60 minutes. There did not appear to be any significant recovery in the renal blood flow towards normal values. In these 2 animals and one other (see appendix 5) blood volume changes were studied; average effects in the 2 animals under consideration are shown in figure 44. Restoration in blood volume was rapid, but was not complete after 150 minutes. The administration of the sustaining infusions probably exaggerated the rate of restoration of total blood volume to some extent (as is demonstrated in Chapter 6).

The results suggest the occurrence of prolonged renal vasoconstriction after haemorrhage. This was seen to be independent of the moderate degree of anaemia produced, in 2 animals in which packed red cells were reinfused after the first 60 minutes' observations. Despite a temporary reversion of arterial pressure,

TABLE 13

Effect of haemorrhage on renal PAH extraction ratio (E_{PAH}) in 4 animals which were bled 15 ml/kg (animals 1 and 2) or 20 ml/kg. (animals 3 and 4).

	Haematocrit %				E_{PAH} %			
Animal No:	1	2	3	4	1	2	3	4+
CONTROL OBSERVATIONS	38.2	38.1	38.0	36.6	94.4	92.3	95.9	91.0
					95.1	94.7	94.8	90.7
BLED for 15 min.								
	31.1	30.3	26.9	24.0	93.3	96.8	95.4	93.9
SERIAL OBSERVATIONS FOR 60 minutes AFTER HAEMORRHAGE						94.0		88.4
			26.8		95.4		94.7	
						93.9		88.2
	28.0	24.0			94.0	97.4	95.9	
			25.9			95.1		
					94.4		90.3	
						95.5		

+ Values in denervated kidney

renal blood flow, GFR and filtration fraction towards control values (see figure 44) the effects of the red cell infusion were not sustained, in agreement with the findings of other workers (36, 152).

To summarize, within 30 minutes after haemorrhage there was an increase in renal vascular resistance and filtration fraction, which suggested renal vasoconstriction affecting predominantly the post-glomerular vessels. In the subsequent 2 hour period, although the animals rapidly replaced their own blood volume towards normal, renal vasoconstriction appeared to continue and was only temporarily relieved by restoring red cell volume. The evidence was against significant accumulation of PAH in the kidney, and the use of the clearance method to measure renal blood flow in hypovolaemic anaemia appeared valid. There was no appreciable difference in the renal responses between animals with ureter catheters and animals with a bladder catheter only.

The remainder of this chapter is concerned with an analysis of the nature of renal vasoconstriction in acute hypovolaemic anaemia. As a number of afferent receptor zones are likely to be affected by haemorrhage, including the arterial baroreceptors and the atrial volume receptors (166), a complex pattern of efferent mechanisms, nervous and humoral, may be involved in the renal vascular response. In the next section, it is shown that "ureter" animals with one kidney denervated and the other kidney intact can serve as useful experimental models for testing for the presence of nervous or humoral effects on the renal circulation; in the last section of the

chapter an attempt is made to analyse the contributions of nervous and humoral factors to the control of the renal circulation during acute hypovolaemic anaemia.

II Demonstration of Nervous and Humoral Effects on the Circulations of the Innervated and Denervated Kidney.

In this section results are presented from 4 "ureter" animals, in 3 of which the left kidney was chronically denervated. The animals were infused with adrenaline and angiotensin and given low oxygen mixtures to breathe; comparison was made between the effects of each of these treatments on the innervated and denervated kidney. It has been demonstrated in several previous studies (13, 100, 103, 172) that the vessels of the denervated kidney are hypersensitive to catecholamines; thus in the present study the response of the denervated kidney to adrenaline would be expected to exceed that of the innervated kidney. Administration of low O_2 mixtures to rabbits with ureter catheters and one kidney denervated has been previously shown to cause a converse effect, the response being greater on the innervated side, due to chemoreceptor stimulation and increased renal vasomotor tone (98). In view of the different responses of the denervated and innervated renal vasculature to humoral and nervous constrictor stimuli, a comparison of the PAH and creatinine clearance ratios of denervated kidney to innervated kidney (D/I ratio) permits analysis of their separate effects. Taking as an example the clearance ratios for PAH, the following patterns of response are possible:-

(1) If there is an increase in D/I clearance ratio above control values as a result of a test procedure, this implies a greater degree of vasoconstriction in the innervated kidney than in the denervated kidney and suggests that nervous factors must be involved in the response, in addition to any humoral constrictor agents which may be present.

(2) A decrease in D/I ratio indicates a greater degree of vasoconstriction in the denervated kidney and suggests that its vessels are exhibiting a hypersensitive response to the test procedure. It may be inferred that an increased titre of adrenaline, or of other hormones with adrenaline-like effects on denervated vessels, has resulted from the test procedure; nervous influences may however be contributing to the renal vasoconstriction.

(3) Unchanged D/I ratios indicate either the absence of nervous or humoral control, or suggest that these may exist together, each nullifying the effects of the other. Another alternative is that humoral factors not producing a hypersensitivity reaction in the denervated kidney are involved in the renal vascular response.

These interpretations could not be extended to D/I ratios for renal blood flow if denervation affected the PAH extraction of the operated kidney. However, in 2 animals it has been shown that the PAH extraction ratio did not change as a result of denervation (table 1). In 2 additional animals with renal vein catheters it was shown that E_{PAH} remained constant despite infusions of adrenaline (1.2 µg/Kg/min) or angiotensin (0.1 µg/Kg/min), in both the innervated and the denervated kidney (Table 19). A similar result

TABLE 19

Effects of intravenous infusions of adrenaline and angiotensin on renal PAH extraction ratio (E_{PAH}) in 2 animals, one of which had a chronic left renal denervation.

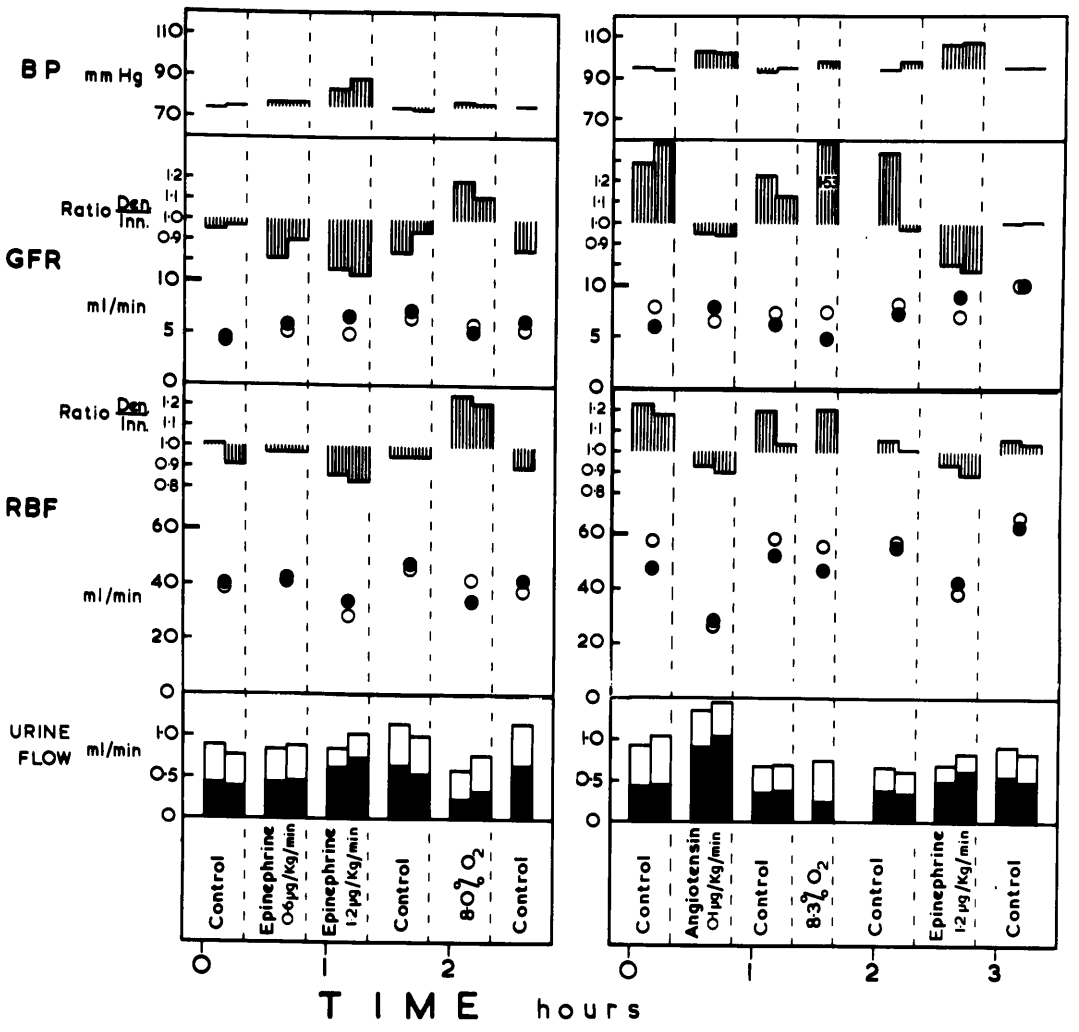
	HAEMOGLOBIN (gm/100ml)		E_{PAH} (%)	
Animal No:	1	2	1	2+
CONTROL	10.9	11.3	97.3 96.4	91.0 90.7
ADRENALINE 1.2 µg/Kg/min	10.4	10.5	96.8 98.0	91.6 91.9
CONTROL	10.3	10.5	97.8	90.6
ANGIOTENSIN 0.1 µg/Kg/min	10.1	9.7	99.1 97.6	92.4 -
CONTROL	9.8	-	97.9	-

+ Values in denervated kidney.

has been reported after moderate doses of adrenaline in men (183). In rabbits breathing low O_2 mixtures, no significant change occurred in E_{PAH} in the intact kidney (98). Thus it is probable that D/I clearance ratios for PAH provide an accurate impression of the D/I renal blood flow ratios during the various treatment procedures to be considered.

Figure 45 and table 20 illustrate the effects of nervous and humoral stimuli in the 4 animals tested. Figure 45 (left panel) shows the design of a typical experiment and the results obtained in one animal. Reduction of D/I ratios was produced by the administration of adrenaline. The effects of adrenaline 0.6 µg/Kg/min were minimal, but a decrease in D/I ratio for GFR suggested hypersensitivity of the afferent arteriole of the denervated kidney. At a dose of 1.2 µg/Kg/min there was a rise in arterial pressure and a reduction in renal blood flow; D/I PAH clearance ratios decreased, indicating hypersensitivity of the denervated kidney to adrenaline. After stopping the adrenaline infusion, arterial pressure fell to its previous level, renal blood flow reverted to a value slightly greater than control, and differences in RBF and GFR between the two kidneys became minimal. Acute hypoxia resulted in a reversible reduction of blood flow and GFR in the innervated kidney, but caused little change in the denervated kidney. The D/I ratios for RBF and GFR illustrated the antagonistic effects of adrenaline and hypoxia on the distribution of blood flow between the innervated and the denervated kidney.

Figure 45 (right panel) shows results obtained in an experiment in which the effects of angiotensin and adrenaline were contrasted


BP
mm Hg
110
90
70
GFR
Ratio Pen/Inn
1·2
1·1
1·0
0·9
ml/min
10
5
0
RBF
Ratio Pen/Inn
1·2
1·1
1·0
0·9
0·8
ml/min
60
40
20
0
URINE FLOW
ml/min
1·0
0·5
0
Control
Epinephrine 0·6µg/Kg/min
Epinephrine 1·2µg/Kg/min
Control
8·0% O2
Control
Control
Angiotensin 0·1µg/Kg/min
Control
8·3% O2
Control
Epinephrine 1·2µg/Kg/min
Control
0
1
2
3
TIME hours

within one animal. In this experiment, the changes produced by hypoxia were not as pronounced as in the other experiment in figure 45, possibly due to the higher level of renal vasomotor tone in the resting animal. Angiotensin 0.1 µg/Kg/min caused an increase in arterial pressure, a marked decrease in renal blood flow, and effects on D/I clearance ratios which were similar to those of adrenaline 1.2 µg/Kg/min.

Table 20 shows results obtained in two other animals tested with adrenaline, low O_2 mixtures and angiotensin. The first of these animals, which was not denervated, showed constant left/right ratios for RBF and GFR in the presence of variations in arterial pressure, total renal blood flow and total GFR. The second animal in table 20 was denervated, and showed responses similar to the animals presented in figure 45.

Total renal vascular resistance increased to values averaging 142$\pm$ 14.6 (SE) % of control after adrenaline 1.2 µg/Kg/min, and to values averaging 203$\pm$ 9.5 (SE) % of control after angiotensin 0.1 µg/Kg/min. These changes were of the same order of magnitude as the changes in renal resistance observed after haemorrhage. Adrenaline 1.2 µg/Kg/min resulted in a significant increase in filtration fraction in both kidneys of 4 animals; a similar effect was observed after angiotensin in 3 animals. The findings indicate that renal vasoconstriction was predominantly post-glomerular, in agreement with numerous other studies of these hormones (e.g. 46, 64, 141). In two out of three denervated animals, D/I ratio for filtration fraction decreased after adrenaline and after angiotensin,

TABLE 20

Values obtained in 2 animals with bilateral ureteric catheters, during constant-rate infusion of adrenaline and angiotensin, and whilst breathing 8.0% O_2. Treatments were administered consecutively with control measurements interspersed, the time scale being similar to that for the experiments shown in Fig. 45.

Animal 1: renal nerves intact
Animal 2: left renal denervation, right renal nerves intact

	Blood pressure mm Hg		Renal blood flow				Glomerular filtration rate			
			Total RBF ml/min		Ratio Left RBF/ Right RBF		Total GFR ml/min		Ratio Left GFR/ Right GFR	
Animal No:	1	2	1	2	1	2	1	2	1	2
	89	79	121	127	0.99	1.11	16.2	15.2	1.00	1.07
CONTROL	90	79			0.99	1.12			0.81	1.07
ADRENALINE	90	89	124	96	1.00	1.09	19.3	16.6	0.99	1.09
0.6 µg/Kg/min	93	89			0.99	1.06			0.99	1.01
ADRENALINE	100	99	122	89	0.99	1.01	21.7	16.2	0.99	1.07
1.2 µg/Kg/min	102	103			0.99	1.02			0.95	1.05
CONTROL	91	70	131	91	0.99	1.11	22.4	16.5	1.10	1.13
	91	68			0.99	1.13			0.98	1.24
HYPOXIA	94	81	109	81	1.01	1.17	18.2	15.7	1.00	1.22
(8.0% O_2)	95	80			0.98	1.38			0.97	1.28
CONTROL	94	70	118	73	0.96	1.05	19.7	14.9	0.91	1.12
	95	69			1.01	1.08			0.99	1.13
ANGIOTENSIN	107	92	74	47	0.97	1.00	18.2	12.8	0.95	0.98
0.1 µg/Kg/min.										

providing evidence that the primary site of hypersensitivity in the denervated kidney was the afferent arteriole, as reported by Koza et al (100), and as would be predicted from the fact that the renal vasoconstrictor nerves terminate near this site (124). In the remaining animal the findings were inconclusive in this regard as the D/I ratios for filtration fraction decreased after angiotensin but increased slightly after adrenaline.

It is concluded that the "ureter" animal with one kidney denervated can serve as an experimental model to test for the presence of nervous effects and certain humoral effects on the renal circulation. In the following section, this model is used to analyse the renal vascular response to haemorrhage, and to assess the contribution of nervous and humoral factors to the control of the renal circulation in acute hypovolaemic anaemia.

III Role of Nervous and Humoral Factors in the Renal Vascular Response to Haemorrhage.

Control experiments

The results obtained in 4 "ureter" animals with intact renal nerves on both sides are shown in figure 46. These animals were sham-operated before experiments, at a time when unilateral renal denervation would have otherwise been performed. There was a marked decrease in diuresis after bleeding 15-20 ml/Kg, but the rates of urine flow from each ureter remained approximately equal, and probably adequate to prevent significant delay of excreted PAH and creatinine in the renal pelvis, as discussed in Section I. Haemodynamic changes following haemorrhage in these experiments have been described in Section I. There were no significant differences in renal blood flow

Figure 46. Time before and after haemorrhage (15-20 ml/Kg) in relation to mean values for urine flow, renal blood flow (RBF), glomerular filtration rate (GFR), blood pressure (BP) and haematocrit (HT) in 4 animals with intact renal nerves. Separate clearance measurements were carried out from each kidney. The ratios of clearance in the sham-denervated kidney to clearance in the normal kidney (Ratio Den/Inn) are shown above the absolute values for RBF and GFR.

Open circles and white columns - values for sham-denervated kidney.

Black circles and black columns - values for innervated kidney.

Control periods: - 40 to 0 minutes.
Acute hypovolaemic anaemia: 0 to 100 min.
Breathing 8.4% O_2 in N_2: 60 to 80 min.

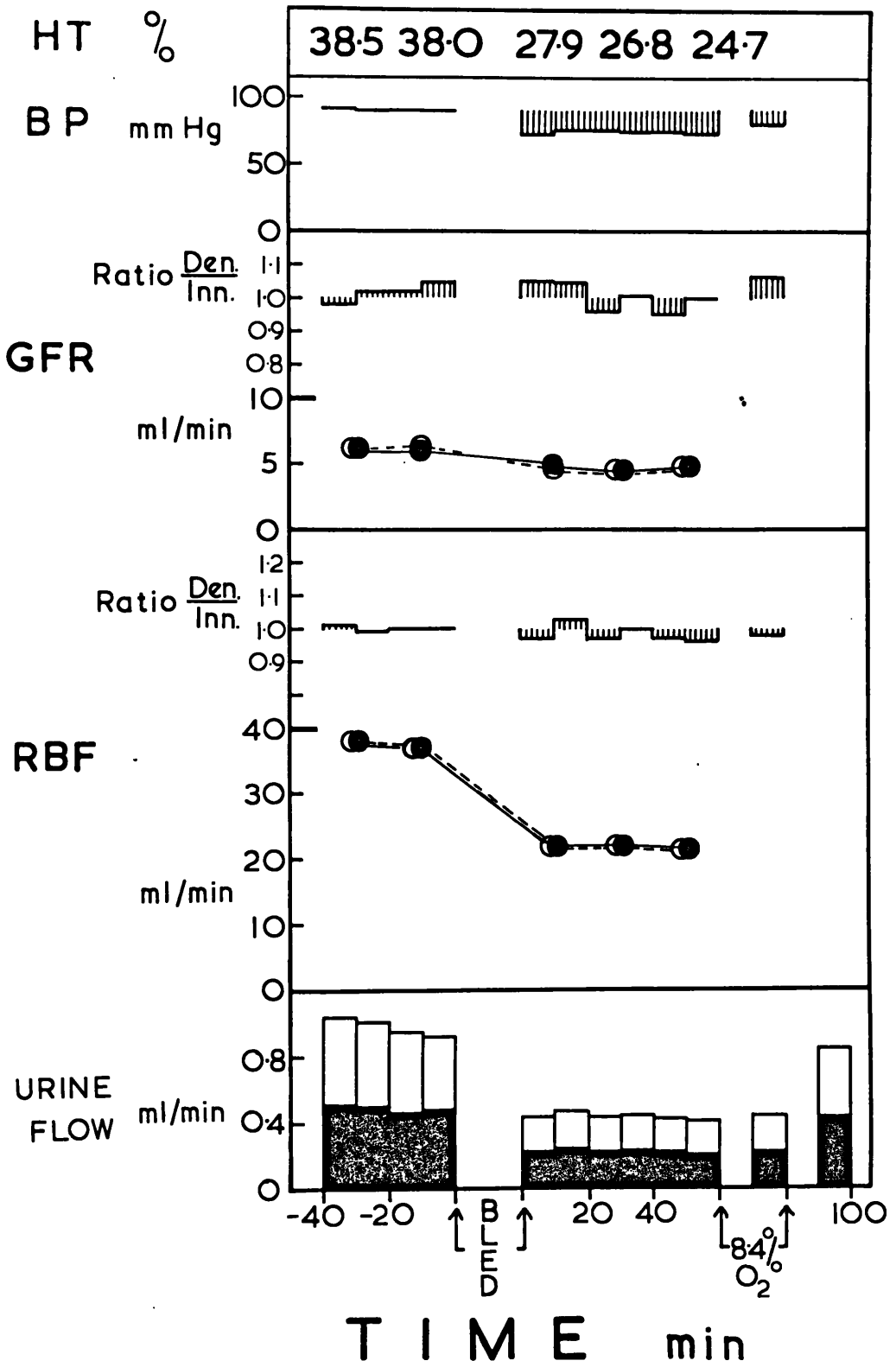


Figure 46: Legend on opposite page.

or glomerular filtration rate demonstrated between the left kidney and the right kidney. Despite the fall in RBF and GFR after haemorrhage the mean left/right ratios did not vary from unity by more than 5% throughout all procedures, including haemorrhage and administration of low O_2 mixtures.

Similar observations were made in the 2 animals in which changes in the renal circulation were observed for 150 minutes after haemorrhage (see figure 44). Left/right ratios for RBF and GFR in these 2 experiments are shown in table 21.

Effects of haemorrhage in unilaterally denervated animals.

The mean effects observed in 4 animals bled 15 ml/Kg are shown in the left panel of figure 47, whilst the results obtained in 4 different animals bled 20 ml/Kg are shown in the right panel of figure 47. During control observations the blood flow in the innervated kidney was 8% lower than in the denervated kidney, indicating the presence of renal vasoconstrictor tone in the resting animal (as has been discussed in Chapter 3). Following haemorrhage, significant reductions in blood flow and GFR, and an increase in renal vascular resistance occurred in both the innervated and the denervated kidney. The differential effects on the two kidneys in each animal are best illustrated by consideration of D/I clearance ratios, rather than by consideration of the absolute clearance values, which are expressed as the average of two consecutive 10 minute clearance periods. The time trends in D/I clearance ratios after haemorrhage were studied by analysis of variance; the results for D/I blood flow (PAH clearance)

TABLE 21

Haematocrit values, ratios of left RBF/right RBF and ratios of left GFR/right GFR obtained in 4 animals with bilateral ureteric catheters under control conditions, during a period of 2½ hours after haemorrhage, and whilst breathing 8% O_2. Each ratio is based on a 10 minute clearance measurement, and the paired values are 30 minutes apart.

Animals 1 and 2: renal nerves intact
Animals 3 and 4: left renal denervation, right renal nerves intact

	Ht				Left RBF/Right RBF				Left GFR/Right GFR			
Animal No:	1	2	3	4	1	2	3	4	1	2	3	4
CONTROL	47.0	38.4	42.9	36.9	0.99	0.97	1.05	-	1.01	0.95	1.03	-
					0.97	1.03	1.08	1.011	0.97	0.91	0.97	0.80
	44.9	36.9	41.0	33.9	0.99	0.97	0.96	0.92	0.98	1.01	1.04	0.92
					0.99	0.98	0.95	1.00	0.98	1.00	0.90	1.04
BLED++												
+ 30-50	-	24.2	26.1	22.3	-	0.96	0.97	0.91	-	0.80	0.92	0.95
					-	0.95	0.88	0.95	-	1.03	0.85	1.02
+ 80-100	31.9	22.0	22.9	20.6	0.96	0.97	1.00	0.94	0.95	1.05	0.99	0.87
					0.97	0.99	1.06	1.00	0.96	0.98	1.02	0.83
+ 130-150	29.9	20.2	20.8	18.9	0.97	0.92	0.92	0.95	0.95	0.81	0.93	0.91
					1.07	0.98	1.01	0.93	1.05	0.88	1.01	0.87
8.0% O_2												
+160-170					-	-	1.44	1.15	-	-	1.55	1.21

\+ Time in minutes after haemorrhage

++ Animals 1-3 bled 15 ml/Kg, animal 4 bled 20 ml/Kg.

ratios are presented in tables 22, 23, and are quoted without further reference in the following discussion.

The animals which were bled 15 ml/Kg tolerated this procedure without apparent distress. After haemorrhage there was a reduction in mean D/I blood flow ratio in the group, but this was due to an effect in 2 animals only and was not statistically significant. During the period between 20 and 60 minutes after haemorrhage the D/I ratio was significantly increased above its control value ($p = 0.05$), suggesting the presence of increased renal sympathetic constrictor activity at this time. Similar time trends were observed in GFR, but the changes were smaller than in RBF, and the D/I ratios did not rise significantly above control values. There was a reduction in D/I ratio for filtration fraction in all four animals, suggesting a relatively greater degree of constriction in the afferent arteriole of the denervated kidney. This behaviour was also observed during the administration of adrenaline and angiotensin (Section II) and is consistent with a report that the afferent arteriole constitutes the primary site of hypersensitivity to adrenaline (100). The results thus indicate that an increase in nervous vasoconstrictor activity contributed to the reduction in renal blood flow in the first hour after moderate haemorrhage, and suggest that humoral factors such as circulating adrenaline or angiotensin may also have been involved.

The animals which were bled 20 ml/Kg (Figure 47, right panel)

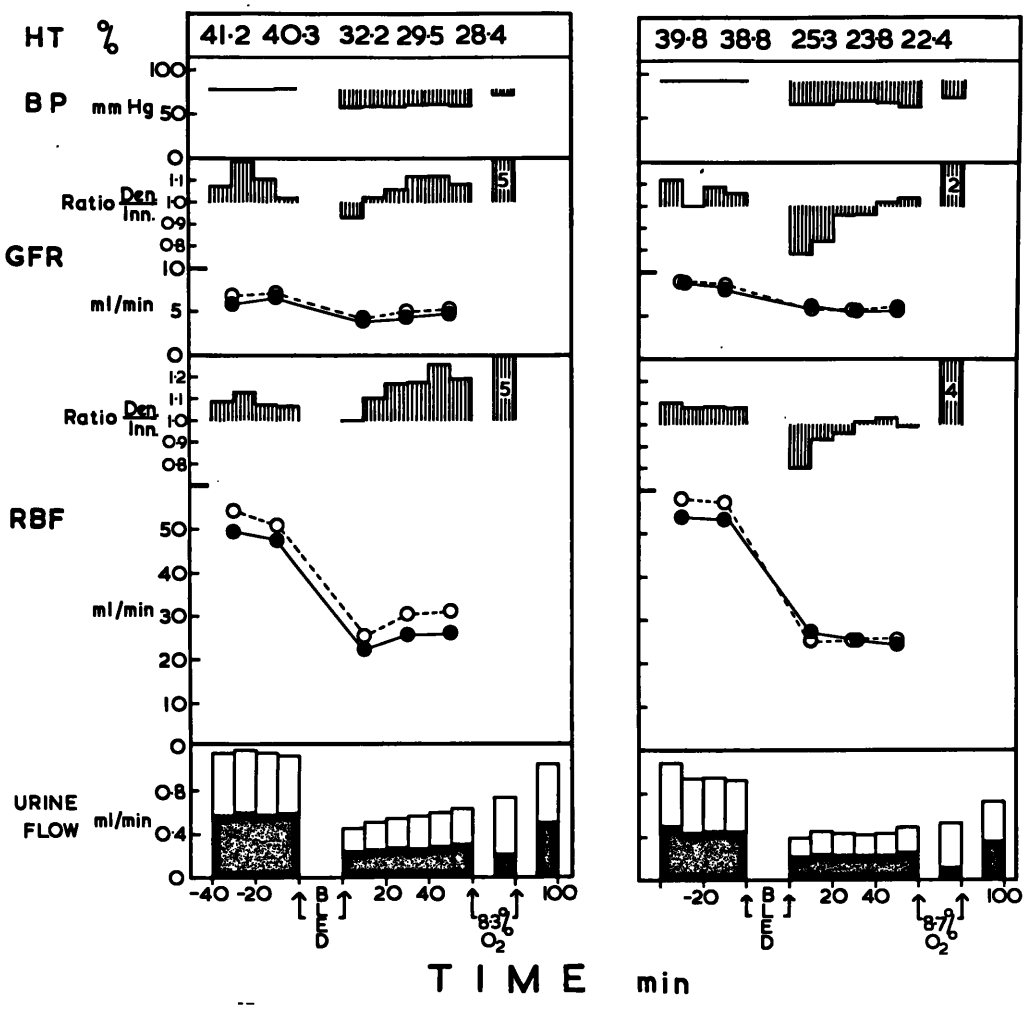


Figure 47. Time before and after haemorrhage in relation to mean values for urine flow, renal blood flow (RBF), glomerular filtration rate (GFR), blood pressure (BP) and haematocrit (HT) in 4 animals bled 15 ml/Kg (left panel) and in 4 animals bled 20 ml/Kg (right panel). Separate clearance measurements were carried out from the left, denervated kidney and the right, innervated kidney.
Open circles and white columns - values for denervated kidney.
Black circles and black columns - values for innervated kidney.

Control periods: - 40 to 0 minutes
Acute hypovolaemic anaemia: 0 to 100 min.
Breathing 8-9% O_2 in N_2: 60 to 80 min.

TABLE 22

Analysis of time trends in D/I ratios for PAH clearance in animals bled 15 ml/Kg. (see Fig.47)

Time (min)	Animal 1	Animal 2	Animal 3	Animal 4
T-40	1.153	1.062	1.033	1.108
T-30	1.161	1.245	1.019	0.093
T-20	1.089	1.089	1.016	1.100
T-10	1.112	1.110	0.940	1.096
(BLED)				
T10	0.515	1.251	0.872	1.398
T20	0.700	1.157	1.183	1.385
T30	0.881	1.223	1.119	1.455
T40	0.862	1.241	1.114	1.494
T50	1.250	1.194	1.116	1.491
T60	1.194	1.192	0.853	1.392

Analysis of Variance

SOURCE OF VARIATION	S.S.	D/F	M.S.
Between animals	0.608	3	0.2026
Between times	0.189	9	0.0210
Error	0.775	27	0.0287
Total	1.572	39	0.0403

Comparison of T30 to T60 with T-40 to T-10:-

$$\text{Mean difference} = \frac{19.069 - 17.416}{16} = 0.1033$$

$$SE = \sqrt{\frac{0.0287}{16}} = 0.042$$

$$t = 2.439$$

$$p = 0.05$$

Comparison of T50 with T10:-

$$\text{Mean difference} = \frac{5.051 - 4.016}{4} = 0.259$$

$$SE = \sqrt{0.0287/4} = 0.0847$$

$$t = 3.047$$

$$p = 0.05$$

TABLE 23

Analysis of time trends in D/I ratios for PAH clearance in animals bled 20 ml/Kg (see Fig. 47)

Time (min)	Animal 1	Animal 2	Animal 3	Animal 4
T-40	1.016	1.105	1.228	1.057
T-30	0.988	1.112	1.137	1.063
T-20	1.058	1.115	1.096	1.055
T-10	1.096	1.107	1.067	1.039
(BLED)				
T10	0.928	0.970	0.389	0.910
T20	0.795	0.980	0.775	1.182
T30	0.804	1.063	0.815	1.164
T40	1.012	1.069	0.876	1.095
T50	1.063	1.037	0.815	1.201
T60	0.983	1.015	0.861	1.096

Analysis of Variance

Source of Variation	S.S.	D/F	M.S.
Between animals	0.2001	3	0.0667
Between times	0.3235	9	0.0359
Error	0.3851	27	0.01426
Total	0.9086	39	0.0233

Comparison of T30 to T60 with T-40 to T-10:-

$$\text{Mean difference} = \frac{17.343 - 15.949}{16} = 0.0871$$

$$SE = \sqrt{0.1426/15} = 0.0298$$

$$t = 2.92$$

$$p = 0.02$$

Comparison of T10 with T-10:-

$$\text{Mean difference} = \frac{4.303 - 3.197}{4} = 0.276$$

$$SE = \sqrt{0.1426/4} = 0.0597$$

$$t = 4.6$$

$$p = 0.02$$

were more disturbed by the procedure, and became restless for a few minutes after haemorrhage. In addition the reduction in blood pressure, GFR and RBF was greater than in animals bled by the smaller amount. The mean D/I blood flow ratio decreased significantly during the first 10 minutes after haemorrhage ($p = 0.02$), and then returned gradually towards control values, although it was still below these values after 60 minutes. Similar changes were observed in the D/I ratios for GFR, but the behaviour of the GFR was somewhat more variable than that of the renal blood flow. The D/I ratios for filtration fraction were also variable, increasing in two animals and decreasing in the other two. The results suggest that a hormonal component contributed to the vasoconstrictor response in this group. The return towards control value in D/I ratios during the first 60 minutes could be the result of diminution in hypersensitivity or hormone levels, or be the result of increased activity in the renal vasomotor nerves on the innervated kidney. Some support for the latter alternative is derived from observation that there was a progressive slight reduction in RBF in the innervated kidney during the first hour after haemorrhage, in contrast to the relatively constant flow in the denervated kidney (see figure 47). Also in one animal bled 20 ml/Kg a definite biphasic response occurred, with initial reduction in D/I blood flow ratios, and subsequent elevation above control values (Figure 48). Thus it is possible that nervous as well as humoral factors played a part in the renal vasoconstrictor response to severe haemorrhage, but the evidence for the presence of certain humoral factors is more definite.

Figure 48: Time before and after haemorrhage in relation to urine flow, renal blood flow (RBF), glomerular filtration rate (GFR), blood pressure (BP) and haematocrit (HT) in one animal bled 20 ml/Kg. Separate clearance measurements were carried out from the denervated kidney and innervated kidney.

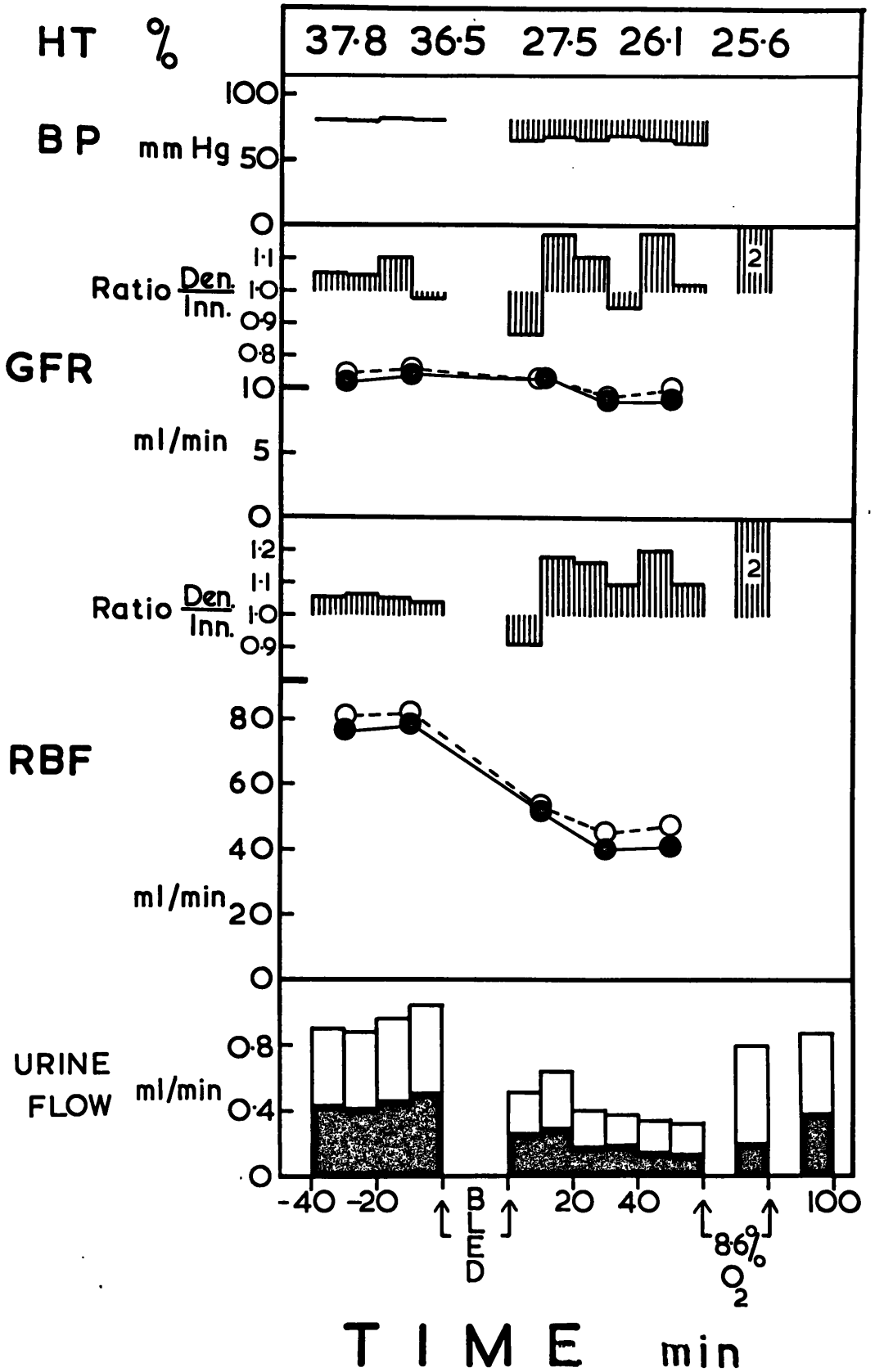


Figure 48: Legend on opposite page.

Table 21 shows results obtained in 2 additional animals with unilateral renal denervation which were observed for 150 minutes after haemorrhage. The D/I ratios for blood flow and GFR were below control values in the first 30 minutes after haemorrhage (observation in one animal only), but returned to values close to the initial control values during the period between 30-150 minutes after haemorrhage.

At the conclusion of each experiment, the efficacy of renal denervation was tested by administering low O_2 mixtures (Figures 47, 48). In each of the 9 animals tested, there was an immediate increase in D/I ratios, indicating that denervation was probably adequate (98). In the animals subjected to bleeding the administration of 8% O_2 resulted in a far greater vasoconstrictor response in the innervated kidney than had been observed in animals not subjected to bleeding. Thus in 9 animals bled 15-20 ml/Kg the average D/I blood flow ratio during a 10 minute period of breathing 8% O_2 was 3.9 ± 1.3 (SE) whilst in 3 animals not subjected to bleeding (Figure 45 and Table 20) it was 1.24 ± 0.14 (SE).

DISCUSSION

The changes in the renal circulation observed in the rabbit following haemorrhage included marked reduction in renal blood flow and an increase in renal vascular resistance, in agreement with the results observed in man and the dog. The changes appeared to be related to the reduction in blood volume rather than the reduction in haematocrit, since no significant changes in RBF were observed during normovolaemic anaemia of similar degree (as

established in Chapter 4). Although the restoration of the rabbit's blood volume after bleeding is initially rapid (43), there was no evidence of recovery in renal haemodynamics during the first 150 minutes after haemorrhage. In Chapter 6 it will be shown that in animals rendered "chronically" anaemic by repeated bleeding, although restoration of the blood volume was still not quite complete 18-24 hours after the last bleed, the renal circulation had partially recovered at this stage from the immediate effects following haemorrhage. The demonstration of continued renal vasoconstriction following infusion of packed red cells further supports the role of hypovolaemia as the initiating factor in renal vasoconstriction after haemorrhage, and suggests that delayed replacement of blood does not appreciably mitigate the severity of the changes in the renal circulation during the early period after blood loss; similar findings have been reported in man (107).

Analysis of the changes in D/I clearance ratios demonstrate qualitatively the participation of nervous and humoral factors in the prolonged renal response to haemorrhage. In animals bled 15 ml/Kg or 20 ml/Kg humoral factors appeared to be most prominent in the period immediately after haemorrhage. In animals bled 15 ml/Kg nervous factors probably contributed towards the constrictor response during the subsequent period of observation. However, in these animals the demonstration of a relatively greater fall in filtration fraction on the denervated side is consistent with a simultaneous contribution by humoral constrictor factors, in view of evidence that the afferent arteriole is the site of hyper-

sensitivity (100).

Following the more severe degree of haemorrhage, humoral factors were more prominent, and this may have been in part due to the greater distress produced by this degree of blood loss. Humoral agents which are known to be released into the circulation after haemorrhage include ADH (71), catecholamines (68, 180), angiotensin (44), adrenal corticoids (179), and ferritin (85). The present experiments confirmed the hypersensitivity response of the chronically denervated kidney to infusions of adrenaline and angiotensin. The reduction in total renal blood flow produced by these agents was not as marked as the reduction observed following haemorrhage, but in the moderate dosage used, their effects on total renal vascular resistance and filtration fraction, and on D/I ratios, were similar to the effects of severe haemorrhage. The results are consistent with the possibility that these or similar hormones, acting alone or in combination, played a part in the renal constrictor response to haemorrhage.

The return of the D/I blood flow ratios towards control values 60-150 minutes after bleeding are difficult to interpret; the various possibilities have already been discussed. The results provide some support for the concept that when nervous and humoral constrictor factors coexist, they act differently on the innervated kidney and the denervated kidney, tending to restore the D/I ratio towards normal. Thus if an early predominance of humoral effect is followed by a balance of nervous and humoral effects, the degree of vasoconstriction would not necessarily change but D/I ratios may

revert to normal. Such an interpretation is supported by the progressive slight reduction in RBF in the innervated kidney during the first hour after haemorrhage, in contrast to the relatively constant flow in the denervated kidney, and also by the occasional occurrence of a biphasic response in D/I blood flow ratios. The greater differential nervous constrictor response resulting from the inhalation of low O_2 mixtures during hypovolaemic anaemia could also be due to a relatively high baseline impulse traffic in the vasomotor nerves to the innervated kidney.

SUMMARY

In the unanaesthetized rabbit bled without plasma replacement, a condition of hypovolaemic anaemia quickly ensued due to the rapid initial haemodilution characteristic of this species. There was a reduction in renal blood flow and glomerular filtration rate, and an increase in renal vascular resistance and filtration fraction. These effects tended to persist throughout the 150 minute period of observation after haemorrhage, despite partial recovery of total blood volume, and were only transiently relieved by infusion of packed red cells. The renal vasoconstriction during acute hypovolaemic anaemia was shown to be produced by complex nervous and humoral effector mechanisms, which appeared to be initiated by the reduction in blood volume after haemorrhage.

CHAPTER 6

THE RENAL CIRCULATION IN "CHRONIC" POST-HAEMORRHAGIC ANAEMIA

Earlier in this report it has been shown that when the haematocrit was reduced without a decrease in blood volume, moderate anaemia caused no changes in the renal circulation, whilst severe anaemia caused no significant alteration in renal blood flow but other effects on the renal vascular bed which are attributable in part to renal hypoxia and in part to reduced red cell concentration. However, when a reduction in haematocrit was accompanied by decreased blood volume, marked renal vasoconstriction occurred which was thus attributed to the central mechanisms regulating body fluid volume rather than to the concomitant moderate anaemia.

It remained to determine whether the local effects of reduction in haematocrit or the central effects of reduction in blood volume predominated in the renal response when a severe degree of anaemia was accompanied by decreased blood volume. In view of the relevance of this type of anaemia to the condition seen in clinical practice after a patient has suffered recurrent haemorrhage, the present experiments were designed to simulate the clinical situation. Thus severe anaemia was obtained in the rabbit following repeated lowering of its blood volume by haemorrhage over a period of 3 days. The effects of this procedure on the renal circulation and on blood volume are presented in the first section of this Chapter. In the second section the results are compared to those obtained in normovolaemic anaemia. An attempt is made to differentiate the

local effects of severe anaemia from those observed under conditions which require conservation of body fluid volume.

RESULTS

I Effects of "Chronic" Post-Haemorrhagic Anaemia

Figure 49 shows results obtained in 6 animals, studied in each case on two occasions four days apart, and at two levels of haematocrit. There were significant reductions ($p = 0.01$) in renal blood flow, GFR, arterial pressure and filtration fraction as a result of "chronic" anaemia. The decrease in renal blood flow paralleled the fall in arterial pressure, and there was no significant change in renal vascular resistance, which averaged 102 ± 6.0 (SE) % of control.

Total blood volume during "chronic" anaemia averaged 94.7 ± 2.2 (SE) % of control values ($p = 0.07$) in the 6 animals shown in figure 49. In a separate series of 5 animals in which no infusions or renal measurements were carried out, values for total blood volume (Table 24) were slightly lower, and during chronic anaemia averaged 92.8 ± 2.3 (SE) % of control ($p = 0.04$). It is probable that the sustaining infusion exaggerated the degree of restoration of blood volume in the animals used for renal measurements.

Table 25 shows the effects in 8 animals of "chronic" anaemia on tubular excretion of PAH during conditions designed to saturate the renal transport mechanism for PAH. Tm_{PAH} was significantly decreased in chronic anaemia (Table 25 and Figure 50). The changes in renal haemodynamics produced by high plasma PAH levels (see Table 2), were not significantly potentiated in the presence of anaemia. Thus

TABLE 24

The effects of "chronic" anaemia on total blood volume in 6 animals which received sustaining infusions during renal clearance measurements (also shown in Fig. 49), and in 5 animals which received no infusions and in which no renal clearance measurements were made. C = control values. T = values in "chronic" anaemia.

	Haematocrit (%)	Plasma Volume (ml)	Red cell Volume (Cr51) (ml)	Total blood Volume (ml)	Total blood Volume (ml/Kg)
C	31.3	135	42	176	71.8
T	12.3	156	16	172	70.2
C	34.5	113	44	157	65.4
T	9.9	141	12	153	63.8
C	38.1	143	59	202	67.3
T	13.2	177	18	195	65.0
C	39.2	116	51	167	72.6
T	16.1	124	18	141	61.3
C	33.2	118	41	159	61.2
T	9.2	137	12	148	56.9
C	34.9	117	44	162	57.9
T	12.6	143	17	160	57.1
	Mean difference (C - T) = 5.33 ± 2.2 (SE) % (p=0.07) (when infusions given)				
C	34.4	100	37	138	65.7
T	13.4	109	14	122	58.1
C	36.1	106	46	152	59.6
T	14.3	134	18	152	59.6
C	41.8	106	53	159	56.8
T	20.4	126	25	151	53.9
C	37.1	104	45	148	61.7
T	16.5	118	20	138	57.5
C	39.2	101	41	142	61.7
T	12.4	111	13	124	53.9
	Mean difference (C - T) = 7.2 ± 2.3 (SE)% (p = 0.04) (without infusions)				

Figure 49: Haematocrit in relation to renal blood flow (RBF), glomerular filtration rate (GFR), blood pressure (BP), filtration fraction and average total blood volume in 6 animals. Black circles - control values. Open circles - values in "chronic" anaemia obtained 4 days later. Total blood volume was measured as the sum of separately determined Cr^{51} red cell volume (black column) and T-1824 plasma volume (white column). Results from the same animal are joined together by a straight line.

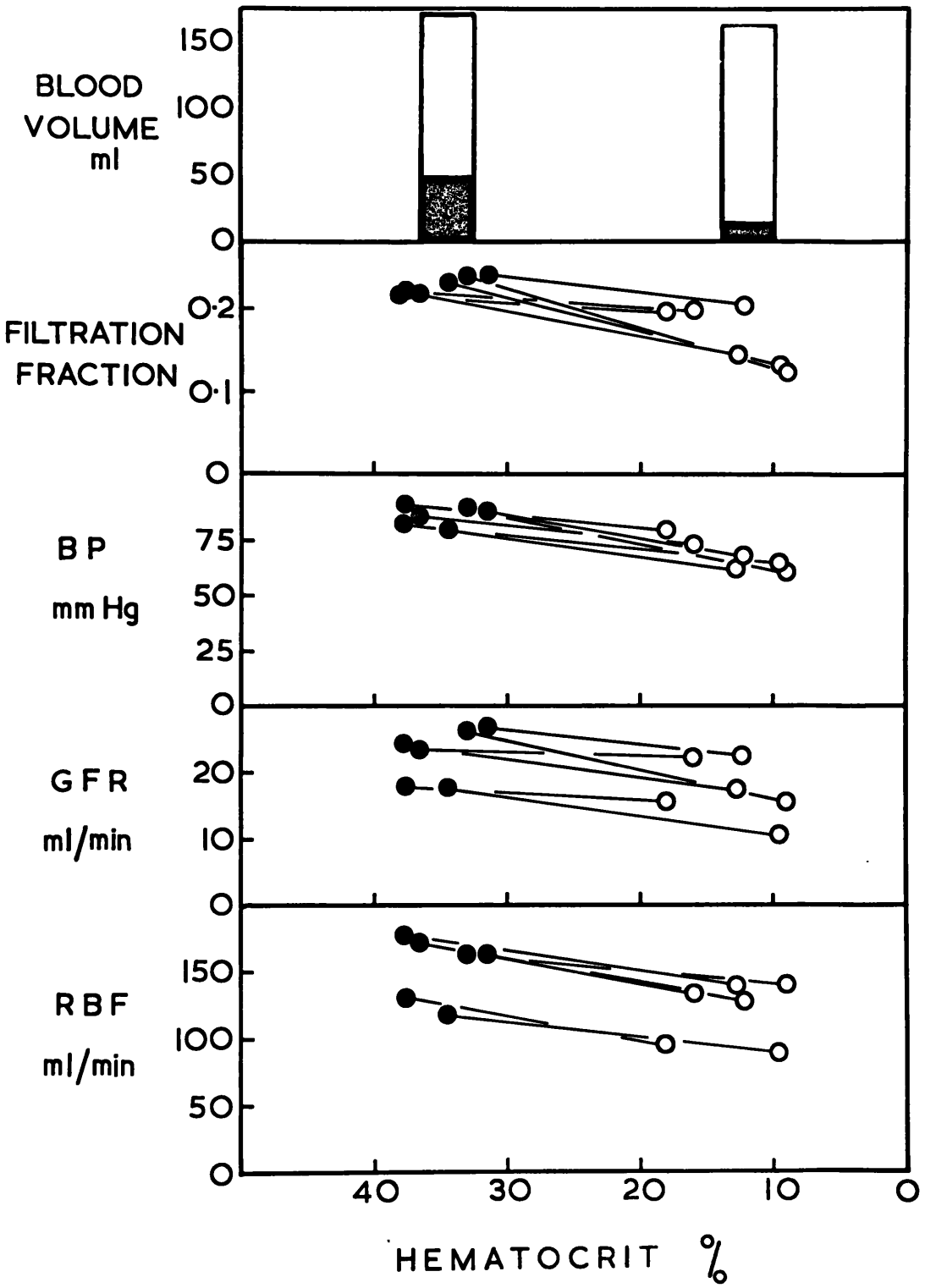


Figure 49: Legend on opposite page.

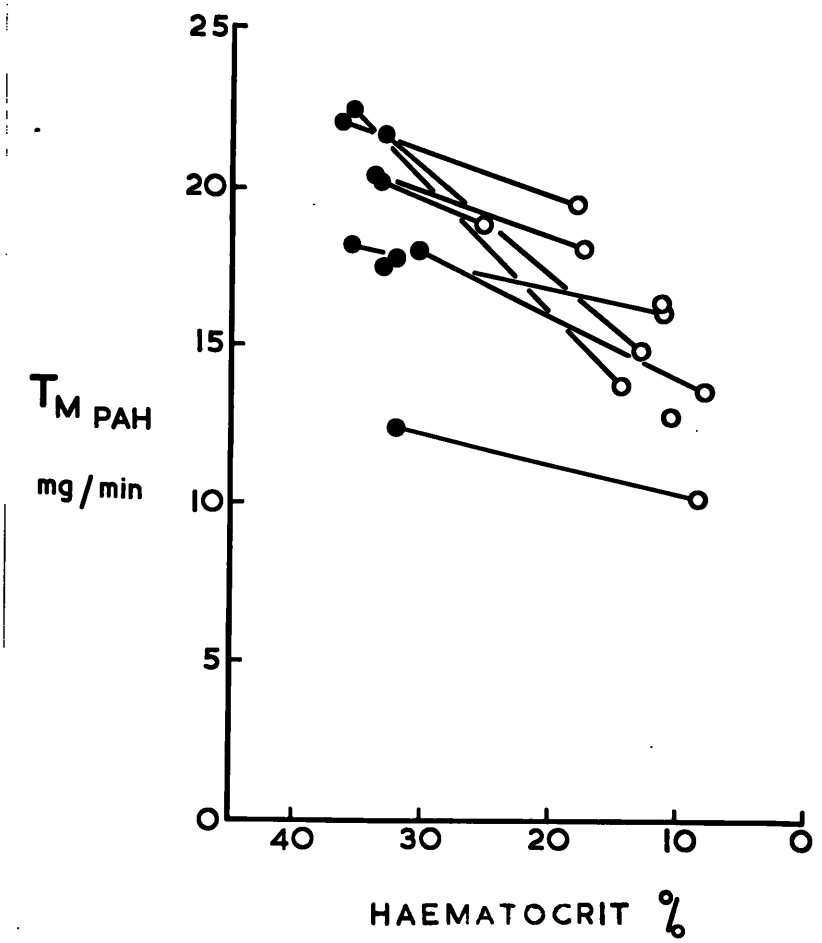


Figure 50. Haematocrit in relation to Tm_{PAH} in 11 animals during control observations (black circles) and in "chronic" anaemia 4 days later (open circles). Results in the same animal are joined by a straight line.

TABLE 25

Mean values obtained in 8 animals with high plasma PAH titres, at normal haematocrits (C), and during chronic anaemia (T). The results shown for renal blood flow (RBF), glomerular filtration rate (GFR) and filtration fraction (FF), are the percentage changes produced when the plasma PAH level was raised from a concentration of 1-3 mg % to the high concentrations shown. The standard errors (SE) shown are based on comparisons within animals.

		C	T	SE
Haematocrit %		33.7	14.3*	± 1.87
Arterial plasma PAH concentration (mg%)		45.9	65.0	±10.6
Lead/T ratio		1.78	4.19	± 1.03
Tm_{PAH} (mg/min)		19.4	15.6*	± 0.93
Effects produced	Δ RBF	+ 0.6%	+16.3%	± 9.4%
in renal circulation	Δ GFR++	-22.1%	-19.1%	± 6.6%
by high plasma PAH levels	Δ FF++	-25.1%	-31.4%	± 4.9%

* Treatment effect significant ($p \leq 0.05$)

++ Effect of elevation of plasma PAH concentration significant both at normal haematocrit and during chronic anaemia.

the change in Tm_{PAH} appeared to be due to anaemia, rather than to toxic effects of PAH on the renal circulation. Renal PAH extraction ratio at low plasma PAH concentrations (1-3 mg %) averaged 93.3 % at normal haematocrit values, and decreased by 7.1 $\pm$ 1.7 (SE) % as a result of "chronic" anaemia. A similar reduction in renal PAH extraction ratio has been observed in severe acute normovolaemic anaemia.

II Comparison of Effects of "Chronic" Anaemia with Effects of Acute Normovolaemic Anaemia.

The differences between renal circulatory effects observed in acute anaemia, produced by bleeding with plasma replacement, and "chronic" anaemia are shown in figure 51. In 17 animals of the group subjected to acute normovolaemic anaemia the haematocrit had been lowered from its normal value to approximately 20% by preliminary bleeding several days previously. The "acute" group did not differ markedly from the "chronic" group as regards preliminary handling and bleeding. Following a reduction of haematocrit, the changes in the two groups were similar for GFR, arterial pressure and filtration fraction. The effects of acute anaemia on renal blood flow, renal vascular resistance and total blood volume were significantly different from those of "chronic" anaemia. In acute anaemia, renal blood flow was unchanged, whilst it fell in "chronic" anaemia. Renal vascular resistance was reduced by 16.5 $\pm$ 4.7 (SE) % of control values in acute anaemia but remained unchanged in "chronic" anaemia. The design of the "acute" experiments was such that total blood volume was maintained or increased; this was confirmed in 5 animals

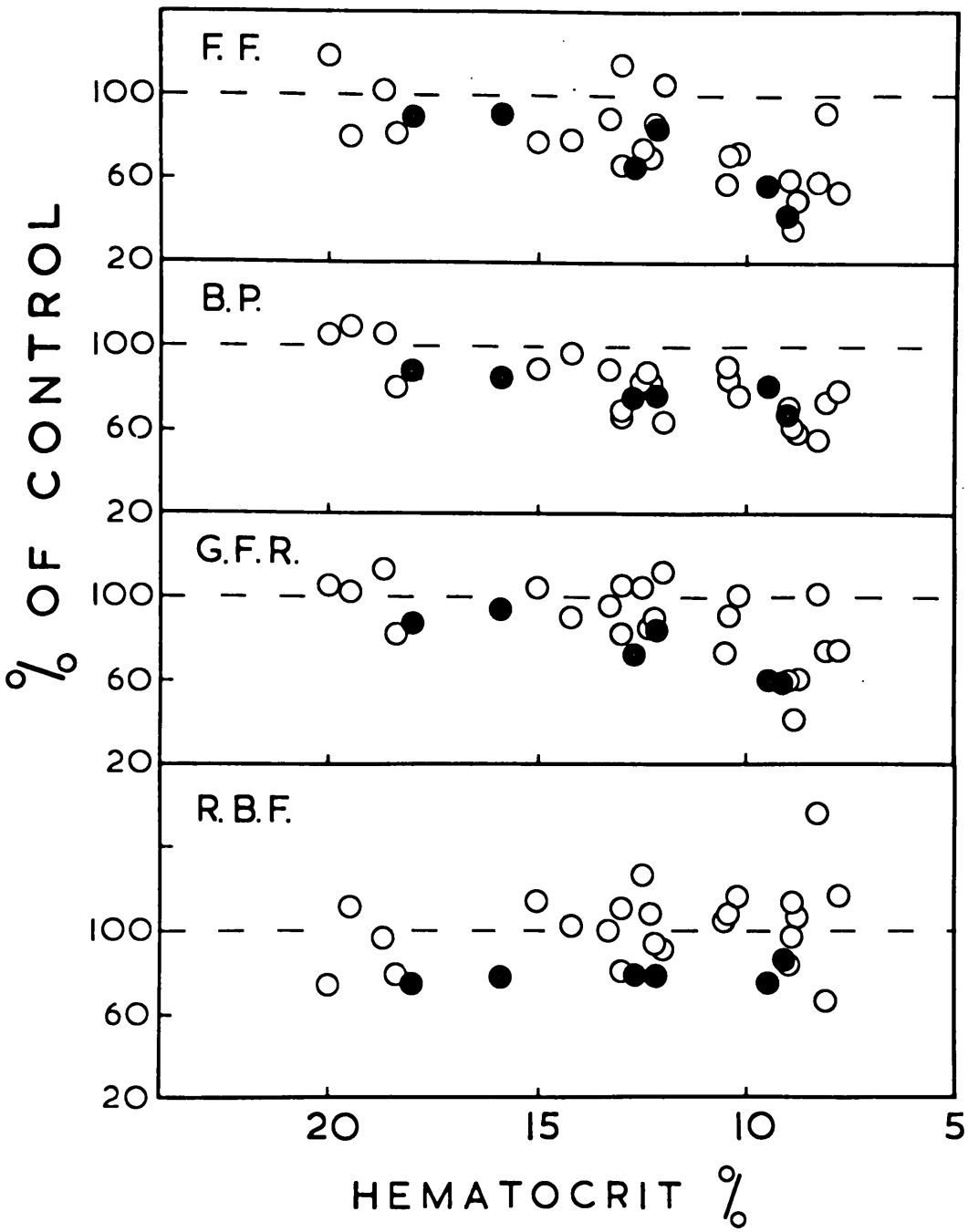


Figure 51. Comparison of "chronic" anaemia (black circles) with acute normovolaemic anaemia (open circles). Each animal is represented by one point expressing the result in anaemia as a percentage of the result during control observations in that animal (see figs. 49 and 37).

of the group (see Figure 42). There was a slight decrease in blood volume in "chronic" anaemia, which could be shown to be statistically significant if due allowance was made for the effect of the sustaining infusion.

DISCUSSION

The main difference between the renal effects of acute normovolaemic anaemia and "chronic" anaemia was that whilst total renal vascular resistance fell in acute anaemia, in "chronic" anaemia it was maintained despite a reduction in filtration fraction. This finding indicates constriction in the afferent arteriole (i.e. the likely neural effector site) in the "chronic" group, and suggests that the renal vasomotor nerves were implicated in the renal response to "chronic" anaemia. The possibility that persistent renal vasoconstriction and equivocal D/I ratios 60-150 minutes after haemorrhage in the "ureter" animals were brought about by combined nervous and humoral effects, has been discussed in the previous chapter. The type of experiment carried out in the present group of animals was not suitable for eliciting the presence of humoral effects on the renal circulation, but it is possible also at this later stage after haemorrhage that a synergy of nervous and humoral constrictor factors was operative.

The difference in blood volume effects between the two groups was small. In the acute anaemia group the blood volume was maintained at normal values or even increased slightly; in the "chronic" group there was a small but significant reduction in blood volume. Despite the known rapid replacement of blood volume

by the rabbit following haemorrhage (43), compensation in total blood volume was apparently not complete in the "chronic" anaemia group.

In the acute anaemia group, the plasma PAH level required to produce depression of renal PAH extraction ratio was considerably lower than at normal haematocrit ratios (see Figure 43) suggesting that in these animals, as in the "chronic" anaemia group, there was reduction in Tm_{PAH}. There was no appreciable difference in renal PAH extraction ratio at low arterial PAH concentration between the two groups, for any level of haematocrit. The effects on filtration fraction were also comparable. These findings suggest similar degrees of impairment of PAH transport and similar intra-renal distribution of blood flow at reduced haematocrit values in the two groups.

It appears that the changes in the renal circulation in acute normovolaemic anaemia represent local effects of tissue hypoxia and reduced red cell concentration, whilst the changes in "chronic" post-haemorrhagic anaemia may represent some degree of extrinsic control of the renal circulation. Immediately following haemorrhage, marked increase in renal vascular resistance was observed, and the participation of nervous and humoral factors in this response was demonstrated (see Chapter 5). The present results suggest that in the "chronic" anaemia group the local renal vasodilatation resulting from severe anaemia was opposed by some degree of renal vasoconstriction associated with blood volume regulation. It appears that in these animals the adjustments to blood loss are prolonged and include effects on the renal vascular bed.

CHAPTER 7

GENERAL DISCUSSION

This investigation concerned the effects on the renal circulation of the unanaesthetized rabbit of three types of experimental anaemia, each of which was characterized by different degrees of reduction in haematocrit and total blood volume. By comparison of the different effects observed in each of these three anaemias, an attempt has been made to analyse the mechanisms by which the renal effects of anaemia are produced, and to consider separately the contributions of reduced red cell concentration, renal hypoxia and hypovolaemia.

In acute normovolaemic anaemia there was no change in renal blood flow, but reduction of haematocrit to low levels resulted in renal tissue hypoxia, and reduction in renal vascular resistance and filtration fraction. This suggested the presence in severe anaemia of hypoxic renal vasodilation, predominantly affecting the post-glomerular vasculature. Renal PAH extraction ratio was significantly decreased, although the effect observed was smaller than in previous series (94, 173), and there was indirect evidence of diminished maximal PAH transport capacity. These findings indicated impairment of tubular cell function in anaemia. PAH extraction was decreased even at very low arterial PAH levels, and thus it is unlikely that the impairment was due to a deficient supply of rate limiting substrates such as acetate (129).

Breathing 100% oxygen increased the oxygen available to the tissues in severe anaemia by about 50%. This proved an apparently effective means of relieving tissue hypoxia without changing red cell concentration, as renal vein O_2 saturation was restored nearly to its level at normal

haematocrit, and renal O_2 consumption was increased. However the effects on renal PAH extraction ratio were equivocal, and although renal vascular resistance and filtration fraction reverted significantly towards their control levels, they were still decreased. These results suggested that whilst the renal effects of anaemia were in part attributable to local hypoxia, other factors associated with the reduction in red cell concentration may have been involved.

The renal haemodynamic findings in normovolaemic anaemia presented a contrast to those observed in other types of hypoxia in which red cell concentration was not reduced. When acute arterial hypoxia was produced in "ureter" animals by administration of low oxygen mixtures, there was an increase in renal vascular resistance and a marked decrease in renal blood flow in the innervated kidney; it has been shown elsewhere that these effects appear to result from increased vasomotor activity in the renal sympathetic nerves and do not occur after chemoreceptor denervation (98). Thus it is probable that the renal response to arterial hypoxia is primarily conditioned by the reduction in arterial pO_2 rather than by decreased renal tissue oxygenation.

In carboxyhaemoglobinaemia, as in anaemia, arterial pO_2 is not appreciably reduced (7). The effects of breathing carbon monoxide are manifested at the tissue level where, due to decreased transport and availability of circulating oxygen, marked hypoxia is produced. Indeed the range of renal venous pO_2 values observed during carbon monoxide inhalation in the present study was much lower than in severe anaemia. However the effects on renal PAH extraction ratio were not as marked: for a given reduction in PAH extraction in the carbon monoxide experiments,

a much greater reduction in renal venous pO_2 than in anaemia was observed. There was no significant effect on filtration fraction, suggesting that the ratio of pre-glomerular to post-glomerular resistance was unchanged. Despite the known increase of cardiac output in rabbits after breathing carbon monoxide (98), there was a reduction in renal blood flow. However this reduction was in proportion to the fall in arterial pressure and may have been a passive response, reflecting a redistribution of blood flow to other regions of the circulation where vasodilatation had occurred. An alternative explanation of the reduction of renal blood flow is that it resulted from the operation of baroreceptor reflexes (98) i.e. this form of hypoxia, like severe normovolaemic anaemia, could tend to produce renal vasodilatation, but the tendency might be antagonised by increased vasoconstrictor activity.

Whilst decreased blood viscosity probably accounted for part of the fall in renal vascular resistance when the haematocrit was reduced from 20% to 10%, the nature and magnitude of its contribution to this effect is difficult to assess. Other workers have demonstrated relatively little change in flow through the isolated perfused hindlimb when haematocrit was altered in this range, although marked effects were produced when the haematocrit ratio was varied at higher levels (186, 109). Pappenheimer and Kinter (136) have postulated that as a consequence of "plasma skimming" in the interlobular arteries and afferent arterioles, the vessels in the periphery of the cortex are perfused with blood of much higher haematocrit than arterial blood. They suggest that when the arterial haematocrit is reduced, alterations in peripheral cortical haematocrit are in a range in which viscosity is greatly decreased, resulting in a steep fall in regional

resistance. Thus reduced red cell concentration in anaemia might give rise to a greater change in total resistance to blood flow in the kidney than in other organs. It is not known whether such alterations in intra-renal haemodynamics occurred in anaemia in the present study.

In attempting to account for the reduction in renal PAH extraction ratio and filtration fraction in severe normovolaemic anaemia, another route for possible redistribution of intra-renal blood flow has been considered i.e. the "juxta-medullary shunt" described by Trueta et al (176). Although it is not apparent how flow through the vasa recta in the medulla is controlled, it has been shown to be relatively independent of the mechanisms regulating cortical flow (1013). There is evidence both morphological (Figs. 34-36) and experimental (11, 29, 54, 122) which suggests that if increased flow through the juxta-medullary glomeruli and vasa recta bundles was brought about by anaemia, it could lead to reduction in filtration fraction and renal PAH extraction ratio. Whether such an increase in medullary flow actually occurs in normovolaemic anaemia is at present a matter for conjecture; the radio-active krypton method of intra-renal blood flow measurement (174) could provide further information. Preliminary work has been carried out on the application of this technique to the rabbit (Figs. 52, 53).

When rabbits in the present series were subjected to bleeding without plasma replacement, a condition of hypovolaemic anaemia resulted in the period immediately following blood loss, due to the rapid initial haemodilution which is characteristic of the species (41, 43). There was a marked fall in the renal clearance of PAH, which remained at a decreased level throughout subsequent observations over a period of 150 minutes

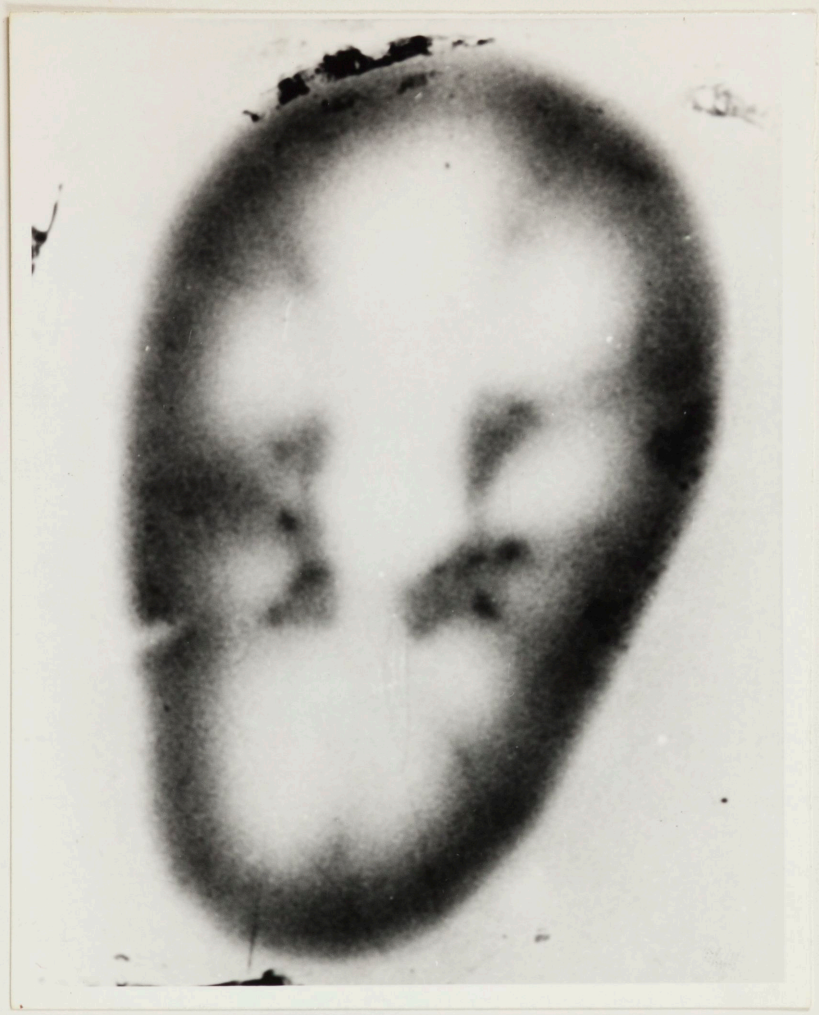


Figure 52. Autoradiograph from a rabbit kidney removed 3 minutes after injection of Krypton^{85} into the renal artery, showing initial distribution of radioactivity in the cortex, blood vessels and hilar fat (c.f. Fig 53). Exposure time of film = 6 hours. (The technique used was similar to that described by Thorburn et al (174) in the dog.) Medulla is relatively free of Kr^{85} at this stage, but kidneys removed at a later stage after Kr^{85} injection showed more activity in the medulla than in the cortex. The rate of appearance and disappearance of Kr^{85} from each region of the kidney appears to depend on the magnitude of regional blood flow (174).

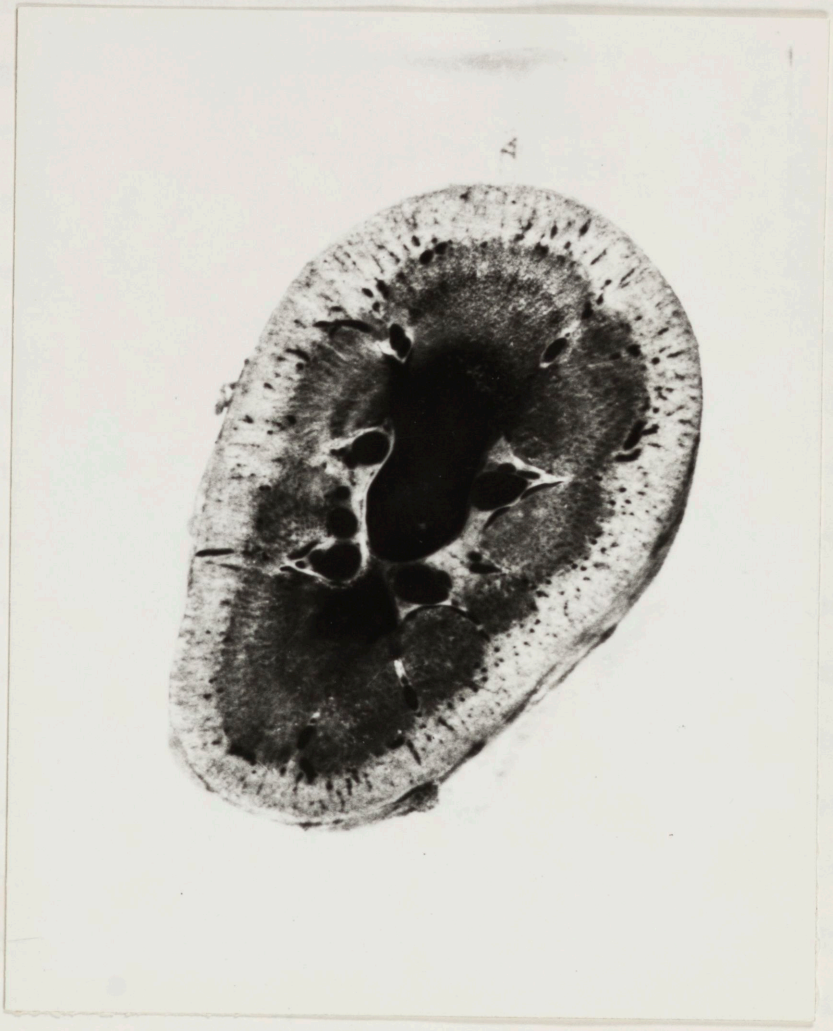


Figure 53. Photograph of tissue section used for autoradiograph (Fig. 52).

despite the partial restoration of total blood volume. Urine flow was also decreased following haemorrhage, but was maintained at a rate still compatible with accurate clearance measurements during hypovolaemic anaemia. Renal PAH extraction ration was unchanged, suggesting that there was no appreciable storage of PAH in renal interstitial tissue. Thus the effect of haemorrhage on the renal circulation in the rabbit was similar to that observed in other species (36, 107, 138) and consisted essentially of vasoconstriction with a marked reduction in renal blood flow. GFR was not reduced as severely as renal blood flow, and filtration fraction was increased, suggesting that the increase in vascular resistance was located mainly in the post-glomerular vessels.

Renal vasoconstriction may have been initiated by reflexes arising from volume receptors or the arterial baroreceptors; its continuance appeared to some extent independent of hypovolaemia, since there was no recovery of renal blood flow with partial restoration of blood volume in the early period after haemorrhage. No evidence regarding the afferent pathways involved in possible constrictor reflexes was provided by the present investigation. However an attempt has been made to identify some of the effector mechanisms which operate on the renal circulation after haemorrhage.

In preliminary experiments on "ureter" animals with one kidney denervated and the other kidney intact it was shown that nervous constrictor factors could be distinguished from certain humoral constrictor factors by studying the seperate renal clearances. Thus breathing low oxygen mixtures resulted in decreased clearance in the innervated kidney with little effect on the denervated side, whereas adrenaline end angiotensin caused a greater decrease in the denervated kidney, apparently due to the hypersensitivity of

its vessels to these hormones.

In further experiments, animals with unilateral renal denervation were subjected to haemorrhage of graded severity so as to test for the presence of nervous and humoral constrictor factors in acute hypovolaemic anaemia. These experiments demonstrated that a complex pattern of nervous and humoral control was acting upon the renal circulation after haemorrhage. The results obtained and the various alternative interpretations possible, have been discussed in detail in Chapter 4. While it cannot be stipulated on the basis of these experiments whether nervous or humoral mechanisms predominate in the renal response to haemorrhage, the following points can be marshalled in favour of an early humoral predominance followed by delayed neurogenic control:-

(i) In animals bled 15 ml/Kg, there was a delayed rise in the ratio of denervated RBF: innervated RBF, in contrast to the immediate converse humoral effect observed in the animals bled 20 ml/Kg.

(ii) After a bleed of 20 ml/Kg, the humoral effect persisted only for 10-20 minutes, and then the decreased D/I ratios returned to normal despite the continuance of renal vasoconstriction.

(iii) There was a progressive slight reduction in RBF in the innervated kidney during the first hour after severe haemorrhage, in contrast to the relatively constant flow in the denervated kidney. In one animal bled 20 ml/Kg, a definite biphasic response occurred, with initial reduction in D/I blood flow ratios, and subsequent elevation above control values.

(iv) When animals breathed low O_2 mixtures after haemorrhage, a much greater constrictor response was observed in the innervated kidney than was observed in animals not subjected to bleeding.

In the third type of anaemia studied each animal was bled repeatedly so as to reduce the haematocrit to a low level over a period of 4 days. No plasma was replaced, but the animal's body fluid control mechanisms were allowed 18-24 hours to operate after the last bleed. At this stage renal blood flow was decreased, although not as severely as in the period immediately after haemorrhage, and total blood volume was not restored completely to its control level. These findings suggested that constrictor factors associated with blood volume regulation might still be acting on the renal circulation. In other respects the renal effects of "chronic" anaemia were similar to those observed in severe normovolaemic anaemia; thus filtration fraction, renal PAH extraction ratio and Tm_{PAH} were each reduced approximately in proportion to the fall in haematocrit ratio. Although cardiac output was not measured in the present series, it is probable from the results of other workers (41, 43) that it was increased in "chronic" anaemia despite the finding of decreased renal blood flow. A parallel can be drawn between "chronic" anaemia and carboxyhaemoglobinaemia in that both these conditions apparently result in hypoxic vasodilation in most regions of the peripheral circulation, whilst the renal circulation may possibly be subject to a degree of extrinsic constrictor control.

SUMMARY AND ABSTRACT

This report concerns the effects on the renal circulation of the unanaesthetized rabbit of three types of experimental anaemia, each of which was characterized by different degrees of reduction in haematocrit and total blood volume.

In acute normovolaemic anaemia there was no change in renal blood flow, but reduction of the haematocrit to low levels resulted in renal tissue hypoxia, and reduction in renal vascular resistance, filtration fraction and renal PAH extraction ratio. Comparison was made with the effects of carboxyhaemoglobinaemia in animals with a normal haematocrit, and with the findings in severely anaemic animals whose blood O_2 transport was increased by breathing 100% O_2. It was concluded that renal tissue hypoxia and reduction in red cell concentration each contributed to the changes in the renal vascular bed and in PAH extraction ratio.

In acute hypovolaemic anaemia renal vasoconstriction resulted in a marked decrease of renal blood flow in the presence of moderate reduction in haematocrit. Filtration fraction was increased, and there was no change in renal PAH extraction ratio. Renal vasoconstriction appeared to be initiated by the reduction in total blood volume after haemorrhage, but persisted despite the initially rapid restoration of blood volume. By analogy with the effects of adrenaline, angiotensin and arterial hypoxia it was shown in animals with one kidney denervated and the other intact that haemorrhage resulted in a complex pattern of humoral and nervous constrictor effects on the renal circulation.

In the third anaemia studied, a severe degree of reduction in haematocrit was accompanied by slight reduction in total blood volume.

This type of anaemia was produced by repeated bleeding, following which the animal's body fluid control mechanisms were allowed 18-24 hours to operate before the effects on the renal circulation were determined. At this stage RBF was decreased, despite a reduction in filtration fraction, E_{PAH} and PAH transport capacity similar to that observed in severe normovolaemic anaemia. It appeared that the tendency to renal vasodilatation in severe anaemia was being modified by persistent nervous or humoral constrictor factors, associated with incomplete blood volume regulation.

In summary, it has been demonstrated that reduced red cell concentration, reduced oxygen transport and hypovolaemia can each be involved in the response of the renal circulation to post-haemorrhagic anaemia.

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APPENDIX

Appendix to Chapter 2.

Effects of Bilateral Ligation of Central Ear Vessels on Body Temperature in the Rabbit (Single experiment)

Treatment	Time	Rectal Temp. (°C)	Environmental Temp. (°C)
Control	0 mins.	40.5	28.2
	20 mins.	40.4	
	40 mins.	40.4	28.3
Ligation R.Ear	0 mins.	40.4	
	10 mins.	40.4	28.2
	20 mins.	40.4	
	40 mins.	40.4	
	60 mins.	40.3	28.4
Ligation L.Ear	0 mins.	40.4	28.6
	10 mins.	40.3	
	30 mins.	40.3	28.7
	2 hours	40.5	
	3 hours	40.55	28.2
Tracheotomy (Local anaesthesia)	0 mins.	40.3	28.1
	10 mins.	40.3	
	20 mins.	40.4	
	30 mins.	40.5	
	40 mins.	40.4	28.8
Radiator at 4 feet.	0 mins.	40.45	28.6
	10 mins.	40.6	35.0
	20 mins.	40.8	39.0
	30 mins.	40.9	43.0

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DATA IN NORMAL RABBITS

Body Weight (Kg)	Kidney Weight (Kg)	RBF (ml/min)	RBF BW	RBF KW	GFR (ml/min)	GFR BW	GFR KW	RPF (ml/min)	Ear artery pressure (min.Hg)	Heart rate (per min)
3.2	20.8	107	33.4	5.14	15.4	4.81	0.740	66.7	90	340
3.1	-	133	42.9	-	-	-	-	97.4	81	260
3.1	25.0	187	60.3	7.48	20.3	6.55	0.812	129	82	260
2.5	18.8	108	41.2	5.48	19.1	7.64	1.016	71.2	91	-
2.9	20.6	102	35.2	4.97	13.9	4.79	0.678	65.3	93	290
2.65	19.0	111	41.9	5.84	12.1	4.57	0.637	77.4	64	285
3.0	19.8	98	32.7	4.95	7.6	2.53	0.384	64.2	84	220
2.75	-	129	46.9	-	14.1	5.13	-	81.3	74	300
2.8	19.4	139	49.6	7.16	8.0	2.86	0.412	86.3	70	290
2.4	-	143	59.6	-	19.5	8.13	-	83.9	85	340
2.2	27.0	111	50.5	4.11	13.6	6.18	0.504	67.9	80	290
2.45	17.5	114	46.5	6.51	26.0	10.61	1.486	79.7	80	270
2.65	16.8	163	61.5	9.70	20.3	7.66	1.208	101	74	260
2.45	19.0	126	51.4	6.63	21.3	8.69	1.121	82.8	80	270
2.45	17.2	163	66.5	9.48	26.8	10.94	1.558	112	88	280
2.4	19.4	117	48.8	6.03	17.7	7.38	0.912	76.7	80	330
3.0	22.5	177	59.0	7.87	24.2	8.07	1.075	110	83	260
2.8	-	144	51.4	-	21.4	7.64	-	94.0	90	260
2.6	21.5	163	62.7	7.58	26.2	10.08	1.219	110	90	300
2.3	22.7	172	74.8	7.58	23.7	10.30	1.044	109	86	320
2.8	21.7	-	-	-	20.7	7.39	0.954	-	85	310
3.0	18.7	130	43.3	6.95	17.9	5.97	0.957	81.1	91	280
2.7	18.1	145	53.7	8.01	21.2	7.85	1.171	90.9	90	320
2.6	-	142	54.6	-	18.5	7.11	-	95.4	102	290
2.55	25.6	143	56.1	5.59	17.7	6.94	0.691	88.4	90	220
2.6	15.5	109	41.9	7.03	17.6	6.77	1.135	67.5	90	250
2.65	15.7	128	48.3	8.15	21.0	7.92	1.337	83.0	76	250
MEAN										
2.69	20.1	134.6	50.6	6.77	18.7	7.10	0.957	87.4	84.0	282
SD										
± 0.26	± 3.06	± 25.0	±10.3	±1.48	± 5.03	±2.15	±0.324	±16.9	± 8.04	± 32.3
n (27)	(22)	(26)	(26)	(21)	(26)	(26)	(22)	(26)	(27)	(26)

DATA IN RABBITS WITH URETER CATHETERS.

Body Weight (Kg)	Kidney Weight (g)	RBF (ml/min)	RBF BW	RBF KW	GFR (ml/min)	GFR BW	GFR KW	RPF (ml/min)	Ear artery pressure (mm.Hg.)	Heart rate (per min.)
2.2	14.3	47	21.4	3.29	7.9	3.59	0.552	29.4	78	200
1.9	12.4	66	34.7	5.32	11.2	5.89	0.903	38.0	85	240
2.06	17.5	75	36.6	4.29	11.6	5.66	0.663	46.6	109	240
2.25	14.1	117	52.0	8.29	18.8	8.35	1.333	74.3	90	230
3.05	16.8	98	32.1	5.83	21.4	7.02	1.274	60.6	86	270
2.7	20.7	87	32.2	4.20	11.2	4.15	0.541	47.0	87	240
2.3	20.0	121	52.6	6.05	16.2	7.04	0.810	70.5	89	250
2.9	20.3	123	42.4	6.06	19.7	6.79	0.970	67.6	90	240
MEAN.										
2.4	17.0	91.8	38.0	5.42	14.8	6.06	0.881	54.3	89	239
SD										
±0.41	± 3.2	±28.0	±10.6	±1.54	±4.92	±1.59	±0.303	±16.4	± 8.9	± 19.6

n (8)

RENAL PAH EXTRACTION RATIO IN 49 NORMAL RABBITS.

(Each value shown is the mean of 2 observations in one animal).

GROUP A - Renal vein catheterized by indirect method: anaesthesia and laparatomy 2-4 hours before observations (see Chapter 2).

GROUP B and C - Renal vein catheterized by direct method: anaesthesia and laparotomy 1-4 days before observations (see Chapter 2). Group B - observations made without mannitol diuresis. Group C - observations made during infusion of 5% mannitol at 0.7 ml/min.

Group A	Haemat-ocrit (%)	E_{PAH} (%)	Arterial plasma PAH conc.(mg%)
1	36	95.6	2.00
2	30	94.4	2.30
3	33	89.3	2.23
4	37	92.8	2.14
5	35	95.5	1.93
6	31	95.4	1.56
n = 6			
MEAN	33.7	93.8	2.03
SD	-	± 2.45	±0.26
GROUP B			
1	39	96.2	1.98
2	32	97.5	2.09
3	41	98.3	1.90
4	38	98.2	1.96
5	37	91.1	1.81
6	42	93.3	2.56
7	33	96.5	1.42
n = 7			
MEAN	37.4	95.9	1.96
SD	-	± 2.71	±0.34
GROUP C			
1	37	93.5	2.05
2	33	88.2	2.09
3	34	95.3	1.83
4	38	89.5	2.62
5	33	95.7	2.38
6	38	93.1	1.60
7	38	94.8	2.04
8	38	95.3	2.24
9	32	94.8	1.06
10	38	97.4	1.66
11	38	96.8	1.37
12	31	96.2	1.52
13	38	98.0	1.46
14	35	98.4	1.85
15	31	96.5	1.63
16	36	98.2	1.61
17	35	98.9	1.37
18	30	96.7	2.14
19	35	95.7	2.00
20	37	97.2	1.37
21	38	88.9	1.87
22	41	95.4	1.39
23	36	94.1	1.26
24	43	94.7	1.15
25	39	94.7	1.18
26	35	94.6	1.38
27	33	93.5	0.97
28	31	97.6	1.01
29	30	95.3	1.33
30	38	95.4	1.08
31	35	95.2	1.25
32	38	84.2	3.25
33	31	94.3	1.57
34	34	94.9	2.55
35	35	95.6	2.01
36	38	95.2	1.93
n = 36			
MEAN	35.6	94.8	1.70
SD	± 3.17	± 3.00	±0.512
Groups A, B and C.			
n = 49			
MEAN	35.6	94.9	1.77
SD		± 2.82	

Tm_{PAH} IN NORMAL RABBITS.

Body Wt. (Kg)	Kidney Wt. (g)	Haemat-ocrit (%)	Tm_{PAH} (mg/min)	Tm/BW	Tm/BW	Load T	Arterial plasma PAH (mg %)
2.4	19.4	31.9	12.4	5.17	0.639	1.91	37.3
2.8	-	32.1	17.8	6.36	-	1.44	25.7
3.0	22.5[x]	35.6	18.2	6.07	0.809	1.23	25.5
2.6	21.5	30.2	18.0	6.92	0.837	1.54	35.0
2.3	22.7	32.9	21.7	9.43	0.956	1.53	31.7
2.8	22.4	33.3	20.2	7.21	0.902	1.56	52.5
2.8	21.7	33.7	20.4	7.29	0.940	2.02	59.7
3.0	18.7	36.3	22.1	7.37	1.182	2.65	74.3
2.7	18.1	35.4	22.5	8.33	1.243	1.71	50.8
2.6	-	29.1	17.5	6.73	-	6.80	100.9
MEAN							
2.7	20.9	33.1	19.08	7.09	0.94	2.24	49.3
SD			± 3.01	±1.18	±0.196		
n							
10	(8)	(10)	(10)	(10)	(8)	(10)	(10)

x Unilateral solitary kidney (? congenital)

RENAL VENOUS O_2 SATURATION ($S_{RV_{O_2}}$) RENAL VENOUS O_2 TENSION ($P_{RV_{O_2}}$) AND RENAL O_2 CONSUMPTION ($V_{R_{O_2}}$) IN 10 NORMAL RABBITS.

(including all data except from animal No. 2 in which an exceptionally high value for $V_{R_{O_2}}$ was obtained)

Body weight (Kg)	Kidney weight (g)	Hb (g %)	$S_{RV_{O_2}}$ (%)	$P_{RV_{O_2}}$ (mm Hg)	$\dot{V}_{R_{O_2}}$ (ml/min)	$\frac{\dot{V}_{R_{O_2}}}{BW}$	$\frac{\dot{V}_{R_{O_2}}}{KW}$
2.55	19.8	9.4	73	44	4.98	1.95	0.252
2.65	15.7	10.2	75	46	4.12	1.55	0.262
2.45	17.5	9.6	63	37	5.12	2.09	0.292
2.2	21.0	10.8	71	43	4.44	2.02	0.211
3.1	20.8	11.7	78	49	4.50	1.45	0.216
2.4	-	10.7	70	42	5.81	2.42	-
2.9	17.4	12.5	72	44	4.13	1.42	0.237
2.65	19.0	9.3	73	44	3.55	1.34	0.187
3.0	19.8	10.7	80	52	2.50	0.83	0.126
2.9	20.5	12.0	84	56	2.33	0.80	0.114
MEAN							
2.68	19.1	10.7	74	46	4.15	1.59	0.21
SD			± 5.8		± 1.22		±0.06
(n)			(10)		(10)		(9)

RENAL PAH EXTRACTION RATIO IN 2 MERINO EWES.

Under halothane anaesthesia, the right renal vein in 2 normal ewe sheep was catheterized with 20 gauge "Transflex" tubing, using a similar technique to that described for the rabbit (see Chapter 2). In both sheep, small peri-hilar tributaries used to introduce the catheter tip into the renal vein stream. Measurements of renal PAH extraction ratio were made 24 hours after the operation, and repeated 2 days later. A priming dose of 100 mg PAH was given, and then a sustaining infusion injected at rates of 0.2-0.7 ml/min into a jugular vein catheter from a motor-driven syringe. The initial sustaining infusion consisted of 2 gm PAH per 100 ml sterile normal saline at a rate of 0.35 ml/min: this resulted in a plasma PAH concentration of 1.4-2 mg%. Subsequently the PAH content and rate of injection of the sustaining infusion were altered to obtain the PAH levels shown in table A_{10}. Arterial haematocrit and plasma PAH concentration were measured by means of an indwelling carotid artery catheter.

In both sheep on the first day of observations, a mild rigor occurred, accompanied by a rectal temperature of 104-105°F (normal body temperature for the sheep = 102°F). The reason for this pyrexia was not established, but it may have been due to pyrogenic contamination of the infusion tubing: it was transient only and did not recur during the second set of observations. Bleeding from the carotid artery wound was responsible for the fall in haematocrit which occurred in one animal, between the first and second set of observations (see Table A_{10}).

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RENAL PAH EXTRACTION RATIO IN THE SHEEP

Values for E_{PAH} obtained in 2 merino ewes at several different arterial PAH concentrations. Each estimate is based on one arterial and one renal venous blood sample. (Plasma blank varied from 0.001 - 0.003 O.D. units in 3 observations using arterial blood and 2 observations on renal venous blood).

	ANIMAL (1)			ANIMAL (2)		
	E_{PAH} (%)	Arterial plasma PAH (mg %)	Ht. (%)	E_{PAH} (%)	Arterial plasma PAH (mg %)	Ht. (%)
DAY 1	Insertion of carotid artery and renal vein catheters.					
DAY 2	97.3	1.39	30.0	95.8	1.78	33.0
	98.2	1.39		96.8	1.57	
				97.5	0.96	
				97 .3	0.91	
				97.1	0.85	
				98.5	0.82	
DAY 4	89.6	6.08	23.0	96.9	1.99	32.0
	83.8	12.20		98.0	4.17	
	81.0	24.65		97.2	6.18	
				96.2	12.70	
				70.9	32.27	

Mean of E_{PAH} values at titres of 1-3%.

(1) 97.8% at 1.39 mg % (2) 96.5% at 1.78 mg %.

DATA FROM REF. 65 (Forster, 1947)

Renal haemodynamics in unanaesthetized rabbits.*

Rabbit No.	BW (Kg)	ERPF (ml/min)	ERPF / BW	GFR (ml/min)	GFR / BW	FF
R54	2.9	47	16.2	15	5.17	.320
R27	3.0	70	23.3	15	5.00	.220
R52	2.2	35	15.9	12	5.45	.340
R63	-	65	-	16	-	.250
R72	-	43	-	12	-	.280
R11	-	40	-	9	-	.220
MEAN		50.0	18.5	13.2	5.21	.272
n		(6)	(3)	(6)	(3)	(6)

* Moderate mannitol diuresis (3g % in infusion at 0.6 ml/min)

DATA FROM REF. 169 (Smith, W.W., 1941)

Renal haemodynamics in unanaesthetized rabbits *

Kidney weight (g)	GFR (inulin) (ml/min/g)	ERPF (diodrast) (ml/min/g)	FF
17.9	0.63	2.32	0.27
18.6	0.74	3.72	0.20
19.3	0.75	2.25	0.33
18.0	0.78	2.90	0.27
19.1	0.57	2.05	0.28
22.8	0.59	1.93	0.30
20.4	0.60	2.33	0.26
15.3	0.65	2.32	0.28
MEAN (n) = 8	0.66	2.48	0.27

* Water loading by stomach tube. Blood samples by cardiac puncture.

DATA FROM REF. 87 (Josephsen and Kallas, 1953)

GFR (creatinine clearance) in unanaesthetized rabbits.[x]

Rabbit No.	BW (Kg)	GFR[xx] (ml/min)	$\frac{GFR}{BW}$
19	5.0	18.3	3.66
20	4.5	12.7	2.82
30	4.4	16.2	3.68
31	3.3	10.6	3.21
32	3.4	13.0	3.82
43	4.3	12.5	2.91
41	4.4	14.3	3.25
42	5.5	18.9	3.44
MEAN	4.3	14.6	3.35
(n)	(8)	(8)	(8)

[x] 40 ml/Kg water load.

[xx] Control measurements only (first 2-3 clearance periods)

DATA FROM REF. 86 (Josephson et al, 1953)

Tm_{PAH} in Blue Wiener rabbits.

From Table 1 (first 3 collection periods)

BW = 4.8 Kg

Arterial PAH plasma conc. = 6.8 m M/litre = 131.6 mg %.

Tm_{PAH} = 140 μ M/minute = 27.1 mg/min

Tm_{PAH}/BW = 5.65 mg/min/Kg,

From Table 4 (lower part - first 3 collection periods)

BW = 3.8 Kg

Arterial PAH plasma conc. = 2.34 m M/litre = 45.3 mg %.

Tm_{PAH} = 125 μ M/minute = 24.2 mg/min

Tm_{PAH}/BW = 6.37 mg/min/Kg

Mean Tm_{PAH} in 2 animals = 6.01 mg/min/Kg

CARBON MONOXIDE EXPERIMENTS (i)

(Animals No. 1 - 5)

% CO breathed	Body wt. (Kg)	Kidney wt. (g)	Haematocrit (%)	E_{PAH} (%)	RBF (ml/min)	GRF (ml/min)	FF	Arterial pressure (mm Hg)	Heart rate (/min)	Haemoglobin (g%)	COHb (%)	$S_{RV_{O_2}}$ (%)	$P_{RV_{O_2}}$ (mm Hg)	$V_{R_{O_2}}$ (ml/min)
0	2.55	19.8	31.2	97.6	158'	-	-	83	290	9.4	-	73	44	4.98
0.3			28.1	91.1	103	20.3	.275	59	320	9.0	55	24	16	2.62
0	2.64	16.8	38.3	95.4	163	20.3	.201	74	260	10.2		64	38	7.67
0.3			34.0	92.3	98'	12.7'	.196'	68	300	10.1	55	27	18	2.39
0	2.65	15.7	35.1	95.2	128	21.0	.252	76	250	10.2	-	75	46	4.12
0.3			32.0	88.0	101	17.4	.254	61	320	10.5	58	24	16	2.67
0	2.8	19.4	38.0	88.9	-	-	-	70	290	12.6	-	76	47	-
0.2			31.1	87.2	-	-	-	64	300	11.2	46	45	34	-
0.26			27.0	78.4	-	-	-	52	260	9.5	49	38	26	-
0	2.45	17.5	30.1	95.3	114	26.0	.326	80	270	9.6	-	63	37	5.12
0.3			29.2	87.3	87	12.3	.200	62	340	9.6	56	26	17	2.01

' Clearance variation $>\pm$ 15% of mean

CARBON MONOXIDE EXPERIMENTS (ii)

(Animals No. 6 - 11)

% CO breathed	Body wt. (Kg)	Kidney wt. (g)	Haemat-ocrit (%)	E_{PAH} (%)	RBF (ml/min)	GRF (ml/min)	FF	Arterial pressure (mm Hg)	Heart rate (/min)	Haemo-globin (g%)	COHb (%)	$S_{RV_{O_2}}$ (%)	$P_{RV_{O_2}}$ (mm Hg)	$V_{R_{O_2}}$ (ml/min)
0	2.2	21.0	39.0	94.7	111	13.6	.200	80	290	10.8	-	71	43	4.44
0.2			36.0	90.3	120	15.8	.205	64	350	10.2	50	33	22	2.94
0.3			28.7	89.0	84'	11.5'	.192'	50	310	8.7	55	24	15	2.11
0	3.1	20.8	43.4	94.7	147'	-	-	81	310	11.7	-	78	49	4.50
0.2			39.0	95.0	123'	-	-	82	340	11.0	49	-	-	-
0.3			29.3	69.6	58	-	-	70	290	19.8	56	-	-	-
0	2.4	-	41.2	95.4	143	19.5	.232	85	340	10.7	-	70	42	5.81
0.18			34.9	93.8	133	19.0	.219	71	330	10.1	19	51	30	5.16
0	2.75	-	36.9	97.2	129	14.1	.173	74	300	11.6	-	-	-	-
0.2			33.0	95.7	124	16.4	.197	60	310	11.0	45	34	22	3.77
0	2.9	17.4	36.9	96.6	95'	-	-	91	320	12.5	-	72	44	4.13
0.2			34.7	95.5	88'	-	-	86	320	11.3	25	35	21	5.29
0.26			30.0	93.0	45	7.1	.228	82	310	9.9	46	23	14	1.87
0	2.8	-	35.9	94.1	-	-	-	83	280	13.4	-	64	38	-
0.17			32.5	91.1	-	-	-	74	320	10.9	42	47	34	-

' Clearance variation $> \pm$ 15% of mean

EFFECTS OF ACUTE NORMOVOLAEMIC ANAEMIA (i)

(GROUP I ANAMALS)

No.	Body wt. (Kg)	Kidney wt. (g)	Haemat-ocrit (%)	E_{PAH} (%)	RBF (ml/min)	GFR (ml/min)	FF	Arterial pressure (mm Hg)	Heart rate (/min)	Haemo-globin (g %)	$S_{VR_{O_2}}$ %	$P_{VR_{O_2}}$	$V_{R_{O_2}}$ (ml/min)
12	2.65	19.0	30.3	96.7	111	12.1	.156	64	285	9.3	73	44	3.55
			18.7	95.7	106	13.7	.159	68	310	5.7	67	40	2.60
13	3.0	19.8	34.7	95.7	98	7.6	.118	84	220	10.7	80	52	2.50
			20.0	95.3	72	8.0	.139	88	280	6.3	72	44	1.61
14	2.9	20.5	35.7	98.2	102	13.9	.213	93	290	12.0	84	56	2.33
			22.2	98.3	131	17.1	.168	91	300	7.2	80	52	2.34
15	3.2	20.8	37.8	96.8	107	15.4	.230	90	340	14.0	-	-	-
			18.4	89.1	83	12.7	.187	72	330	6.3	-	-	-
16	3.1	25.0	31.1	96.2	187	20.3	.158	82	260	10.1	-	-	-
			19.5	96.3	207	20.8	.125	89	290	6.2	-	-	-
17	4.3	26.4	37.7	98.0	179	29.7	.264	88	290	12.4	-	-	-
			23.5	96.6	188	30.9	.215	83	330	7.9	-	-	-

EFFECTS OF ACUTE NORMOVOLAEMIC ANAEMIA (ii)

(GROUP II ANIMALS)

No.	Body wt. (Kg)	Kidney wt. (g)	Haemat-ocrit (%)	E_{PAH} (%)	RBF (ml/min)	GFR (ml/min)	FF	Arterial pressure (mm Hg)	Heart rate (/min)	Haemo-globin (g %)	$S_{VR_{O_2}}$ %	$P_{VR_{O_2}}$	$V_{R_{O_2}}$ (ml/min)
4	2.8	19.4	15.3	89.0	-	-	-	63	320	4.6	72	44	-
			8.2	81.8	-	-	-	44	290	2.6	71	43	-
18	3.2	24.5	28.9	95.2	147	21.1	.202	78	290	7.8	73	44	3.91
			15.0	93.3	163	21.6	.156	71	320	4.1	60	36	3.59
19	3.0	15.5	19.3	95.4	86	-	-	72	310	5.3	75	46	1.71
			8.9	92.2	99	8.9	.099	54	300	2.2	66	39	1.06
20	2.7	20.6	20.8	94.0	138	20.8	.190	69	270	6.3	68	40	3.70
			12.5	91.3	171	21.2	.141	59	300	3.1	63	37	2.74
21	3.0	21.0	20.9	97.7	116	19.4	.211	80	290	6.3			
			13.3	94.3	112	18.1	.186	73	310	3.5			
22	2.8	17.6	24.0	95.4	112	17.5	.206	86	270	7.4			
			9.0	88.5	91	10.1	.122	63	270	2.6			
23	2.8	17.8	25.7	99.0	134	18.9	.190	87	290	7.4			
			13.1	98.0	104	19.5	.216	77	350	3.5			
24	2.4	(16.0)	25.0	88.8	88	11.2	.170	85	300	7.4			
			10.5	84.0	91	7.9	.097	78	290	3.1			

EFFECTS OF ACUTE NORMOVOLAEMIC ANAEMIA (iii) CONTINUED FROM (ii)

(GROUP II ANIMALS)

No.	Body wt. (Kg)	Kidney wt. (g)	Haemat-ocrit (%)	E_{PAH} (%)	RBF (ml/min)	GFR (ml/min)	FF	Arterial pressure (mm Hg)	Heart rate (/min)	Haemo-globin (g %)
25	2.3	16.4	23.1	98.2	105	17.4	.215	79	280	6.7
			13.0	96.8	113	13.9	.141	72	340	3.8
26	2.6	15.1	23.7	95.4	122	21.8	.234	92	260	6.8
			14.2	88.5	122	19.2	.183	91	330	4.2
27	2.8	18.7	25.4	96.0	143	19.9	.187	81	300	7.0
			12.0	87.2	127	21.7	.194	69	370	3.1
28	2.8	16.0	21.9	99.0	96	15.7	.210	75	310	6.4
			12.2	97.4	87	13.7	.180	64	310	3.8
			7.8	93.8	112	11.8	.114	59	320	2.3
29	2.6	21.2	20.9	98.2	-	-	-	87	290	-
			14.6	95.9	-	-	-	76	330	-
30	3.3	-	23.2	97.5	-	-	-	80	330	8.7
			14.7	95.1	-	-	-	78	330	4.5

EFFECTS OF ACUTE NORMOVOLAEMIC ANAEMIA (iv)

(GROUP II ANIMALS)

No.		Body wt. (Kg)	Kidney wt. (g)	Haemat-ocrit (%)	E_{PAH} (%)	RBR (ml/min)	GFR (ml/min)	FF	Arterial pressure (mm Hg)	Heart rate (/min)	Haemo-globin (g %)	$S_{VR_{O_2}}$ %	$P_{VR_{O_2}}$	$V_{R_{O_2}}$ (ml/min
	C	2.0	16.1	19.7	92.3	103	20.2	.243	82	295	6.1	56	34	3.64
31	T_1			8.8	77.3	107	11.7	.120	50	325	2.3	34	28	2.34
	T_2			8.5	78.8	88	12.1	.151	61	325	2.4	74	45	2.44
	C	2.3	15.8	23.3	92.5	153	23.0	.196	83	260	7.1	64	38	5.11
32	T_1			12.3	88.2	160	19.0	.136	73	320	3.7	53	33	3.74
	T_2			11.4	90.0	141	17.3	.139	68	275	3.8	71	43	4.77
	C	2.2	14.3	22.6	94.2	91	13.5	.192	76	320	6.7	60	36	3.17
33	T_1			10.2	91.8	107	13.3	.139	59	320	2.8	44	32	2.31
	T_2			9.6	89.9	90	13.4	.165	65	320	2.7	71	43	2.69
	C	2.9	18.6	22.6	95.1	141	23.8	.216	77	305	6.9	65	38	4.47
34	T_1			12.2	94.6	190	22.6	.133	60	320	3.3	59	35	3.55
	T_2			10.7	93.3	-	-	-	58	300	3.1	86	48	-
	C	-	17.6	15.3	88.6	95	7.5	.092	75	275	4.0	37	29	3.27
35	T_1			8.9	88.6	90	2.9	.032	47	335	2.2	33	28	1.87
	T_2			7.6	88.7	113	8.3	.080	53	300	2.1	85	59	2.58
	C	2.4	18.0	23.6	98.0	143	22.3	.205	78	300	7.0	56	34	5.76
36	T_1			10.4	95.5	150	19.6	.146	67	320	3.0	49	32	3.18
	T_2			10.2	97.8	128	18.3	.158	70	310	2.8	78	49	3.51

EFFECTS OF ACUTE NORMOVOLAEMIC ANAEMIA (V)
(GROUP II ANIMALS)

CONTINUED FROM (1V) T_1 = Breathing room air T_2 = Breathing 100% O_2

No.	Body wt. (Kg)	Kidney wt. (g)	Haemat-ocrit (%)	E_{PAH} (%)	RBF (ml/min)	GFR (ml/min)	FF	Arterial pressure (mm Hg)	Heart rate (/min)	Haemo-globin (g %)	$S_{VR_{O_2}}$ %	$P_{VR_{O_2}}$	$V_{R_{O_2}}$ (ml/min)
	C 2.2	19.0	17.0	90.0	70	7.7	.133	71	285	5.0	44	31	2.63
37	T_1		8.3	86.9	108	7.6	.077	55	270	2.3	47	32	1.90
	T_2		7.2	87.6	110	9.0	.087	58	290	2.3	83	55	2.67
	C 2.55	19.8	22.0	96.7	-	-	-	72	280	5.9	56	34	-
1	T_1		10.4	89.1	-	-	-	44	300	3.0	44	32	-
	T_2		9.2	90.1	-	-	-	53	300	3.0	72	44	-
	C 2.64	16.8	22.2	94.2	-	-	-	81	250	6.7	63	37	-
2	T_1		11.1	85.9	-	-	-	67	310	3.4	47	32	-
	T_2		10.9	91.9	-	-	-	72	290	3.3	76	47	-
	C 2.65	15.7	18.9	90.6	-	-	-	65	280	4.7	37	29	-
3	T_1		9.6	85.4	-	-	-	50	310	2.5	23	22	-
	T_2		10.6	88.2	-	-	-	55	330	2.3	67	40	-

EFFECTS OF HYPOTENSIVE AGENT (ARFONAD)

C = control period T = Arfonad infusion (2mg/min)

No.		Body wt. (Kg)	Kidney wt. (g)	Haemat-ocrit (%)	E_{PAH} (%)	RBF (ml/min)	GFR (ml/min)	FF	Arterial pressure (ml/Hg)	Heat rate (min)	Haemo-globin (g%)	$S_{VR_{O_2}}$ (%)	$V_{R_{O_2}}$ (ml/min)
38	C_1	2.45	19.0	34.5	94.6	126	21.3	.257	80	270	10.6	69	4.1
	T			30.4	96.0	123	21.2	.247	64	350	9.9	70	3.7
	C_2			30.5	96.8	118	23.7	.289	80	280	9.8	67	4.1
39	C_1	2.25	16.3	32.8	93.5	-	-	-	69	220	10.4	80	-
	T			28.2	94.4	-	-	-	50	280	9.0	76	-
	C_2			-	93.8	-	-	-	66	240	-	-	-

BLOOD VOLUME EXPERIMENTS

Animals 40 - 44 Details are given in Figure 42

APPENDIX TO

CHAPTER 5

EFFECTS OF HAEMORRHAGE (ACUTE HYPOVOLAEMIC ANAEMIA)

(i) "NORMAL ANIMALS (see Fig. 44)

C= -30 to 0 min. T_1= 0 to 30 mins, T_2 = 30 to 60 mins

RED CELL INFUSION = 60 to 80 min

T_3= 120 to 150 min, T_4= 150 to 180 min

No.		Body wt. (Kg)	Kidney wt. (g)	4t (%)	E PAH (%)	RBF (ml/min)	GFR	FF	Arterial pressure (min/Hg)	Heart rate (/min)	Haemo-globin (g%)
45 (Bled 15ml/kg)	C	2.55	25.6	38.2	94.8	143	17.7	0.200	90	220	11.1
	T_1			31.1	94.4	102	19.7	.281	80	260	9.3
	T_2			28.0	94.2	94	18.9	.280	78	270	8.8
	T_3			34.1	92.7	124	18.3	.224	84	230	11.0
	T_4			32.8	93.1	115	17.8	.231	80	260	10.7
46 (Bled 20ml/kg)	C	2. 6	15.5	38.0	95.3	109	17.6	0.261	90	250	10.8
	T_1			26.9	95.1	75	14.6	.266	83	260	8.5
	T_2			24.0	93.0	69	15.5	.294	86	260	-
	T_3			34.0	87.0	98	17.3	.268	91	280	10.6
	T_4			32.3	92.1	70	14.8	.314	90	280	10.1
47 (Bled 15 ml/Kg)	- E_{PAH}, etc only - no clearances (see Table 18)										

EFFECTS OF HAEMORRHAGE
(ii) "URETER" ANIMALS - INNERVATED (figs 44,46)

C_1=-40to-20min, C_2=-20to0, T_1=0to20, T_2=20to40, T_3=40to60min

No.		Body wt. (kg)	Kidney wt. (g)	Ht (%)	HG (g%)	RBF (ml/min) R	RBF (ml/min) L	GFR (ml/min) R	GFR (ml/min) L	Arterial pressure (min Hg)
48	C_1	2.2	14.3	37.1	11.9	23.7	23.0	3.9	4.0	78
	C_2			36.9	11.6	21.9	21.5	3.7	3.7	80
(Bled 15ml/kg)	T_1			27.7	8.7	11.5	11.3	2.6	2.7	67
	T_2			25.6	8.2	10.8	10.2	2.8	2.3	68
	T_3			25.2	7.9	11.0	10.7	2.8	2.7	66
49	C_1	1.9	12.4	42.4	13.3	34.0	32.0	5.9	5.3	85
	C_2			42.0	13.3	32.5	29.9	6.3	6.0	85
(Bled 15ml/kg)	T_1			30.2	9.2	17.3	14.5	4.4	4.0	66
	T_2			29.8	8.5	16.9	14.0	4.6	3.7	69
	T_3			24.8	7.8	16.5	14.0	4.3	3.5	69
50	C_1	2.05	17.5	37.5	11.5	35.8	38.8	5.8	5.9	109
	C_2			37.1	11.2	36.6	39.9	5.3	6.1	107
(Bled 20ml/kg)	T_1			25.1	7.8	19.4	22.7	3.9	4.7	86
	T_2			24.9	7.3	21.3	22.8	3.7	4.2	86
	T_3			22.8	6.3	21.1	22.8	4.8	5.2	88
51	C_1	2.25	14.1	36.8	11.0	57.6	58.4	9.0	9.8	90
	C_2			35.9	10.6	56.9	58.1	9.2	9.7	87
(Bled 15ml/kg)	T_1			28.5	8.3	39.2	40.2	7.9	8.3	79
	T_2			26.7	6.6	38.5	41.9	7.4	8.4	82
	T_3			25.9	7.6	38.2	38.0	7.5	7.9	76

Animals 52,53 ($2\frac{1}{2}$hour period after bleed) are shown in Table 21 and Fig. 44.

Total blood volume values (ml):-

	At T-30	Amount bled	At T+30	At T+150
52	150	46 ml	120	134
53	133	39 ml	117	131
54	129	31 ml	111	-

EFFECTS OF HAEMORRHAGE
(iii) "URETER" ANIMALS BLED 15ml/kg- LEFT DENERVATION (Fig 47)

C_1=-40to-20, C_2=-20to0, T_1=0to20, T_2=20to40, T_3=40to60min.

No.		Body wt. (kg)	Kidney wt. (g)	Ht (%)	Hb (g%)	RBF (ml/min) R	RBF (ml/min) L	GFR (ml/min) R	GFR (ml/min) L	Arterial pressure (min Hg)
55	C_1	2.4	20.3	38.9	13.2	53.9	62.3	6.1	7.2	80
	C_2			38.3	13.0	47.6	52.5	6.0	6.8	77
	T_1			28.1	9.0	11.7	7.5	1.9	1.1	54
	T_2			25.2	7.9	21.4	18.5	2.8	2.4	56
	T_3			24.4	7.1	15.8	19.3	2.6	2.6	59
56	C_1	2.25	19.2	43.7	14.3	42.8	46.4	6.7	8.0	75
	C_2			41.3	13.6	46.5	51.1	7.9	8.9	82
	T_1			32.6	9.6	20.7	24.6	4.6	5.0	59
	T_2			28.0	8.8	25.8	31.9	5.9	6.5	61
	T_3			27.0	8.8	27.2	32.5	6.2	6.9	59
57	C_1	1.9	15.9	41.3	13.8	49.8	51.1	4.7	5.6	67
	C_2			41.2	14.0	46.9	45.9	4.8	4.6	66
	T_1			34.0	11.1	26.0	26.7	4.0	4.1	53
	T_2			31.8	10.3	28.2	31.5	4.4	4.3	53
	T_3			31.0	10.0	33.2	32.3	4.6	4.8	54
58	C_1	2.6	19.7	40.9	12.8	51.9	57.2	6.2	6.7	85
	C_2			40.4	12.8	49.2	54.0	8.5	8.5	85
	T_1			33.9	10.5	30.9	43.0	6.2	7.5	70
	T_2			33.0	10.3	27.2	40.1	5.0	7.1	70
	T_3			31.1	9.7	28.0	40.4	5.9	7.3	72
59	Observed $2\frac{1}{2}$ hours after bleeding - Shown in Table 21.									

EFFECTS OF HAEMORRHAGE

(iv) "URETER" ANIMALS BLED 20ml/kg - LEFT DENERVATION (Fig 47)

No.		Body wt. (kg)	Kidney wt. (g)	Ht. (%)	HG (g%)	RBF (ml/min) R	RBF (ml/min) L	GFR (ml/min) R	GFR (ml/min) L	Arterial pressure (mm. HG)
60	C_1	2.15	17.9	41.9	12.6	52.7	51.7	9.5	8.4	86
	C_2			39.8	12.8	45.8	49.3	6.0	7.0	87
	T_1			26.6	8.1	23.0	19.8	5.7	5.7	64
	T_2			25.0	7.6	18.0	16.4	4.6	4.8	61
	T_3			23.4	7.1	17.6	18.0	4.1	5.5	63
61	C_1	2.4	15.0	43.0	13.8	38.7	42.9	7.1	7.3	103
	C_2			42.8	14.1	40.9	45.4	7.1	8.1	101
	T_1			27.1	9.0	17.8	17.3	5.0	5.7	62
	T_2			25.4	8.3	15.6	16.5	4.9	4.8	68
	T_3			23.3	7.5	16.1	16.5	5.0	5.5	62
62	C_1	2.4	17.2	36.2	12.2	47.5	56.1	8.2	8.9	100
	C_2			36.2	11.8	47.7	51.4	7.5	7.1	103
	T_1			19.9	6.3	16.9	11.1	2.7	1.1	72
	T_2			18.7	6.0	28.6	23.9	4.1	4.0	80
	T_3			17.2	5.5	24.2	20.3	4.8	2.9	74
63	C_1	2.35	21.2	37.8	11.6	76.4	81.0	10.6	11. 2	81
	C_2			36.5	11.2	78.5	82.2	11.1	11.6	81
	T_1			27.5	8.6	51.5	53.7	10.8	10.9	66
	T_2			26.1	8.2	40.1	45.2	9.2	9.4	67
	T_3			25.6	7.9	41.1	47.3	9.2	10.0	64

64 Observed $2\frac{1}{2}$ hours after bleeding - Table 21.

EFFECTS OF ADRENALINE, ANGIOTENSIN.

Animals 65-70 Fig. 45, Tables 20, 19.

APPENDIX TO

CHAPTER 6

EFFECTS OF "CHRONIC" ANAEMIA (i)

C = control period T = observations in anaemia 4 days later

No.		Body wt. (Kg)	Kidney wt. (g)	Ht (%)	E_{PAH} (%)	RBF (ml/min)	GFR (ml/min)	FF	Arterial pressure (mm.Hg)	Total blood volume (ml)	Tm PAH (mg/min)
71	C	2.45	17.2	31.4	94.3	163	26.8	.240	88	176	-
	T			12.2	84.1	127	22.6	.203	68	172	12.8
72	C	2.4	19.4	34.4	94.9	117	17.7	.231	80	157	12.4
	T			9.5	88.2	89	10.6	.131	65	153	10.2
73	C	3.0	22.5	37.7	95.2	177	24.2	.219	83	202	18.2
	T			12.7	92.1	139	17.4	.144	62	195	16.1
74	C	2.6	21.5	33.0	88.2	163	26.2	.239	90	159	18.0
	T			9.0	71.4	140	15.7	.123	61	148	13.6
75	C	2.3	22.7	36.6	93.5	172	23.7	.218	86	167	21.7
	T			15.9	91.5	134	22.2	.198	73	141	14.9
76	C	3.0	18.7	37.6	89.5	130	17.9	.221	91	-	22.1
	T			18.0	84.4	96	15.5	.197	80	-	19.5
77	C	2.8	25.6	34.1	95.3	-	-	-	76	162	-
	T			12.6	86.2	134	19.2	.198	66	160	16.4

EFFECTS OF CHRONIC ANAEMIA (ii)

No.		Body wt. (Kg)	Kidney wt. (g)	Ht. (%)	E PAH (%)	RBF	GFR (ml/min)	FF	Arterial pressure (mm.Hg)	Total blood volume (ml)	Tm PAH (mg/min)
78	C	2.8	21.7	38.0	95.3	-	20.7	-	85	-	20.4
	T			18.3	91.4	128	24.4	.232	87	-	18.1
79	C	2.8	22.4	33.8	-	-	-	-	-	-	20.2
	T			25.3	-	-	-	-	-	-	18.8
80	C	2.7	18.1	35.4	93.4	145	21.2	.233	90	-	22.5
	T			14.3	-	-	-	-	69	-	13.8
OTHER Tm_{PAH} DETERMINATIONS											
81		2.8	-	34.5	95.6	144	21.4	.228	90	156	17.8
82		2.6	-	33.0	95.7	142	18.5	.205	102	-	17.5

ADDITIONAL BLOOD VOLUME EXPERIMENTS.

Animals 83 - 87 shown in Table 24.