PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The Effect of Water and of Isotonic Saline Administration on the Renal Plasma and Glomerular Filtrate Flows in the Dog, with Incidental Observations of the Effects on these Flows of Compression of the Carotid and Renal Arteries

R. V. Sellwood and E. B. Verney

Phil. Trans. R. Soc. Lond. B 1955 **238**, 361-396
doi: 10.1098/rstb.1955.0003

THE EFFECT OF WATER AND OF ISOTONIC SALINE ADMINISTRATION ON THE RENAL PLASMA AND GLOMERULAR FILTRATE FLOWS IN THE DOG, WITH INCIDENTAL OBSERVATIONS OF THE EFFECTS ON THESE FLOWS OF COMPRESSION OF THE CAROTID AND RENAL ARTERIES

By R. V. SELLWOOD* and E. B. VERNEY, F.R.S.

Pharmacology Laboratory, University of Cambridge

(*Received* 8 *June* 1954—*Revised* 20 *July* 1954)

CONTENTS

* Research Scholar, Australian National University, Canberra.

The effects of water and of isotonic saline administration on the renal plasma and glomerular filtrate flows of the living dog are described.

The plasma extraction ratio for *p*-amino-hippurate (PAH) during renal passage has been directly determined under the conditions of our experiments; and it is shown that this ratio gives, at low arterial plasma levels of PAH, a reliable measure of plasma flow. This also holds after water administration and during the large rise in blood pressure produced by bilateral carotid occlusion.

In control experiments, serial determinations of renal plasma and filtrate flows over a period of about 2 h showed that these flows remained remarkably constant.

After water administration the renal plasma and filtrate flows increased, the increases being related to the dose of water given. The filtration fraction was little altered in animals with intact renal nerve supply.

Isotonic saline, in the same dosage as water, also increased the filtrate flow, but the increase in plasma flow was minimal or absent in animals with intact renal nerve supply. The filtration fraction therefore rose.

When the renal nerve supply had been interrupted the plasma flow increased after saline was given, and the plasma-flow response to water was greater than it was when the renal nerve supply was intact. Renal denervation had no significant effect on the filtrate-flow response to water, but increased that to saline. Renal denervation, therefore, had no significant effect on the filtration fraction after saline administration, but caused a fall in the filtration fraction after water administration.

The increases in plasma and filtrate flows preceded the increases in urine flow. They were not the result of alterations in blood pressure, nor was their trend affected by antidiuretic hormone in a dosage sufficient to inhibit the diuresis.

Any immediate changes in the plasma and filtrate flows that may have occurred when the blood pressure to the kidney was suddenly and reversibly altered by bilateral occlusion of the common carotid arteries or by partial obstruction of the renal artery, were rapidly neutralized by vascular readjustment within the kidney.

Large variations in plasma and filtrate flows between successive fifteen minute periods were observed in one animal made hypertensive by chronic partial constriction of the artery to one kidney at a time when, the arterial pressure being normal, the other kidney had become grossly shrunken and fibrotic as a result of arterial thrombosis. Denervation of the kidney with the mechanically constricted artery largely abolished these variations.

The results are discussed, and a hypothesis is advanced which, in order to account for the changes observed in plasma and filtrate flows after water and saline administration to 'innervated' and 'denervated' animals, postulates a functioning relationship between the intratubular pressure at some undetermined site and the resistance of the preglomerular vessels.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Introduction

Two methods are available for estimating changes in renal blood flow in the dog after the peroral administration of water and during the subsequent diuresis, the thermostromuhr method of Rein (1928, 1929*a*, *b*, 1931) and the method involving the Fick principle applied to the excretion of a particular substance present in or introduced into the blood. Results with the former method have been conflicting. Janssen & Rein (1928) reported no change in flow in decerebrated animals; no details of the experiments are given. Walker, Schmidt, Elsom & Johnson (1937), using a modification of Rein's instrument, found 'no consistent parallelism between blood and urine flows enduring throughout an entire experiment'. The variations in blood flow were large: in the four experiments recorded, the maximum flow during the period of observation exceeded the minimum flow by 43 %, 33, 15 and 25 % of the latter value. The stromuhr unit was applied under paraldehyde or pentobarbitone anaesthesia, and observations were made 15 to 17 h later. Handovsky & Samaan (1937), on the other hand, recorded increases in blood flow of between 30 and 70 % that were consistently related to diuresis, an increase being evident 5 min after the water (20 to 30 ml./kg body wt.) was given, and progressing to a plateau maximum well in advance of the rising urine flow. In their experiments a Rein-unit was applied under ether anaesthesia to the left renal artery by the lumbar route, the wound being closed around the emergent leads and the rubber tube that conveyed the urine from the cannulated ureter, an operation that 'could be easily completed within 10 to 20 min.' Observations were made from 4 h to 4 days after the animal had recovered from the anaesthetic. Rein (1929*a*), in the description of his method, had emphasized the importance of ensuring constancy in thermal contact between the thermocouples and the vessel wall, and this was largely achieved in the survival experiments of Cowan, Verney & Vogt (1938). Here the galvanometer readings were irregular in the first few days after operation and then became much steadier, a change that was probably associated with the development of a thin fibrous capsule which, when the animals were later killed, was seen snugly enclosing the stromuhr and immobilizing it by merging with the adventitia of the renal artery where the vessel entered and left the unit. At such times variations in flow of the order of 10 % were, however, encountered even when the animal was standing still; and no significant change in blood flow resulted from the giving of water (20 ml./kg body wt.) or from the intravenous injection of small doses (e.g. 4 mU)* of post-pituitary extract. The record of an experiment where 30 ml. water/kg body wt. were given shows, however, a small increase in blood flow which preceded the peak of diuresis and was maintained during the marked and prolonged inhibition by post-pituitary extract (Cowan *et al.* 1938, fig. 9; cf. fig. 8).

The second method theoretically involves the intravenous infusion of a non-toxic substance having a high, known and constant renal extraction ratio, and estimation of the period output of the substance by the kidney and the mean concentration of the substance in the arterial plasma during the same secretory period. The substance which, up to now, has been found most suitable for estimation of renal blood and plasma flow

* 1 milliunit (mU) is the specific antidiuretic activity corresponding to that yielded by 0·5 μg of the International Standard Preparation (Dry Pituitary, Posterior Lobe, Powder) when extracted by the prescribed method.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

is sodium *p*-amino-hippurate (PAH). It was introduced for this purpose by Smith, Finkelstein, Aliminosa, Crawford & Graber in 1945, and we have used it in the present studies. Smith *et al.* (1945) state that 'excessive hydration...tends to cause renal hyperaemia in the dog', but they give no details.

Simultaneously with PAH we have infused creatinine in order to obtain serial measurements of the glomerular filtration rate; we have assumed that creatinine is eliminated quantitatively in the dog by a process of glomerular filtration. Shannon (1936), working on the dog, states that 'the rate of glomerular filtration is essentially unrelated to the rate of water excretion within the range of ordinary experimental urine flows....At rates of urine formation above 4·0 ml./min. there is a variable tendency for the filtration rate to increase....Conversely, when the urine is below 0·5 ml./min, the filtration rate tends to fall.' And in a later paper (Shannon 1942, table III) figures are given which show a decrease in filtration rate during the subsidence of a diuresis from water by stomach tube in a dose of 5·0 % of body weight.

No systematic studies have yet been made with a view to following the renal plasma and filtrate flows simultaneously in the living dog after the peroral administration of water on the one hand and isotonic sodium chloride on the other; and we felt that if this were done some light might be thrown on the nature of the accompanying intrarenal events. Our object, therefore, has been to follow by means of PAH and creatinine infusions the renal plasma flow and glomerular filtration rate in trained bitches over periods of some 3 h, to determine whether the peroral administration of water or of isotonic sodium chloride affects these values, and finally to see whether the behaviour of the animal in these respects is changed as a result of denervation of the kidneys or decentralization of the sympathetic system. During the course of this work opportunity arose of making a few observations of the effects on plasma and filtrate flows of carotid occlusion and of temporary and chronic partial obstruction of the renal artery; the results are briefly described. The animals used were fully grown, healthy bitches (wt. 10 to 20 kg) that had been perineotomized in order to make catheterization of the bladder simple and undisturbing, and trained to lie on the side for 3 h or more under the conditions of the actual experiments. All operations were performed under ether anaesthesia and with full aseptic precautions; recovery was rapid and uneventful. We shall first give our methods, operative (including the operation histories of the animals), experimental and chemical, and then proceed to a description of the experiments we have made on the living animal and to a discussion of their results.

II. Methods

A. *Operative*

(1) *Carotid loops* (Van Leersum 1911)

These were made by the technique described by Verney & Vogt (1938), the carotid sinus in some animals being denervated at the same operation.

(2) *Removal of the thoracic and abdominal sympathetic chains*

The technique adopted was that described by Verney & Vogt (1938). The thoracic chains were removed in two stages, the abdominal in one. Removal of a thoracic chain

included that of the stellate ganglion above and resection of the thoracic roots of the splanchnic nerves below; and removal of an abdominal chain included that of ganglia $L1$ above and $S1$ below, and resection of the abdominal parts of the splanchnic nerves. The terms 'thoracic sympathectomy' and 'abdominal sympathectomy' will be used to connote the removals here described.

(3) *Renal artery loop and subcutaneous emplacement of the renal vein (left)*

The artery loop was made by the method of Lockett, O'Connor & Verney (1942). A simple modification of this method allows the renal vein to lie in a subcutaneous position where it is available later for the collection of renal venous blood. Some months after the first stage of the artery-loop procedure has been completed, and when the skin over the region of the renal pedicle has become freely mobile on the underlying fascia, a transverse incision some 8 cm long is made from the depths of the groove near the aorta and about 2 cm caudad to the hilum of the kidney, and a subcutaneous pocket is prepared for the later reception of the kidney. The incision is then continued through the fascia to expose the kidney and its pedicle structures. These are freed from the surrounding tissue, the vein is cleaned, and any tributaries, e.g. the ovarian vein, are divided between ligatures. At this stage the kidney may be denervated by cutting and carefully stripping the sympathetic filaments from the pedicle structures. A longitudinal strip of fascia, about 1·5 cm wide and 5 cm long, is now cut from the medial end of the cranial edge of the first incision; its cranial attachment is thus anterior to the hilum of the kidney. The strip is threaded deep to the renal vein and superficial to the renal artery, pelvis and ureter, so as to separate the vein from the rest of the pedicle and thus to give it deep support when attempts are later made to obtain blood from it. A small hole is cut where the renal vein passes between the two edges of the fascia, so as to ensure no obstruction, and the end of the fascial strip is sutured back to the caudal edge of the original incision. The remainder of that incision is then closed. The lateral edge of the strip of fascia is now sutured to the fascia beneath the kidney, but a large hole is left for the artery and ureter. The medial edge of the strip is left free. Sutures are then put through the skin and fascia so as to hold the skin closely against the vein, one suture craniad and lateral, and one caudad and medial to the vein. The subcutaneous course of the vein is marked by two horsehair sutures put through the skin where the vein leaves the hilum and again where it passes through the fascia medially. These spots are later marked by the intradermal injection of indian ink.

(4) *Chronic and partial obstruction of the renal artery*

This was effected by the implantation around the renal artery of a constricting clip of silver by the method of Verney & Vogt (1938).

(5) *The animals and their operation histories*

No. 345; wt. 18·5 kg. 9 October 1947: perineotomy. 1 November 1947: left carotid loop; denervation of left carotid sinus. 20 November 1947: left abdominal sympathectomy. 6 April 1948: first stage of renal artery loop. 21 February 1949: second stage (completion) of renal artery loop. 2 May 1949: left thoracic sympathectomy. 27 May 1949: right

thoracic sympathectomy in part. 21 October 1949: right thoracic sympathectomy completed. 8 March 1950: right abdominal sympathectomy; demarcation, by skin sutures, of anterior border of renal vein. This animal is, at the time of writing, in excellent health (wt. 18·75 kg); and there has been no consistent change in the interpolar length of the left kidney (67 to 77 mm as measured transcutaneously) over the past 5 years.

No. 356; wt. 12·2 kg. 27 May 1948: right carotid loop. 27 October 1948: perineotomy. 4 November 1949: abdominal sympathectomy (left splanchnics divided, not excised); fundus of bladder excised (see Rydin & Verney 1938). 22 November 1950: denervation of right carotid sinus.

No. 357; wt. 20·0 kg. 27 October 1948: perineotomy. 9 March 1949: first stage of left renal artery loop. 29 March 1949: left carotid loop; denervation of left carotid sinus. 13 June 1949: second (completion) stage of renal artery loop. This was a failure as the renal artery thrombosed, and the kidney gradually shrank to about the size of a walnut and became very hard and fibrous. 12 May 1950: partial obstruction of right renal artery by the application of a silver clip. 22 June 1950: denervation of right kidney. 20 November 1950: collateral blood supply to right kidney arrested. This was effected by dividing between ligatures the attachments of the kidney; several small vessels running to the kidney were thus divided. A largish tortuous vessel on the ventro-lateral aspect of the ureter was also tied. The upper and lower poles of the kidney were then sutured back into their normal positions.

No. 370; wt. 15 kg. 1 March 1950: left carotid loop. 4 April 1950: perineotomy. 14 April 1950: first stage as for left renal artery loop. 22 November 1950: right carotid loop. 7 March 1951: subcutaneous emplacement of left renal vein and partial denervation of kidney by cleaning the artery and vein. 30 May 1951: excision of left abdominal splanchnic nerves and removal of left abdominal sympathetic chain anterior and posterior to origin of renal artery. This animal is, at the time of writing, in excellent health (wt. 16·7 kg), and there has been no certain change in the interpolar length of the left kidney (about 75 mm as measured transcutaneously) over the past 3 years.

No. 395; wt. 11·8 kg. 17 October 1951: perineotomy. 31 November 1951: left carotid loop. 15 February 1952: denervation of both kidneys.

B. *Experimental*

(1) *The heart-lung double-kidney preparation*

The technique was as described by Verney (1929), with some slight modifications. After the animal had been eviscerated, the kidneys freed from their peritoneal attachments, the ureters divided and the lumbar vessels in the renal area cut between ligatures, the aorta was double ligatured and divided well below the renal arteries, and two loose ligatures were placed around the aorta well above the origins of these vessels. The ligatures were then quickly tied, the aorta divided between them and, after the vena cava had been divided above and below the renal veins, the kidneys were rapidly transferred to the heart-lung circuit by tying the arterial cannula into the lower end of the excised segment of aorta. Heparin was used as anti-coagulant. The kidneys lay on a Perspex tray in a container of the same material, and this was shaped as a half cylinder or trough with a drainage outlet at one end. The tray had enough holes in it to allow free drainage of the

venous blood, and its container was held at an angle to ensure that no stagnation of blood occurred within it. Each ureter was cannulated, and the urine from each was collected separately. Renal blood flow could be measured directly by allowing the venous blood to drain into a graduated cylinder. The lower end of this was connected by rubber tubing with the venous inflow of the heart-lung preparation, the renal venous blood being thus returned by gravity to the main circulation. The infusion of PAH and creatinine was made into the tube between the peripheral resistance and the blood reservoir of the heart-lung. Arterial blood samples were obtained by puncture of the renal arterial tube or, more simply, by pipette from the blood reservoir. When the kidneys had been connected, a solution of urea 5 g in 20 ml. 0·9 % NaCl was added to the circulating blood.

(2) *The techniques used for observations on the living animal*

The routine procedure. The animal was fed routinely 20 h before the beginning of each experiment, and a standard diet for each animal was maintained throughout the series of experiments. From $4\frac{1}{2}$ to 5 h before, and usually again $2\frac{1}{2}$ h before each experiment, the animal was given water by stomach tube. These doses ranged from 300 to 150 ml. of water warmed to body temperature. In general an animal would be given the same doses of water on each occasion before experiment, the first being usually the larger, but the doses for different animals varied with their weight.

All observations were made in a room (see Verney 1947) essentially similar in construction and equipment to that described by Hart & Verney (1934). The room temperature was not thermostatically controlled but always lay between 19 and 22° C. Just before each experiment the animal was placed in a Pavlov stand, catheterized, and the bladder emptied. The animal was then laid on its side on a warmed table, and the catheter was cut short at the vulva and connected to a glass T-piece with a short piece of rubber tubing on the side arm and a longer piece that led to the collecting vessel. This arrangement allowed the bladder to be washed out at the end of each urine-collection period. The longer rubber tube was connected to a glass tube that was held in a clamp at a constant level below the bladder and delivered into a 10 ml. graduated cylinder with a tap below. This cylinder emptied in turn directly into the urine-collection flask. The rate of urine flow could thus be measured at any time, and an accurate check made on the recovery of bladder washings. The leg was now shaved over the course of the malleolar vein, and the carotid loop was similarly treated. In all experiments a sample of arterial blood and one of urine were collected before the intravenous administration of PAH and creatinine was begun; and a systolic arterial pressure reading was taken by the cuff method (Verney & Vogt 1938) in those experiments in which this pressure was to be followed. Samples of blood were between 5 and 6 ml., and clotting was prevented by washing the syringe with a saturated solution of potassium oxalate. The volume of fluid left in the syringe and needle was never more than 2 % of the volume of blood taken, and the error was a constant one and applied equally over the whole series of results. A sample was taken from a carotid artery about the middle of each urine-collection period (see below), and in some experiments 5 to 6 ml. of blood were similarly withdrawn from the renal vein or from a foreleg vein.

The priming solution was such as was calculated to raise the plasma level of PAH to about 2 to 3 mg and that of creatinine to about 10 mg/100 ml., and the infused solution was of sufficient concentration to maintain these levels. After a little experience it was a relatively simple matter to arrive at the approximate dosage for a particular animal. The priming doses were of the order of 15 mg PAH/kg body weight, and of 80 mg creatinine/kg body weight; and the infusion of PAH was at the rate of approximately 0·25 mg/kg/min and of creatinine at about 0·4 mg/kg/min. The infused solution was approximately isotonic with blood and almost neutral in reaction (pH about 6). When necessary the pH was adjusted by the addition of a few drops of 10 % NaOH. The priming solution was injected into the malleolar vein as soon as the samples for 'blank' estimations had been taken. As soon as the injection was completed the syringe was disconnected from the needle, and the tubing from an infusion syringe connected to it. This syringe was of about 25 ml. capacity, and the plunger was driven by a screw powered by a constant-speed motor (Verney 1947). The rate of infusion in all experiments was 8·0 ml./h, so that the infusion could continue for 3 h. It was run for 20 min before the first urine-collection period was started so as to allow equilibrium of the infused solutes between cells and body fluid to become reasonably complete and the blood level to become constant.

The urine-collection periods varied from 10 to 30 min, but were mostly 15 to 20 min. They were accurately timed to the nearest second by stop-watch. The periods followed each other directly so that the end of one period was the beginning of the next. In order to recover all the PAH excreted in the urine during a period, the following procedure was adopted. At the beginning and end of the first period and at the end of each subsequent one, the bladder was drained as completely as possible and the clip on the side tube near the catheter was transferred to the delivery tube. Ten ml. of warm sterile 0·85 % NaCl were then injected into the bladder via the side tube, the clip transferred back, and the bladder contents were drained into the graduated cylinder so that complete recovery of the wash-fluid could be checked. The bladder-wash was repeated with a second 10 ml. of saline, and the time when this entered the bladder was taken as the beginning and end of the periods. After drainage of this fluid the catheter was clamped, and the delivery tube washed through with 5 ml. saline followed by air. The urine volume was derived from the total volume of fluid in the flask. After the flask had been changed, the clip was transferred from catheter to side tube, and urine collection proceeded as before.

Arterial blood sampling was timed to fall as near as possible at the mid-point of the period when the urine was actually secreted. It was possible to assess roughly the expected rate of urine flow in each collection period, and the volume of the kidney pelves and of the ureters was taken as 1 ml. in all experiments. In this way an attempt was made to estimate the middle of the secretion period, but our primary aim has been to maintain the blood levels of PAH and creatinine steady; if these are rapidly varying as a result of rapid changes in the rates of secretion, clearly no single blood sample can give an accurate measure of the mean levels of these substances during the period in question.

The animals were treated in exactly the same way in each experiment: the same routine was followed in the collection of samples; the same assistant sat with the animal and supported the head during the carotid punctures, he noted the exact times at which the blood samples were taken, and maintained an independent check on the duration of the

urine-collection periods. When it was desired to change the conditions during an experiment in order to investigate the effects of a single variable, this was usually done after two or three 'normal' collection periods that served as a control of the later ones. Most experiments lasted for seven or eight periods, with urine collections over about 2 h. The results that we shall be describing are based on fifty-eight such successfully conducted experiments.

When the effects of a rise in arterial pressure were to be determined, this was produced by clamping the two carotid loops with large 'bulldog' clips. During such periods carotid blood samples were obtained immediately after momentary release of the carotid clamp.

Temporary constriction of the renal artery. This was achieved by means of an adjustable metal clip placed around the renal artery loop (figure 1).

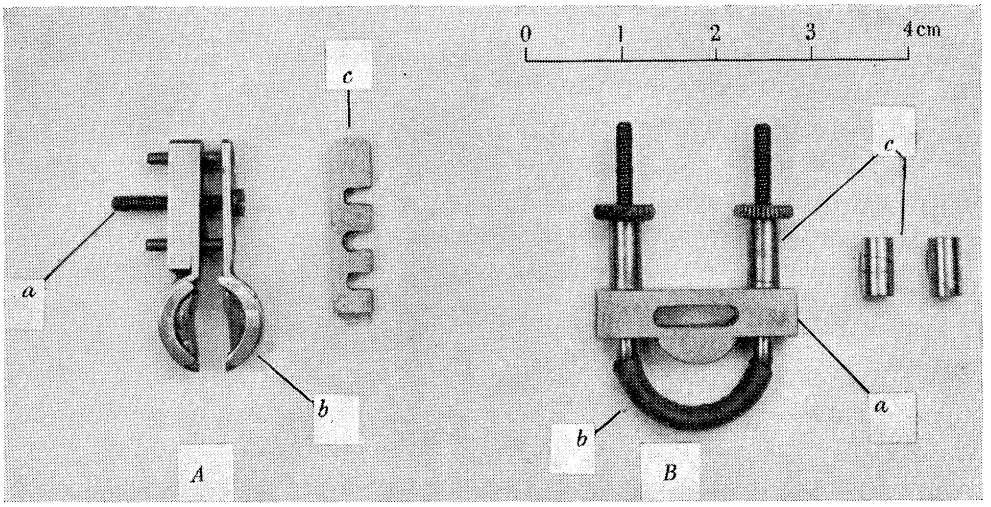


FIGURE 1. Two types of clamp that have been used to produce in the living animal temporary compression of the renal artery as it lies in a loop of skin. *A* is the type used in the experiments reported here. On the inside of each jaw is a mobile distance piece *b*. The jaws are closed on to the arterial loop by means of the screw *a*. A suitable distance plate *c* is slipped between the uprights of the clamp, the centre slot accommodating the screw *a*, and the other two the metal guides above and below. When the screw is tightened a fixed and reproducible degree of compression is thus produced. It has been found convenient to have a series of distance plates *c* of thickness 0·50, 0·75, 0·95, 1·25 and 1·5 mm.

The second type of clamp (*B*) consists of a brass U covered below by a thin rubber tube *b*. The ends of the brass limbs are threaded for an equal distance. The U is passed under the loop and the brass plate *a* slipped on to the limbs of the U. This is followed by a pair of distance tubes *c* selected from a paired series of varying length. The nuts are then tightened, thus compressing the loop between *a* and *b*.

(3) *Treatment of blood samples*

The percentage of packed red cells was determined on every blood sample. If the PAH content of whole blood was to be estimated, 1 ml. was reserved for this. The remainder was centrifuged at 1500 *g* for 10 min, and the plasma pipetted off. The plasma proteins were precipitated in the intervals between the collections of blood samples. Renal venous blood samples were invariably in the centrifuge within 5 min of their collection, and this

was usually so with arterial samples too. The purpose of rapid separation of cells from plasma was to minimize changes in cell/plasma ratio of PAH in the drawn blood.

(4) *Treatment of urine samples*

After measurement, the samples were made up to 100 ml. Estimation then of substances as mg % gave directly the amounts in mg excreted during the period.

C. *Chemical*

PAH was estimated by the modification of the method of Bratton & Marshall (1939) described by Smith *et al.* (1945). Creatinine was estimated by Peters's (1942) modification of the Folin & Wu (1919) technique, utilizing the Jaffé reaction. Plasma proteins were precipitated with cadmium sulphate as described by Smith *et al.* (1945), 1:30 filtrates being used throughout. It was found that with 1:15 filtrates there was frequently incomplete recovery of creatinine from dog plasma. Recovery of both substances from urine was always 100 % at suitable dilutions. Full recovery of PAH from whole blood was obtained only when 1:60 filtrates were used. The depth of colour developed was measured with a 'Spekker' absorptiometer, 605 (yellow green) gelatin filters being used for PAH and 604 (green) filters for creatinine.

III. Results

A. *Passage of* PAH *into and out of the red cells of the dog*

As any exchange of PAH between plasma and red cells would introduce an error into the calculated results for renal blood flow, it was important to investigate this point by both *in vitro* and *in vivo* observations. The method was to determine the volume of packed red cells, and then to estimate PAH in both whole blood and plasma. The amount of PAH in the red cells was then calculated.

When PAH is added to whole blood *in vitro* the concentration in the plasma falls only very slowly while the blood remains at room temperature. For example, in one experiment with an initial plasma-level of 2·0 mg/100 ml., only 6·4 % of the added material had disappeared from the plasma after 4 h. If the blood is incubated at 38° C the plasma level becomes fairly constant after about 35 min. After PAH has been introduced into the red cells in this way and the cells are then separated, mixed with fresh plasma or saline, and incubated as before, equilibrium is again reached in about 35 min. Under these circumstances the cell/plasma ratio of PAH is lower when the cells are mixed with 0·85 % NaCl than when mixed with plasma or 0·95 % NaCl. This is illustrated in table 1. It became clearly necessary to investigate this exchange under *in vivo* conditions, and especially to determine whether it occurred to any significant degree during the period of renal passage and the subsequent separation of the renal venous plasma.

B. *Exchange of PAH and of creatinine between cells and plasma under perfusion conditions and* in vivo, *and the extraction of PAH from the plasma during its passage through the kidney*

Phillips, Dole, Hamilton, Emerson, Archibald & Van Slyke (1946) estimated the PAH content of whole blood and of red cells directly in the dog, applying corrections to their figures because the *in vitro* recovery rates were 94 and 92 % respectively. In dogs in which

the left renal vein had been made accessible by a modification of Rhoads's (1934) technique for transplanting the kidney to a position under the skin of the flank, and to which continuous intravenous infusions of PAH were being given, Phillips *et al.* (1946) found that renal venous cells contained less PAH than arterial cells, a difference that increased as the blood stood at room temperature. They introduced a correction of 5 % to the observed plasma extraction ratio for PAH to allow for the PAH that had diffused from the renal venous cells during collection and centrifugation of the blood, and in three experiments on three such animals they found an average figure of 0·865 for the corrected extraction

TABLE 1

To 45 ml. of blood 5 ml. 0·9 % NaCl containing PAH 100 mg/100 ml. were added, and the whole was incubated at 38° C for 56 min. The cells then contained 5·45 mg PAH/100 ml. Four separate amounts of this blood, each 8 ml., were centrifuged, and all the plasma removed. To the cells were added 5 ml. of fresh plasma (from the same animal) or of saline, and the mixtures incubated at 38° C for 30 min. PAH estimations in mg/100 ml.

no. of sample	fluid added to cells	'blood' PAH	'plasma' PAH	cell PAH	cell/ plasma	haematocrit, %cells
1	5 ml. plasma	2·55	2·86	2·01	0·70	36·2
2	5 ml. plasma	2·52	2·85	1·93	0·68	36·2
3	5 ml. 0·95 % NaCl	2·52	2·85	1·96	0·69	37·2
4	5 ml. 0·85 % NaCl	2·52	3·03	1·78	0·59	41·0

The figures show the effect of the constitution of the fluid environment of red cells on the diffusion of PAH from cells to 'plasma' *in vitro*. It can readily be calculated that the total amount of PAH diffusing across the cell membrane is approximately the same in all cases, the lower cell/plasma ratio in no. 4 being accounted for by the larger fluid content of the red cells.

ratio. In the experiments in which the cell contents of PAH were determined, the arterial plasma concentration of PAH was much larger than that used by us in similar experiments, and it is seemingly of importance to know whether this correction to the extraction ratio is always justified.

Heart-lung-kidney preparation

The first observations were made on the heart-lung-kidney preparation. This allowed a number of checks. The blood flow could be measured directly; it could be estimated by the Fick principle using the renal extraction of PAH from whole blood; and the red cell content of PAH in arterial and renal venous blood could be determined. The results of an experiment of this kind are given in table 2. The blood flows as determined by the Fick principle applied to whole blood (column *m*) tallied fairly closely (except in the last period) with the average of those measured directly (column *l*), and the same applies of course to the plasma flows derived from these blood flows by means of the haematocrit values (columns *o* and *n*). The plasma flows derived from the plasma extraction ratios (column *p*), however, fell definitely and consistently below those calculated from direct measurements of blood flow. This meant that the $A-V$ difference for plasma was too great. Now it will be observed (table 2, columns *f* and *c*) that the cell content of PAH in renal venous blood was greater on all occasions than that in corresponding arterial blood. This appeared to show that PAH passed rapidly into the red cells as they became venous during renal passage, and passed rapidly out again as they became oxygenated. This raised PAH content of the renal venous cells was precisely the opposite to what had been expected.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

It is of interest that the highest cell content of PAH in the renal venous blood (0·62 mg/100 ml.) was found during period 3; at the beginning of this the arterial supply to the kidney was obstructed to such degree that the perfusion pressure fell to 50 mm Hg, and the blood flow to 151 ml./min. The obstruction was partially released 10 min. later, and the pressure then rose to 80 mm Hg, the blood flow to 210 ml./min; and subsequently the renal venous cell PAH fell a little in spite of an accompanying rise in the arterial cell

TABLE 2

Heart-lung double-kidney preparation. The kidneys were taken from a young dog weighing 9·9 kg. PAH determinations are expressed in mg/100 ml.

period no.	*a* arterial blood PAH	*b* arterial plasma PAH	*c* arterial cell PAH	*d* renal venous blood PAH	*e* renal venous plasma PAH	*f* renal venous cell PAH	*g* plasma extraction ratio, PAH	*h* haematocrit, % cells
2	1·21 (4)	2·64	0·00	0·30	0·39 (6)	0·22	0·85	54·0
4	1·44	2·94	0·17	0·60	0·62 (7)	0·58	0·79	54·0
5	1·50	3·00	0·22 (5)	0·60	0·67 (6)	0·54	0·77	54·0

period no.	*j* perfusion pressure (mmHg)	*k* PAH excretion in urine (mg/min)	*l* blood flow direct (ml./min)	*m* blood flow, Fick (ml./min)	*n* plasma flow from *l* and *h* (ml./min)	*o* plasma flow from *m* and *h* (ml./min)	*p* Plasma flow from $100k/bg$ (ml./min)	*q* glomerular filtration rate (ml./min)
2	106	1·71	$\left.\begin{matrix}171\\197\end{matrix}\right\}$ 184	193	85	89	76	13·3
4	80	2·08	$\left.\begin{matrix}212\\209\end{matrix}\right\}$ 210	217	97	100	91	8·9
5	80	1·51	$\left.\begin{matrix}204\\203\end{matrix}\right\}$ 203	174	93	80	65	7·6

The renal venous cells consistently contain more PAH than the arterial cells. Periods 2, 3, 4 and 5 were 20, 23, 15 and 22½ min respectively. At the beginning of period 3 (not shown in the table) the renal arterial tube was constricted: the perfusion pressure fell to 50 mm Hg and the urine flow dropped to zero in one kidney. The constriction was partially released 10 min later. The urine flows (two kidneys) during periods 2,3,4 and 5 were 1·62, 0·38, 0·64 and 0·54 ml./min respectively. At the end of the experiment (period 7, not shown), the perfusion pressure being 105 mm Hg, the hilum structures were suddenly tied. They were then divided on the aortic side of the ligatures, and the kidneys weighed. Weight of the two kidneys = 113 g. They contained 836000 glomeruli as determined by a maceration method (Sellwood & Verney 1955) applied to one kidney. Histological examination disclosed no gross abnormality in structure.

PAH (table 2, columns *f* and *c*). Clearly, under the artificial conditions of this preparation, the plasma extraction ratio for PAH is too high (and the derived plasma flow too low) because of the increase in the cell content of PAH during the passage of blood through the kidney, an effect which is not fully compensated by any diffusion of PAH from cells to plasma that may occur during the time required for separation of plasma from cells. Unfortunately it has not been possible to obtain renal venous blood during short-term constriction of the renal artery in the living animal, so no information is available about the effect of such constriction on the plasma extraction ratio of PAH.

Observations on the living animal

Attention was then directed to a comparison of the partition of PAH between cells and plasma of arterial blood on the one hand and that between cells and plasma of systemic venous blood (from a limb vein) and of renal venous blood on the other, in the living animal under the conditions already described. It was found that the values often varied

considerably as between arterial and systemic venous blood obtained almost simultaneously, and that the variations were not always in the same direction or of the same degree. It was, however, observed in all experiments that the cell content of PAH in topical systemic venous blood was always at least as great as and usually greater than the arterial cell content. The results of three such experiments are given in table 3.

TABLE 3

Bitch no. 370. Comparison of plasma and cell contents of PAH in arterial with those in systemic venous blood. PAH determinations are expressed in mg/100 ml.

expt. no. and date	period no.	arterial plasma PAH	arterial cell PAH	arterial cell/ plasma	venous plasma PAH	venous cell PAH	venous cell/ plasma
9	9	2·14	0·39	0·18	1·89	0·67	0·36
28 Feb. 1951	10	2·14	0·43	0·20	1·95	0·65	0·33
11	7	2·37	0·66	0·28	2·14	0·70	0·32
3 Apr. 1951	8	2·50	0·62	0·25			
12	7	1·80	0·50	0·28	1·53	0·68	0·44
12 June 1951	8	1·81	0·45	0·25			

With renal venous blood, too, the cell content of PAH in this is usually a little higher than that in arterial blood (table 4, columns *f* and *c*). It is clear that in these experiments there is little evidence of passage of PAH from cells to plasma in renal venous blood; indeed, it appears that the opposite has occurred in exp. 11 and in the early part of 12 (table 4). Under the conditions of our experiments, therefore, the plasma extraction ratio for PAH as determined from analyses of *arterial* and of renal venous plasma is fairly reliable for the calculation, in association with the urinary excretion rate of PAH, of renal plasma flows. This is further illustrated in table 4 by comparison of the values for plasma flow so derived (column *p*) with those calculated from the haematocrit and the application of the Fick principle to whole blood (column *o*). The agreement is close, and such divergence as does occur is owing to the plasma extraction ratio being too high rather than too low. The difference between these results and those of Phillips *et al.* (1946) quoted above may well have been the result of using different plasma concentrations. If the levels of PAH are high, more of the intracellular fraction may remain in a diffusible form. With small concentrations much of the intracellular PAH may be linked with protein and so be relatively indiffusible. The fact that the corrected figure given by Phillips *et al.* (1946) for the plasma extraction ratio, i.e. 0·865, agrees so well with the uncorrected figures reported here (table 4, column *g*) may thus be accounted for. In expt. 11 (table 4) a rise of arterial pressure to 195 mm Hg was effected by bilateral carotid occlusion during period 6—in this animal the pressure with one carotid occluded was 145 mm Hg; the resting pressure could not, for obvious reasons, be measured by the method used, but was probably about 120 mm Hg—and in expt. 12 (table 4) 500 ml. water were given towards the end of period 4. Neither of these manoeuvres affected the plasma extraction ratio (column *g*), nor did they alter significantly the degree of agreement between the plasma flows as determined by the Fick principle applied to whole blood and those as determined by the extraction ratio and urinary excretion rate of PAH (columns *o* and *p*). In the results that follow we have, therefore, used throughout a plasma extraction ratio of 0·85 to calculate the renal plasma flow.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

TABLE 4

Bitch no. 370. Left renal vein subcutaneous, and left kidney partially denervated. To show that the plasma PAH extraction ratio is not altered during the rise in arterial pressure from carotid occlusion or by water administration, and that there is close agreement between the plasma flows as determined from the Fick principle and haematocrit (column *o*) and those as determined from the plasma extraction ratio and the urinary excretion of PAH (column *p*). We have assumed that the two kidneys are behaving in the same way; as already stated, this animal is, at the time of writing, in excellent health, and there has been no certain change in the interpolar length of the left kidney (75 mm. as measured transcutaneously) during the three years which have now elapsed since the operation. PAH determinations are expressed in mg./100 ml. The plasma blank has not been subtracted from the figures in column *b*, but for the calculation of the values in column *p* the figures corrected for this blank have been used.

expt. no. and date	period no.	*a* arterial blood PAH	*b* arterial plasma PAH	*c* arterial cell PAH	*d* renal venous blood PAH	*e* renal venous plasma PAH	*f* renal venous cell PAH	*g* plasma extraction ratio, PAH	*h* haematocrit, % cells	*k* PAH excretion in urine, (mg/min)	*m* blood flow, Fick, (ml./min)	*o* plasma flow from *m* and *h* (ml./min)	*p* plasma flow from 100*k*/*bg* (ml./min)	*q* glomerular filtration rate (ml./min)
10	5	1·94	2·64	0·76					37·1	4·72			213	58·6
28 Mar. 1951	6	1·87	2·38	0·96	0·55	0·37	0·87	0·85	36·2	4·10	326	207	207	56·4
	7	1·78	2·29	0·90	0·57	0·36	0·93	0·85	37·5	4·05	326	206	209	55·0
	8	1·78	2·31	0·93	0·56	0·37	0·86	0·84	38·6	4·09	332	206	211	—
11	5	1·81	2·46	0·69	0·55	0·37	0·87	0·85	36·3	4·20	333	212	206	59·0
3 Apr. 1951	6	1·79	2·43	0·70	0·54	0·34	0·88	0·86	36·2	4·32	346	221	209	69·6
	7	1·78	2·37	0·66	0·55	0·33	0·96	0·86	34·6	3·96	322	211	197	61·2
	8	1·80	2·50	0·62	—	—	—	—	37·2	4·30	—	—	—	68·5
12	4	1·47	1·98	0·24	0·34	0·27	0·50	0·86	29·0	4·67	414	293	277	76·5
12 June 1951	5	1·50	1·98	0·34	0·37	0·28	0·60	0·86	29·2	5·10	447	316	300	85·9
	6	1·50	1·95	—	—	—	—	—	27·2	4·51	—	—	272	90·5
	7	1·44	1·80	0·50	0·31	0·24	0·50	0·86	27·9	4·50	412	296	291	87·4
	8	1·41	1·81	0·45	—	—	—	—	28·9	4·50	—	—	289	82·0
mean of the means of the three experiments		1·70	2·25	0·65	0·48	0·33	0·77	0·85 (5)	34·0	4·37	362	241	233	68·6

In expt. 11 both common carotid arteries were occluded during period 6: the B.P. during this period was 195 mm Hg. The arterial blood sample was obtained from the carotid after momentary release of its occluding clamp. In expt. 12, 500 ml. water were given towards the end of period 4; the urine flows during periods 5, 6, 7 and 8 were 0·4, 2·2, 3·9 and 3·3 ml./min respectively. There was difficulty in obtaining the first renal venous blood sample, and the animal was disturbed by the procedure. Between expts. 11 and 12 the left splanchnic nerves and the portions of the left abdominal sympathetic chain above and below the origin of the renal vessels had been removed (30 May 1951).

No systematic information was collected with respect to the penetration of red cells by creatinine other than the fact that equilibrium between plasma and cells is attained much more slowly than with PAH. In an experiment in which the arterial blood and plasma levels of these two substances were being followed during their continuous intravenous infusion it was found that, although the levels of blood PAH and plasma PAH became parallel within half an hour from the start of the infusion, the levels of blood creatinine and plasma creatinine were not parallel even after 2 h. With creatinine, then, the cell/plasma ratio is unlikely to undergo any significant change during the collection and centrifugation of the blood sample.

C. *Plasma levels of PAH and of creatinine*

After a single intravenous injection of PAH and creatinine, the plasma levels fell steeply with time (figure 2*A*), so that although the curves were smooth it was impossible to assess accurately the mean plasma levels during each period. With continuous infusion, however, the PAH and creatinine plasma levels in a control experiment (figure 2*B*), varied by as little as 4 and 10% respectively over 2 h, while the variation from one experimental period to the next was very slight indeed; this slight variation was presumably a reflexion of the variation with time in the rate of passage of material into and out of the cells of blood and tissues. In this experiment the renal plasma flow and glomerular filtration rate remained steady throughout, the maximum variation being 7·5 and 5% respectively.

Various experimental procedures, however, frequently altered the shape of the plasma concentration curves quite markedly. Generally speaking the excretory rate during constant infusion tends to remain approximately equal to the rate of infusion, changes in plasma flow and glomerular filtration rate being reflected mainly in changes in plasma level which rises or falls in inverse relation. It is clear that the plasma level needs to be followed closely if accuracy in the assignment of values to the plasma flow and glomerular filtration rate is to be achieved, and that it is not a good practice to calculate these for a particular period unless a blood sample has been taken near the middle of the period, and only then if the trend of the plasma levels before and after is known. It is also necessary to be sure that the infusion rate is constant, a problem which becomes more serious when very small infusion volumes are used in order to avoid altering the total body-water significantly. The technique here employed seems to satisfy these requirements.

D. *The effect of pituitary (posterior lobe) extract on renal plasma and glomerular filtrate flows during water diuresis; and observations on the arterial blood pressure during an experiment's course*

Before describing the effects of water and saline administration on the renal plasma flow (R.P.F.) and glomerular filtration rate (G.F.R.) it will be convenient to dispose of two sets of observations made in the course of this work, viz. the effect of pituitary (posterior lobe) extract on the R.P.F. and G.F.R., and the course of the systolic blood pressure during our experimental procedures. The intravenous injection of the extract in sufficient dosage (e.g. 2 mU) to inhibit water diuresis had no obvious effect on the trend of either the R.P.F. or the G.F.R. This held whether the innervation of the kidneys was intact (two experiments) or not (three experiments). In all the experiments the R.P.F. and G.F.R. were diminishing

when the extract was given (i.e. at the height of the diuresis), and they continued to fall at the same apparent rate afterwards.

In an animal (no. 356) which had been subjected a year previously to abdominal sympathectomy (with division but not excision of the left splanchnic nerves) the blood pressure varied by only 8 mm Hg during a control experiment, and the R.P.F. and G.F.R.

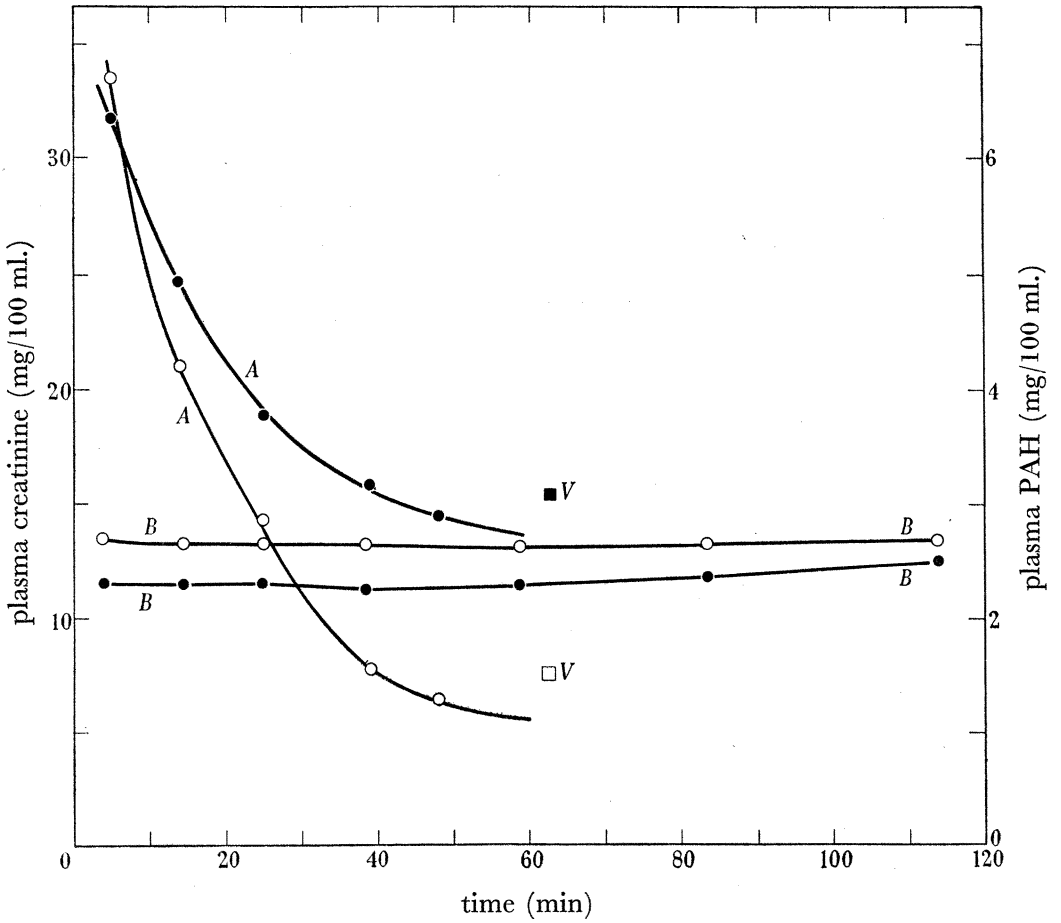


FIGURE 2. Bitch no. 356. The arterial plasma levels of PAH (○—○) and of creatinine (●—●) after a single intravenous injection at $-4\frac{1}{2}$ min (graphs *A*). The injection contained 78 mg PAH/kg body wt. and 312·5 mg creatinine/kg body wt. The points *V* represent the concentrations in systemic venous plasma: they fall considerably above the extrapolated curves for the arterial plasma levels of PAH and creatinine. The graphs *B* give the arterial plasma levels after an injection at −20 min of PAH 12·5 mg/kg body wt. and creatinine 45·8 mg/kg body wt. followed immediately by a continuous infusion of PAH 0·233 mg/kg/min and creatinine 0·467 mg/kg/min. Urine collection began at zero time.

remained constant within a variation of 10%. At the conclusion of the experiment the blood pressure rose when the animal was excited, suggesting that the left splanchnic nerve had regenerated. In another animal (no. 345; completely sympathectomized) there was a variation of only 6 mm Hg during the whole course of an experiment in which water (2·5% of body wt.) was given, and in which the R.P.F. and G.F.R. were being measured; these followed the typical pattern (see below). In a third animal (no. 357, normally innervated) a rise in blood pressure of 30 mm Hg was recorded early in a diuresis experiment and before water was given; there was a small and temporary (one period) fall in

R.P.F. and G.F.R. (10 and 13 % respectively), and these then followed the general pattern of change after water administration, while the blood pressure fell steadily to its original level. These observations show that any small changes in blood pressure that may have occurred during the diuresis experiments are not causally related to the changes in R.P.F. and G.F.R. that we shall now proceed to describe.

E. *Renal plasma flows and glomerular filtration rates during control experiments*

In figure 3 are given the percentage variations from the mean value for R.P.F. and G.F.R. during four experiments on three normally innervated animals and five experiments on three animals with interrupted renal nerve supply, under conditions in every way similar to those of the later diuresis experiments except that a stomach tube was not passed and no fluid was given. The R.P.F. and G.F.R. remain remarkably constant. In sixty-three periods of R.P.F. measurement the variation from the mean was never greater than 10 % and rarely greater than 5 %. In the same number of periods of G.F.R. measurement the variation from the mean only exceeded 10 % on one occasion. The filtration fraction (G.F.R./R.P.F.) was also remarkably constant. In the denervated animals, however, there was a slight tendency for the filtration fraction to be low early in the experiments and to recover later. After the time when the stomach tube would, in the diuresis experiments, have been passed, the values for R.P.F. and G.F.R. were even steadier, and rarely varied by more than 5 %. The interrupted lines represent the mean percentage variations in R.P.F., G.F.R. and filtration fraction after the passing of a stomach tube on two animals with interrupted renal nerve supply, but without the administration of fluid. There were apparently some slight differences from the control experiments in the same animals, but the variations were less than 10 %. In these experiments in which a stomach tube was passed, and in those that follow, it is the variations from the mean of the first three control periods, i.e. before any experimental interference, which are illustrated. In the control experiments cited above (figure 3) there was of course no experimental interference, and so the variations from the mean of all estimations of R.P.F. and from the mean of all estimations of G.F.R. are shown.

F. *Renal plasma flows and glomerular filtration rates after the administration of water to animals with intact renal innervation*

Included in this group are all experiments in which water administration to animals with normally innervated kidneys produced a diuresis of 'normal' magnitude and time course. Water diuresis is known to be readily inhibited by stimuli of various kinds (Rydin & Verney 1938; O'Connor & Verney 1942, 1945; Verney 1947), and a normal diuresis can therefore be used as an indication that the experimental procedures, e.g. collection of arterial blood, washing out the bladder, are not disturbing the animal 'emotionally'.

(1) *Water dose 1·5 to 2·0 % of body weight*

Figure 4 shows that the changes were minimal, there being a slight increase in both R.P.F. and G.F.R., of the order of 5 %, as compared with the values found in the control experiments, while the filtration fraction showed no significant change.

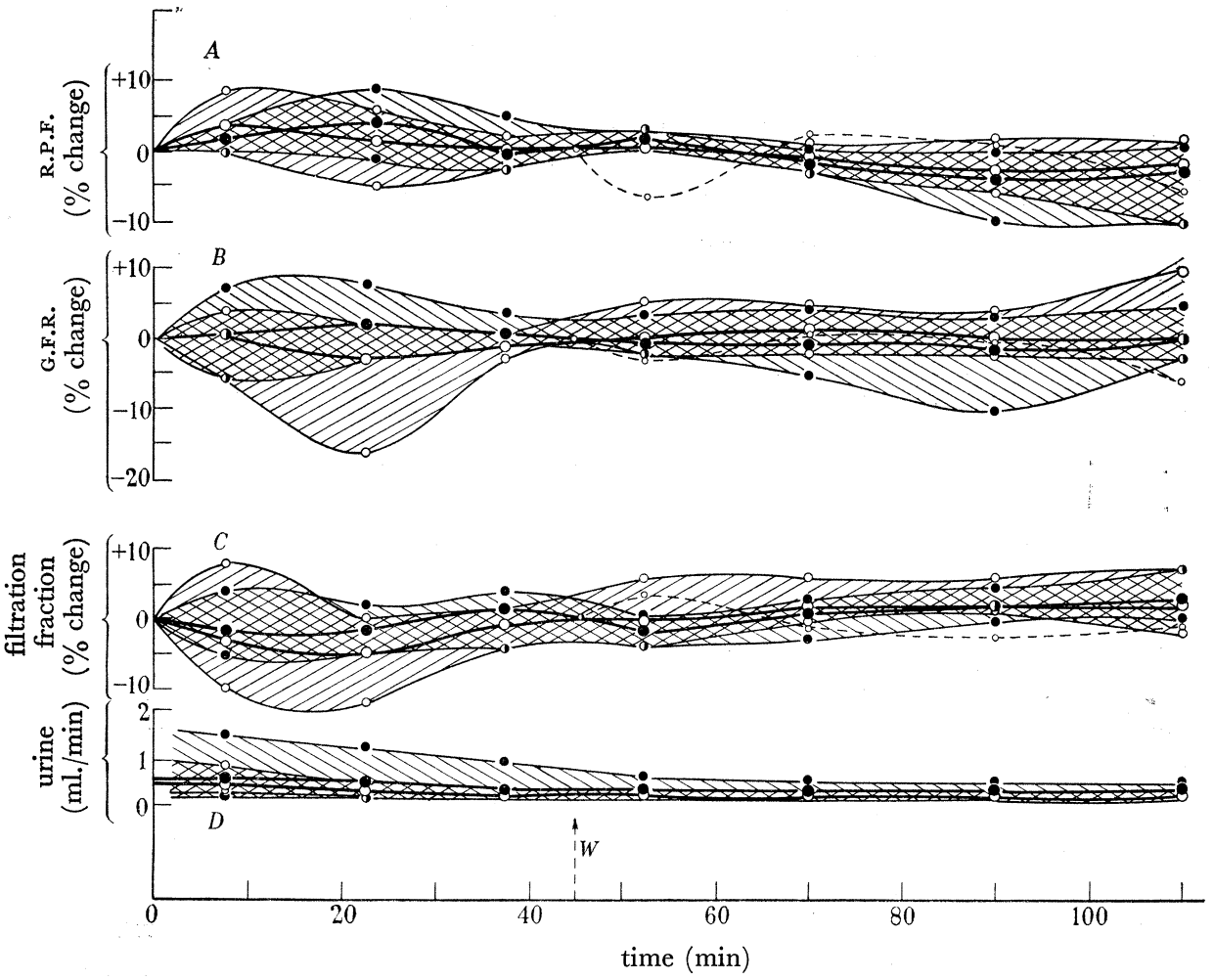


FIGURE 3. The results of nine control experiments on renal plasma flow (R.P.F.) and glomerular filtration rate (G.F.R.): four experiments on animals with intact renal innervation (nos. 357, 370 and 395), and five experiments on animals with interrupted renal nerve supply (nos. 345, 356 and 395).

A = renal plasma flow; *B* = glomerular filtration rate; *C* = filtration fraction; *D* = urine flow, ml./min. The results are represented as percentage differences from the mean R.P.F., G.F.R. or filtration fraction throughout the whole experiment.

The time at which water or saline was usually given (*W*) is taken as a fixed point 45 min after urine collection began. The curve for each experiment was drawn out and the values read at $7\frac{1}{2}$, $22\frac{1}{2}$, $37\frac{1}{2}$, $52\frac{1}{2}$, 70, 90 and 110 min after urine collection began. From these values the mean for each experiment was found, and the deviation of each value from the mean was expressed as a percentage. The points plotted are the mean of the deviations for each group at each particular time; and the extremes of the deviations at these times are also shown. The mean values for each experiment are represented as zero in each instance.

●—● = means and extremes of observations on the 'innervated' group of animals. ○—○ = means and extremes of observations on the 'denervated' group of animals. The variations are small, the deviations of the extreme values for R.P.F. and G.F.R. never exceeding ± 10 % after the time at which water or saline was given by stomach tube in other experiments. The interrupted line gives the mean percentage deviations in two experiments in which a stomach tube was passed on two animals with denervated kidneys but no water given: here the zero in each instance is the mean of the three preceding values i.e. those at $7\frac{1}{2}$, $22\frac{1}{2}$ and $37\frac{1}{2}$ min.

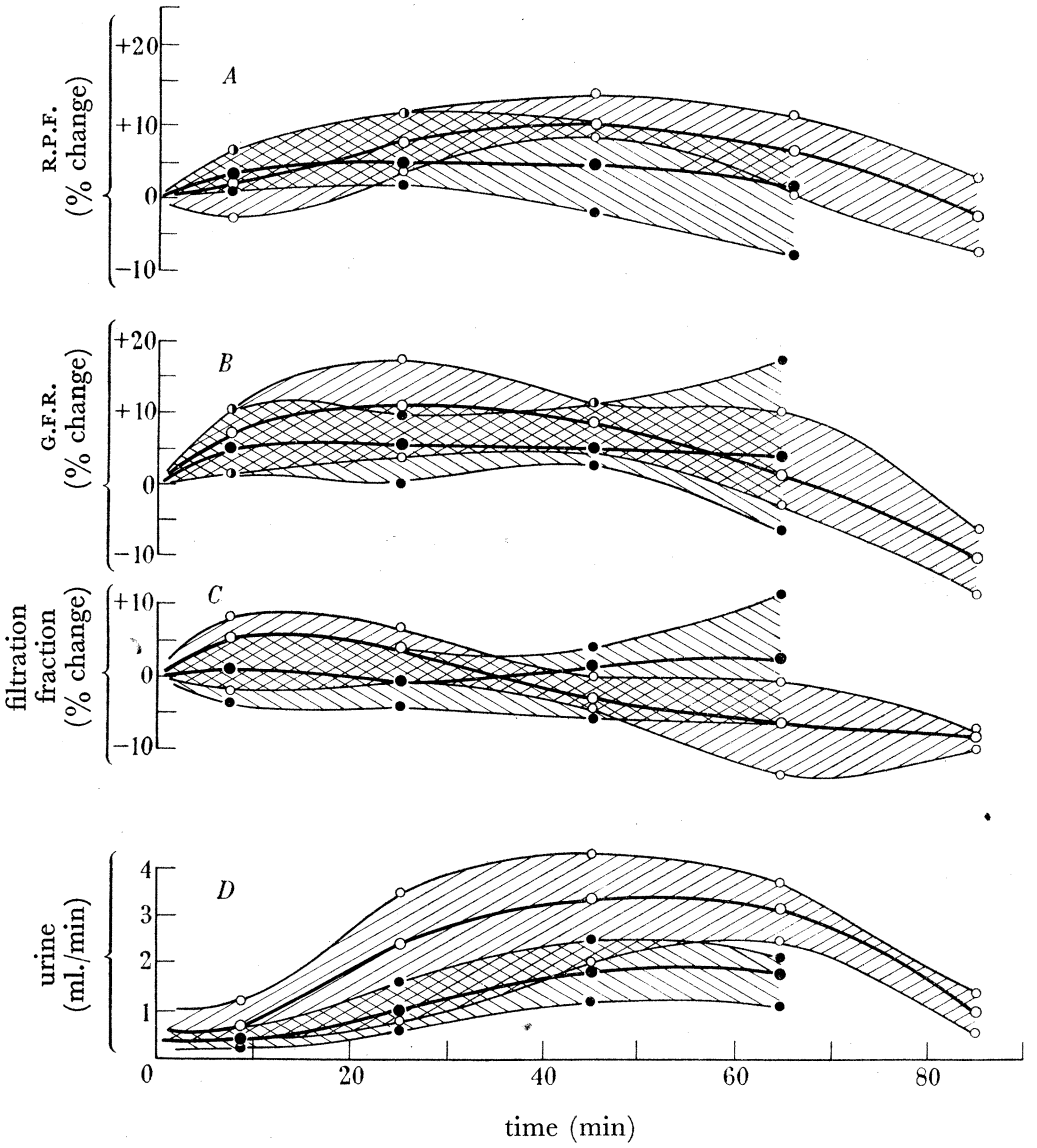


FIGURE 4. The effects on R.P.F. (*A*), G.F.R. (*B*), filtration fraction (*C*) and urine flow (*D*) (ml./min) of giving water by stomach tube in doses of 1·5 to 2·0 % of body weight at zero time. Results for R.P.F., G.F.R. and filtration fraction are expressed in each instance as percentage variations from the mean of three control periods before water was given. The mean and extreme values in 3 experiments (●—●) on normally innervated animals (nos. 357, 370 and 395) and in three experiments (○—○) on 'denervated' animals (nos. 345 and 395) are illustrated. The results should be compared with the control observations to the right of the arrow *W* in figure 3.

(2) *Water dose* 2·5 *to* 3·5 % *of body weight*

Figure 5 shows that both R.P.F. and G.F.R. were considerably increased by these doses of water, the changes in G.F.R. being perhaps a little larger than those in R.P.F., with a resultant slight increase in filtration fraction. These increases in R.P.F. and G.F.R. began before the onset of the diuresis—some increase was indeed noticeable within 15 min of the water being given—and had practically attained their maximum values well before the maximum urine flow was reached.

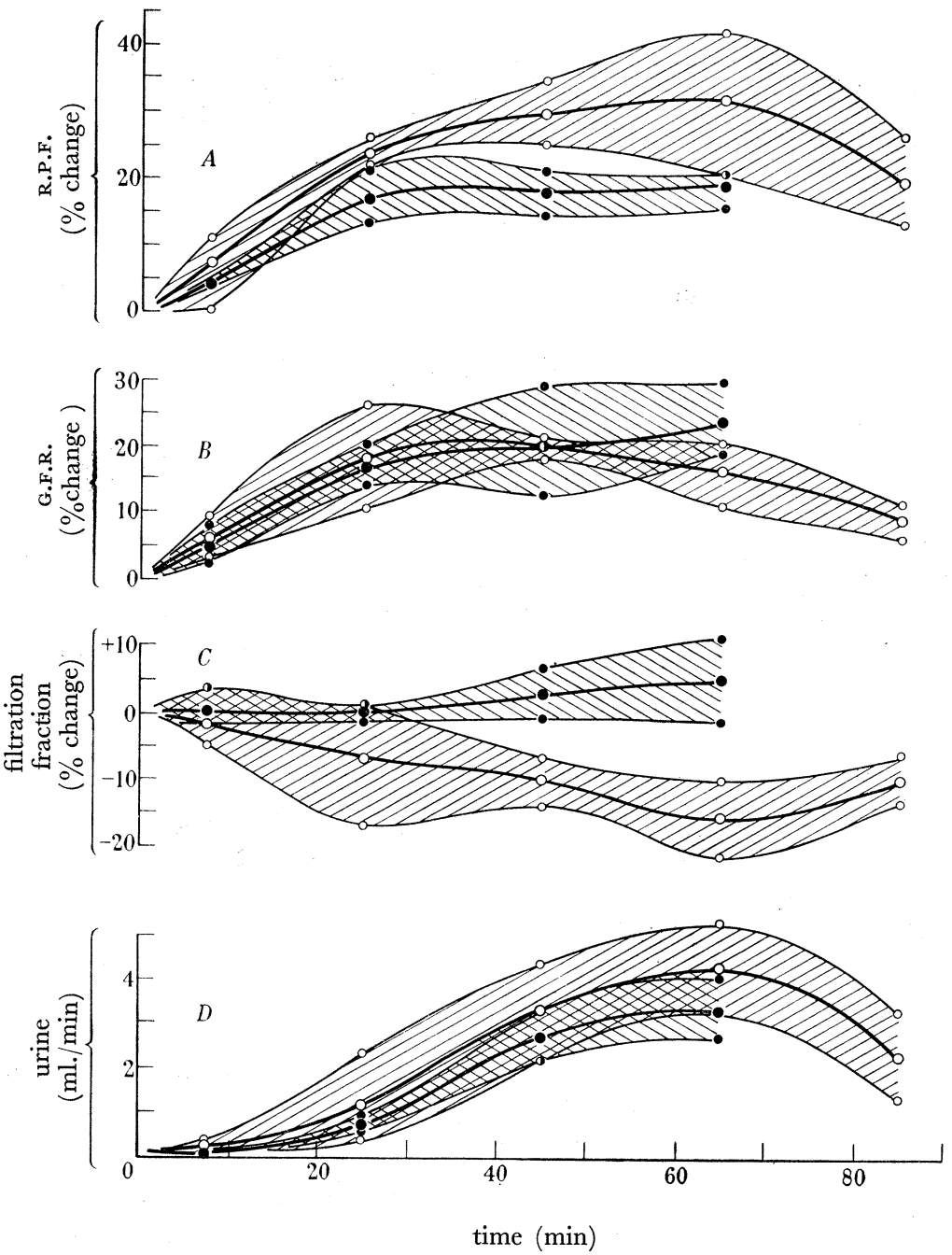


FIGURE 5. The effects on R.P.F. (*A*), G.F.R. (*B*), filtration fraction (*C*) and urine flow (*D*) (ml./min.) of giving water by stomach tube in doses of 2·5 to 3·5 % of body weight at zero time. Results for R.P.F., G.F.R. and filtration fraction are expressed in each instance as percentage variations from the mean of three control periods before water was given. The mean and extreme values in two experiments (●—●) on normally innervated animals (nos. 370 and 395) and in three experiments (○—○) on 'denervated' animals (nos. 345, 356 and 395) are illustrated. The results should be compared with those in figure 4, and with the control observations to the right of the arrow *W* in figure 3.

G. *Renal plasma flows and glomerular filtration rates after the administration of water to animals with interrupted renal innervation*

Included in this group are all experiments in which water administration to animals with an interrupted renal nerve supply produced a diuresis of 'normal' magnitude and time course.

(1) *Water dose* 1·5 *to* 2·0% *of body weight*

Reference to figure 4 will show that the changes in R.P.F. and G.F.R. were greater than those seen in normally innervated animals given the same dose of water. The G.F.R. reached its maximum before the R.P.F., and the filtration fraction, after an initial rise, fell progressively to well below the control value.

(2) *Water dose* 2·5 *to* 3·5 % *of body weight*

With these larger doses of water the changes in R.P.F. and G.F.R. were greater, but the course of each curve was essentially similar to that obtained with the smaller doses. The G.F.R. reached its maximum (20 % increase) before the R.P.F. (34 % increase), and the fall in filtration fraction was more pronounced (see figure 5).

Comparison with the curves obtained from 'innervated' animals which had also been given the larger doses of water, shows that the changes in G.F.R. were practically the same in the earlier periods, but the increase was better maintained in the animals with normal innervation. On the other hand, the increase in R.P.F. was greater in the animals with interrupted renal nerve supply, and so the filtration fraction fell. These points are perhaps better illustrated in figure 7 where comparison is made of the response of the same animal to the same dose of water (3·5 % of body weight) before and after the kidneys had been denervated by removing all visible sympathetic twigs from the structures in the renal pedicle. As in the grouped results given in figure 5 the increases in R.P.F. and G.F.R., both before and after renal denervation, precede the increases in urine flow.

H. *Renal plasma flows and glomerular filtration rates after the administration of* NaCl 0·875 % *to animals with intact renal innervation*

The dose of saline was 2·5 to 3·5 % of the body weight. The values plotted in figure 6 are the means and extremes of a total of two experiments on two animals. There was no significant change in the R.P.F., but a definite increase (20 %) in the G.F.R. and filtration fraction.

J. *Renal plasma flows and glomerular filtration rates after the administration of* NaCl 0·875 % *to animals with interrupted renal innervation*

Again the dose of saline was 2·5 to 3·5 % of the body weight. The values plotted in figure 6 are the means and extremes of a total of two experiments on two animals. There was a large increase in the G.F.R. (35 %) and a smaller increase in the R.P.F., so that both were considerably above the corresponding values obtained from normally 'innervated' animals. The filtration fraction increased as in the 'innervated' group. This was in marked contrast with the results after giving water to the same animals.

The results of the grouped experiments on the effects of giving saline to 'innervated' animals on the one hand and 'denervated' on the other (figure 6) are confirmed by the observations made on one and the same animal (figure 7) before and after the kidneys had been deprived of their nerve supply.

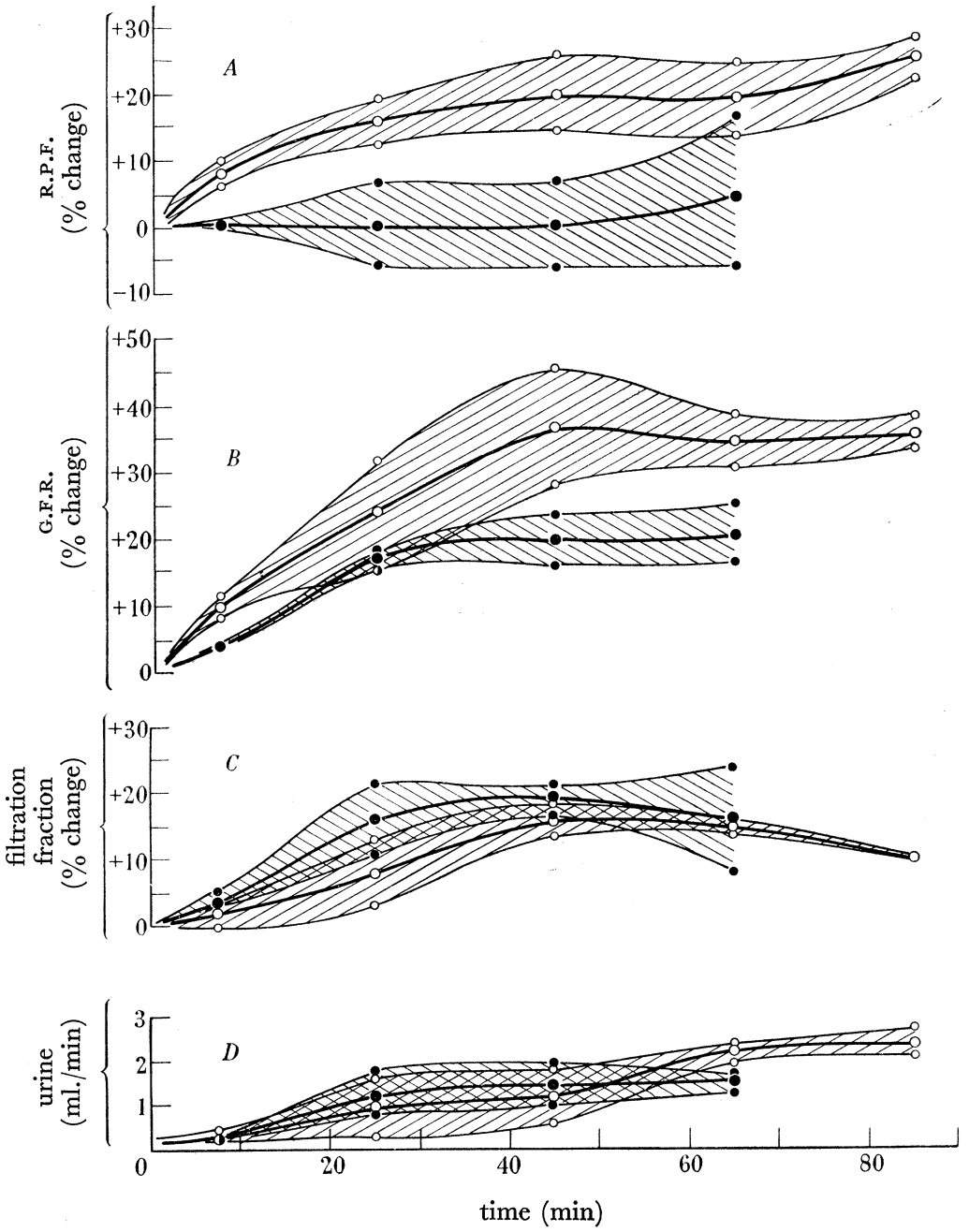


FIGURE 6. The effects on R.P.F. (*A*), G.F.R. (*B*), filtration fraction (*C*) and urine flow (*D*) (ml./min) of giving 0·875 % NaCl by stomach tube in doses of 2·5 to 3·5 % of body weight at zero time. Results for R.P.F., G.F.R. and filtration fraction are expressed in each instance as percentage variations from the mean of three control periods before the saline was given. The mean and extreme values in two experiments (●—●) on normally innervated animals (nos. 370 and 395) and in two experiments (○—○) on 'denervated' animals (nos. 345 and 395) are illustrated. The results should be compared with those in figure 5.

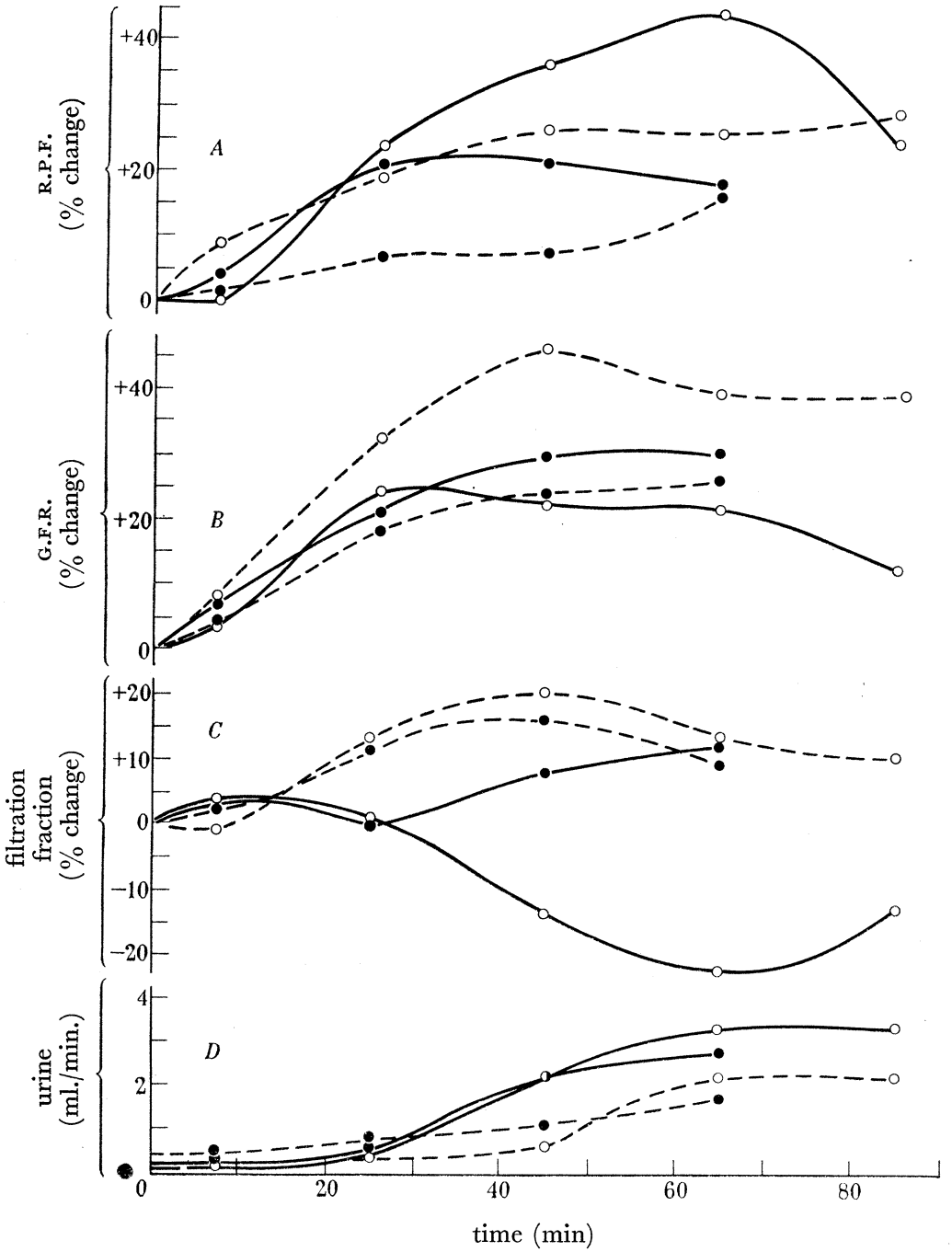


FIGURE 7. Bitch no. 395. Comparison of the effects on R.P.F. (*A*), G.F.R. (*B*), filtration fraction (*C*) and urine flow (*D*) (ml./min) of giving water (———) and 0·875 % NaCl (– – –), in a dose of 3·5 % of body weight, before (●) and after (○) denervation of the kidneys. Results for R.P.F., G.F.R. and filtration fraction are represented in each instance as percentage variations from the mean of three control periods before the water or saline was given. The results are comparable with the grouped results in figures 5 and 6. Plasma flow is increased more by giving water than by giving saline, and both increases are greater after denervation of the kidneys. Filtration rate is much the same in all cases except when saline is given to the 'denervated' animal, and then the increase is much greater. Filtration fraction is increased to much the same degree by saline both before and after renal denervation; but with water, while the filtration fraction rises slightly when the renal innervation is intact, it falls when the kidneys have been denervated.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

K. *Contrast and comparison of the results of water and saline administration to 'innervated' and 'denervated' animals*

After interruption of the renal nerve supply the administration of either water or saline produced a greater increase in the R.P.F. than before such interruption. The increases in G.F.R., on the other hand, were appreciably the same in three groups of experiments, viz. those in which water was given to 'innervated' animals, those in which water was given to 'denervated' animals and those in which saline was given to 'innervated' animals. In the fourth group, viz. those experiments in which saline was given to 'denervated' animals, the increase in G.F.R. was appreciably greater than in the other three. The main difference observed between the responses to the administration of water as opposed to that of saline was that while saline produced an increase in filtration fraction on all occasions, water produced a decrease in animals in which the renal nerve supply had been interrupted.

L. *Incidental observations of the effects on renal plasma and glomerular filtrate flows of compression of the carotid and renal arteries*

We have already emphasized the fact that the changes in plasma and filtrate flows which we have described are independent of any small change in arterial pressure which, in animals that had not been sympathectomized, may have occurred during the experiments. We had opportunity, however, of making a few observations of the effects of changes in the renal arterial pressure on these flows. No experimental analysis of the observations was attempted, but as they were made on the living animal they are perhaps worthy of record. The first involve the effects of bilateral carotid occlusion, the second the effects of temporary and partial occlusion of one renal artery, the third the effects of chronic and partial occlusion of the artery of a sole remaining kidney.

(1) *The effects of bilateral carotid occlusion*

These were observed on bitch no. 370. The sinus of each carotid loop was innervated, and while occlusion of one carotid artery raised the systolic pressure to 145, occlusion of both raised it to 195 mm Hg, as determined by the cuff method. The resting blood pressure could not, for obvious reasons, be measured by this method, but it was probably about 120 mm Hg. Three experiments were made. When the mean plasma and filtrate flows during two 10 min periods both before and after a 20 min period of bilateral carotid occlusion were compared with the flows during the period of occlusion no certain changes were detected. The mean increase in R.P.F. during carotid occlusion in the three experiments was 3·5 % (extremes, 0·5 and 9 %) and in G.F.R. 5·5 % (extremes, −1·5 and 10 %). In one similar experiment after partial denervation of one kidney the increases were 2·3 and 10·5 % respectively. The possibility that reflex vasoconstriction and reflexly released adrenaline contributed to these results was not investigated, but it seems likely that the absence of definite change in plasma and filtrate flows in the face of so large a change in arterial pressure was largely owing to an intrinsically developed increase in the resistance of the preglomerular vessels. This has frequently been reported in anaesthetized dogs (Hartmann, Ørskov & Rein 1936; Unna 1935; Selkurt 1946); and Winton (1932) has demonstrated and measured the increase and decrease in resistance of the vascular bed of the isolated kidney of the dog when the perfusion pressure is raised and lowered

respectively. More recently Brull & Louis-Bar (1950), using the ingenious method devised by Brull (1950) for perfusing, without anticoagulant, a pair of transplanted kidneys by a mechanical system that allows wide variations in perfusion pressure, have shown 'that the venous output is very independent of the arterial pressure'. In ten of sixteen experiments increases in pressure of between 30 and 200 mm Hg within the range 100 to 300 mm Hg produced either no change in blood flow or a fall. Moreover, as has been shown by Selkurt, Hall & Spencer (1949), the glomerular filtration rate (as well as the renal

TABLE 5

Bitch no. 345. The effects of mechanical compression of the left renal arterial loop.

period		renal plasma flow	glomerular filtration rate	filtration fraction	urine flow	interpolar length of left kidney
no.	duration (min)	(ml./min)	(ml./min)	(%)	(ml./min)	(mm)
3	14·37	267	72·5	27·2	0·42	75
4	15·06	252	71·7	28·4	0·30	75
5	15·17	188	40·3	21·4	0·26	67, 67, 73
6	19·93	241	69·6	28·9	0·35	76, 76
7	20·38	236	68·8	29·1	0·36	76
8	19·97	230	66·1	28·8	0·37	75, 76

The clamp (see figure 1) on the renal artery loop was screwed up so as to produce partial obstruction of the artery at the beginning of period 5 (the pulse was still palpable peripheral to the clamp) and was not touched again until the end of period 7, when it was released. The interpolar length was reduced by the obstruction from 75 to 67 mm. It remained at this level for 5½ min, then rapidly recovered to 73 mm 3 min later (period 5). The values in the table, other than those in the last column, are for both kidneys combined. We have assumed that the plasma extraction ratio for PAH was not affected by the manoeuvre.

plasma flow) is well maintained when, in the anaesthetized dog, the arterial infusion pressure to the kidney is reduced, by graded aortic narrowing, from about 150 down to 100 mm Hg.

(2) *The effects of temporary and partial occlusion of one renal artery*

The renal artery loop seemingly affords a means of producing a simple fall in the renal arterial pressure in the living animal, and two experiments of this nature were made on the sympathectomized bitch no. 345. When in such a preparation the renal artery is partially occluded by means of a screw-operated clamp (figure 1), the interpolar length of the kidney diminishes; but after a time that varies with the degree of occlusion, the kidney rapidly recovers its size without there being any alteration of the mechanically imposed compression of the renal artery loop, and this recovery is independent of any change in the general arterial pressure (Verney 1946*b*). The results of one experiment on bitch no. 345 are given in table 5. Accompanying the arterial constriction there was temporary diminution in the interpolar length of the kidney, in the rate of urine flow, in the renal plasma flow, in the glomerular filtration rate and in the filtration fraction. After a few minutes all these values abruptly recovered despite the continuing compression of the renal artery loop; and the subsequent removal of the clamp had no apparent effect on these values (period 8, table 5). In a second experiment the obstruction of the renal artery was apparently more severe: the interpolar length fell from 75 to 67 mm, remained at that level for some 16 min (about three times as long as in the previous experiment), and

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

then rapidly recovered to reach its original value some 3 min later. It seems probable that in this experiment the renal circulation was arrested during the period of reduced kidney size, seeing that then the renal plasma flow and the glomerular filtration rate were about halved and the filtration fraction not appreciably altered. Levy, Robinson & Blalock (1938) have reported that in a preparation in which a kidney had been transplanted to the subcutaneous tissues of the neck by anastomosing the renal and external jugular veins on the one hand and the renal and carotid arteries on the other (a carotid loop having been previously made), partial occlusion of the carotid by means of a clamp applied to the carotid loop is followed by a gradual recovery of renal blood flow and of renal arterial pressure peripheral to the obstructing clamp, and they attribute these changes to a gradual thinning of the wall of the artery at the site of the constriction. We feel it to be unlikely that such thinning is the sole explanation of our findings seeing that once the recovery in kidney size begins it progresses rapidly to completion: it would seem more likely that as the compression of the artery is increased the resistance of the preglomerular vessels falls due to active relaxation, and that only when this fall has reached maximal does the increasing compression of the artery lead to a rise in the total arterial and arteriole resistance and therewith a fall in renal blood flow, in glomerular capillary pressure and in kidney size. These quantities will then be controlled more critically than before by the mechanically imposed resistance, and a slight thinning of the arterial wall may then lead to their rapid return to normal values. Subsequent removal of the obstructing clamp is then immediately followed by a commensurate increase in the resistance of the preglomerular vessels in response to the increase in perfusion pressure to which they are now being subjected. If this be a correct interpretation of the phenomena under discussion the pressure in the renal artery beyond the clamp, at a time when the size of the kidney, the renal blood flow and glomerular filtration rate have all recovered, will still be lower than the general arterial pressure. Unfortunately we have not succeeded in measuring and following the course of the arterial pressure distal to the constricting clamp; the underlying events remain for the time being indeterminate.

(3) *The effects of chronic and partial occlusion of the artery of a sole remaining kidney*

One other series of observations made during the course of this work seems worthy of record, viz. the gross and rhythmic changes in plasma flow and glomerular filtration rate that appeared in an animal (no. 357) after the renal artery of a sole remaining kidney had been constricted by the implantation of a silver clip. These and subsequent observations on this animal are summarized in table 6. Before operation the systolic blood pressure was 123 mm Hg; a control experiment gave stable values for plasma and filtrate flows, the means in seven observations being 145 (S.D. $\pm$ 3·6) and 46·1 (S.D. $\pm$ 1·5) ml./min respectively; and in another experiment the typical changes that result from water administration were seen. Ten days after operation the arterial pressure was 168 mm Hg, and a control experiment at that time showed that the plasma and filtrate flows were less stable than before, the means and standard deviations in seven consecutive observations being 154 $\pm$ 41 and 47·3 $\pm$ 6·2 ml./min respectively. When similar observations were made 8, 15 and 24 days later, the arterial pressure readings during these three experiments ranging between 180 and 210, 188 and 205, and 200 and 218 mm Hg, the variations in plasma and filtrate

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

TABLE 6

Bitch no. 357. Water was allowed *ad libitum*. We have assumed that the plasma extraction ratio for PAH was 0·85 throughout.

date	operation	diet	no. of observations	renal plasma flow, mean and S.D. (ml./min)	glomerular filtration rate, mean and S.D. (ml./min)	systolic arterial pressure (mm Hg)	remarks
20 Apr. 1950	—	bread and milk	7	145 ± 3·6	46·1 ± 1·5	123	—
12 May 1950	constriction of renal artery by silver clip	—	—	—	—	—	—
22 May 1950	—	bread and milk	7	154 ± 41	47·3 ± 6·2	168	—
30 May, 6 and 15 June 1950	—	bread and milk	23	139 ± 62	42·3 ± 15·0	180–218	—
22 June 1950	denervation of kidney	—	—	—	—	—	on the 1st and 2nd day after operation the carotid pulse was at times completely irregular, suggesting paroxysmal auricular fibrillation
27 June 1950	—	bread and milk	7	130 ± 7·4	47·8 ± 4·3	200–218	—
19 Oct. 1950	—	bread and milk	7	167 ± 15·8	41·3 ± 5·7	150	—
26–31 Oct. 1950	—	addition of meat ½ lb. + NaCl 5 g	—	—	—	—	—
1 Nov. 1950	—	meat ½ lb. + NaCl 10 g, 2 h before expt.	7	245 ± 19·7	68·0 ± 4·5	155–172	—
11 and 12 Nov. 1950	—	bread and milk	—	—	—	—	—
13 Nov. 1950	—	bread and milk	7	172 ± 11·6	50·0 ± 3·1	148–170	—
20 Nov. 1950	division of collateral blood supply to kidney	—	—	—	—	—	—
24 Nov. 1950	—	bread and milk	7	124 ± 3·6	38·7 ± 1·3	180–190	—
29 Nov. 1950	—	bread and milk	7	155 ± 6·7	49·2 ± 1·6	182–192	—

flows during the course of each experiment were very great; in a total of twenty-three observations the means and standard deviations were, for plasma flow 139 ± 62, and for filtrate flow $42{\cdot}3 \pm 15$ ml./min. We could detect no correlation between these variations and those in the general arterial pressure. The results of one of these experiments are given in table 7; they suggest that an increasing vasoconstriction builds up within the kidney, and that when this has reached such degree that a marked fall in filtrate flow has occurred the vessels suddenly relax, and then undergo a further period of increasing constriction. The period of such rhythmic change in this experiment and the two others varied between 30 and 80 min. It is, we think, of interest that the mean values of plasma and filtrate flows during the four experiments (thirty observations) after the renal artery

TABLE 7

Bitch no. 357. A silver constricting clip had been placed on the renal artery of the sole remaining kidney on 12 May 1950. The results shown below were obtained in an experiment made on 6 June 1950. We have assumed that the plasma extraction ratio for PAH was 0·85 throughout.

period		renal plasma flow	glomerular filtration rate	filtration fraction	urine flow,	systolic arterial pressure
no.	duration (min)	(ml./min)	(ml./min)	(%)	(ml./min)	(mm Hg)
2	15·03	122	45·5	36·5	1·3	195
3	15·00	70	21·0	30·0	0·8	—
4	15·35	222	41·6	18·7	0·7	188
5	15·23	126	43·2	34·3	0·9	205
6	19·30	70	14·4	20·6	0·3	—
7	19·95	216	46·8	21·7	0·5	198
8	20·57	119	43·9	36·9	0·5	188

had been constricted were 142 and 43·5 ml./min respectively, i.e. although the variations were very large the mean values were not appreciably different from the stable values which obtained before operation viz. 145 and 46·1 ml./min (see above).

After these observations had been made the kidney was denervated, and an experiment five days later showed that the rhythmic changes in plasma and filtrate flows were now very much less: the extreme values in a consecutive series of seven periods were, for plasma flow 119 and 140 (mean 130; S.D. $\pm 7{\cdot}4$) and for filtrate flow 43·9 and 55·4 (mean 47·8; S.D. $\pm 4{\cdot}3$) ml./min. The arterial pressure varied between 200 and 218 mm Hg. Subsequently the pressure gradually fell, and four months after the kidney had been denervated the pressure was 150 mm Hg. In an experiment made at that time (seven observations) the plasma and filtrate flows were 167 (S.D. $\pm 15{\cdot}8$) and 41·3 (S.D. $\pm 5{\cdot}7$) ml./min. respectively: the flows were less stable than during the previous experiment, but the gross variations which had been encountered before the kidney was denervated did not reappear, nor did they do so during the reversible increases in plasma and filtrate flows produced by the addition of meat and sodium chloride to the animal's previous diet of bread and milk, nor after subsequent division of the collateral blood supply to the kidney (table 6).

IV. DISCUSSION

We propose to consider first the results of water and saline administration in animals with normal renal innervation; secondly, those in animals with denervated kidneys; and thirdly, certain ancillary observations which we have already reported and which we feel to be of interest in connexion with the reactivity of the preglomerular vessels.

The administration of water to dogs was followed by increases in the renal plasma flow and glomerular filtration rate. These increases were independent of changes in arterial pressure; indeed, in the animals which had been subjected to abdominal or complete sympathectomy no certain change of pressure was detected during the course of the observations. Were the increase in plasma flow the result of a fall in blood viscosity or of an expansion of the glomerular capillary surface accompanying an increase in blood volume, this—contrary to what we have observed—should have been more effective when saline than when water was given. The increases in plasma flow and filtration rate are, therefore—in the absence of any intermediating hormonal influence on the renal blood vessels—of intrinsic renal origin. These increases preceded the increase in urine flow, and they were sometimes decreasing again before the rate of urine flow had reached its peak. They are, then, seemingly associated with the changes in water load (see Klisiecki, Pickford, Rothschild & Verney 1933) which follow the oral administration of water. If we assume that the accompanying slight increase in the water content of the arterial blood is not the immediate cause of a fall in resistance of the preglomerular vessels, we are driven to conclude that the slight increase in filtration pressure and therewith filtration flow is the factor which is primarily responsible for the change in vascular resistance expressed in the observed increase in plasma flow. We infer, therefore, that an initial increase in pressure within the tubule—acting in some unknown way and at an undetermined site—causes relaxation of the afferent arteriole and therewith a rise in glomerular capillary pressure. The initially effective increase in 'intratubular pressure' must on this hypothesis be extremely small, smaller than the mean fall (from plasma dilution) in the osmotic pressure of the plasma proteins throughout a glomerular capillary loop, though the percentage change in 'intratubular pressure' may well be greater than the percentage change in the mean filtering head of pressure within the loop. But if we accept this increase in 'intratubular pressure' as effective, cause and effect will progress to the production of plateau values for plasma and filtrate flows, and at this stage the rise in 'intratubular pressure' is such that the difference between the mean filtering head of pressure on the one hand and the pressure in Bowman's capsule on the other is remaining greater than before the administration of the water. The increase in intracapsular pressure may well be greater than would be required to deal with the increased volume of filtrate were the course of reabsorption through the whole tubule remaining percentually parallel to what it was before. The excess pressure in Bowman's capsule would thus be an expression of a relatively large volume residue in the tubule as a result of the absorption of isotonic solution from the hypotonic filtrate. And the fact that the increase in glomerular filtration rate precedes the increase in urine flow implies that with the increase in filtration rate after water administration there is a concurrent increase in the volume of hypotonic fluid presented to a more distal part of the tubule, an increase which becomes translated into an increase in urine flow only as and when the falling concentration of antidiuretic hormone in the blood diminishes the water-reabsorptive activity of the distal tubule cells. Now the volume flow of fluid into the distal tubule will be greatest at the peak of the water load, and will be falling as the urine flow approaches its maximum, so that a diminishing flow into the distal tubule will now be associated with a proportionally greater failure of distal reabsorption, two processes that will oppose each other in their effect on the 'intratubular pressure'.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Thus at this stage there may be little change in this pressure, with the result that the increase in plasma flow and its dependent increase in filtrate flow are maintained. Finally, as the water content of the plasma and the volume of residue from the isotonic reabsorbate progressively decline, the 'intratubular pressure' which at some point is, we imagine, controlling the tone of the afferent glomerular arterioles falls; and this fall, accentuated by the increasing volume of water now being reabsorbed from the distal tubule under the influence of the mounting release of antidiuretic hormone, leads to constriction of the afferent vessels and the gradual return of the kidney to its original state. In connexion with the sequence of hypothetical events which we have just outlined, two series of observations are of interest. First, Theobald & Verney (1935) found that if in dogs with exteriorized ureters a pressure of 60 mm Hg were applied to one ureter during water diuresis, the rate of urine flow from that kidney might be as high as one quarter of the normal rate at the peak of diuresis; unfortunately, the general arterial pressure was not measured during the course of such experiment. Secondly, Brodie & Cullis (1906) observed that in decerebrate dogs with innervated kidneys the volume of urine secreted, during sulphate diuresis, by a kidney working against a small ureter pressure (7.5 to 22 mm Hg) was commonly greater than that secreted by the opposite kidney.

But to return to the discussion of our own observations. When saline is given in doses comparable with those of water, the increases in plasma flow are less than, but the increases in filtrate flow not appreciably different from, those when water is given. It may be that with saline administration the rise in pressure in the tubule from the increase in effective filtration pressure accompanying haemodilution is less because the filtered fluid is isotonic and suffers more complete reabsorption than does the hypotonic filtrate resulting from water administration. The conditions then are such as to facilitate filtration to a greater degree than plasma flow, with the result that the filtration fraction, as we have observed, increases. A volume of fluid smaller but of higher tonicity than in the case of water is therefore being presented to the distal tubule; and since the administered isotonic saline affords no stimulus to the release of antidiuretic hormone and has, by its absorption, lowered the concentration of the hormone in the plasma, it may well be that this concentration now slowly and progressively falls still further and sodium and chloride reabsorption from the distal tubule concurrently increase. Thus a diuresis smaller but more prolonged than that after water administration appears (figure 7). Initially this diuresis will be of the water type, and later, as the osmotic pressure of the plasma rises and the concentration of antidiuretic hormone in the blood progressively increases, the diuresis shifts to the salt or osmotic type which will continue until the osmotic pressure of the plasma has returned to normal. This argument gives an intelligible explanation to the classical observations of Haldane & Priestley (1916) and of Priestley (1916) on the effects in man of drinking water or isotonic salt solution in equal volume. They state that with the salt solution 'the diuresis was more prolonged and less intense than when an equal volume of water was drunk', and again 'It was expected that the urine secreted during the diuresis would have a salt concentration no lower than that of the salt solution absorbed, and would be practically a filtration product.... Actually, however, the urine secreted during the diuresis had at first a specific gravity much lower than that of the salt solution and afterwards much higher.' And they concluded that 'although both water and salt were,

relatively to other consitituents, in excess in the blood, the excretory processes by which the excesses of these two substances were eliminated were separate...'. We turn now to the effects of denervation of the kidneys on the changes in plasma and filtrate flows after the administration of water and of isotonic solution of sodium chloride.

The main effect of denervation of the kidneys was to allow a greater increase of plasma flow in response to the same dose of water, but with no parallel effect on the glomerular filtration rate; under these circumstances the filtration fraction therefore fell. These observations could be accounted for by postulating an increased sensitivity, as a result of denervation, of both afferent and efferent glomerular arterioles (and of each to appropriate degree) to some intrinsically evoked dilator agency, or alternatively such an increased sensitivity of the afferent arterioles—as postulated at the beginning of this discussion—coupled with a rise in glomerular capsular pressure which is greater than that in the 'innervated' animal seeing that the filtration rate after water administration is not appreciably altered by denervation of the kidneys. Such a view implies an increased resistance somewhere in the tubule, an increase that might possibly be supplied by pressure on a peripheral part of the nephron, e.g. the collecting ducts, this pressure resulting from an expansion, with the large increase in blood flow, of the medullary vascular bundles. Such pressure would be operating in the same sort of way as the 'intrarenal' pressure as defined and measured by Winton (1933). The potential increase in filtration rate following the administration of water to the 'denervated' animal, over and above the rate pertaining under similar circumstances in the 'innervated' animal, is thus self-limited by a rising pressure in Bowman's capsule. Such a postulated increase in sensitivity of the afferent arteriole to a dilator agency after denervation does not necessarily imply, in the absence of administered water, a greater plasma and filtrate flow simply as a result of denervation, and *a fortiori* a greater urine flow. It is known that functional and unilateral division of the splanchnic nerves under local anaesthesia in the living dog does not affect the close parallelism in the rates of flow and in the composition of the urine from the two kidneys (Klisiecki, Pickford, Rothschild & Verney, 1931). Such change in plasma and in filtrate flow as does occur after denervation is, however, in the direction of an increase. Three sets of observations in our own series of experiments support this. First, a definite increase occurred in bitch no. 370 after the left kidney was completely denervated (table 4, cf. expts. 11 and 12). The second piece of evidence comes from bitch no. 356. When the abdominal sympathectomy was performed the left splanchnic nerves were divided but not excised. From the seventh to the ninth week after operation an obvious change occurred in the animal's plasma and filtrate flows. This change is given in brief in table 8. Seeing that after the last experiment the blood pressure rose from 116 to 140 mm Hg when the animal was excited by a mild faradic stimulus, it is tempting to associate the changes recorded in table 8 with an effective regeneration of the left splanchnic nerves. Thirdly, the observations made on bitch no. 395 before and after denervation of the kidneys have been collected: in fifteen control periods before operation (three in each of five experiments) the average plasma flow was 148 (S.D. $\pm$7) ml./min and the average filtration rate 36·3 (S.D. $\pm$4·2) ml./min, and in a similar series of observations after operation these rates were 152 (S.D. $\pm$7) and 39·6 (S.D. $\pm$3·0) ml./min respectively.

With saline administration the effect of denervation was to cause larger increases in

plasma and filtrate flows, each percentually to about the same degree so that the rise in filtration fraction followed closely that obtaining in the 'innervated' animal. Thus the additional effects of denervation on the plasma and filtrate responses to saline were similar to the responses to water in the 'innervated' animal. We infer therefore that with the increased sensitivity of the afferent arterioles which we have postulated to be a result of denervation, the increase in the controlling 'intratubular pressure' when saline is given to the 'denervated' animal is less than when water is given to the 'innervated' animal. The large excess of isotonic filtrate following saline administration to the 'denervated' animal is apparently so efficiently reabsorbed by the proximal tubule that the volume flow and composition of the fluid reaching the more distal parts of the tubule are little different from those that obtain in the 'innervated' animal. Thus the course and nature of the diuresis are similar to those that we have already discussed.

We have dealt at some length with hypothetical aspects of our observations with a view to their possible explanation in terms of processes that do not involve active participation

TABLE 8

Bitch no. 356. Operation 4 November 1949. The operation comprised removal of the abdominal sympathetic chains from ganglia *L*1 to *S*1 inclusive, resection of the abdominal parts of the right splanchnic nerves with simple division of the left abdominal splanchnic nerves.

	before 21 Dec. 1949: two control experiments; 11 observations	after 3 Jan. 1950: two control experiments; 14 observations
renal plasma flow (ml./min)	always > 164 average: 185	always < 164 average: 150
glomerular filtration rate (ml./min)	average: 40·7 limits: 36·0 and 43·7	average: 38·8 limits: 37·2 and 40·6

of the efferent and other post-glomerular blood vessels. If, indeed, there is a site in the tubule which, through changes in internal pressure, is controlling the tone of the afferent glomerular vessels, we can only surmise where this may be. It would at first sight be tempting to attribute such function to the macula densa region, i.e. the region where the distal tubule is in intimate contact with the afferent arteriole of its affiliated glomerulus; but the fact that post-pituitary extract has no appreciable action on the plasma and filtrate flows during water diuresis probably makes such a view untenable. Bowman's capsule, however, might function in the way we envisage, the capsular membrane itself being a pressure-sensitive zone through which changes in glomerular blood flow are effected. But whatever the sequence of events which underlie our actual observations may prove to be, and the considerations put forward in this discussion are highly speculative, it seems that the renal nerve supply in some way limits the increase in plasma flow that follows water or saline administration and the increase in filtrate flow that follows saline administration. The factors involved in changes in filtration fraction are under further investigation by one of us (R.V.S.) who has found that, at least for the rabbit kidney in the presence of hexamethonium iodide, there is a quantitative relationship between sodium reabsorption in the tubule, renal oxygen consumption and the filtration fraction.

The experiments that we have just discussed show, as we believe, that water administration to the living animal causes a fall in the resistance of the preglomerular vessels; and we have put forward a tentative view of the intermediating events. The change in the re-

sistance of the renal vascular bed with change in infusion pressure, illustrated by the results of carotid occlusion and of temporary compression of a renal artery loop, probably exemplifies a second property of the preglomerular vessels, viz. an intrinsic change in resistance which runs parallel with a change in renal arterial pressure. The increase in the resistance of the renal vascular bed when, by carotid occlusion in the living animal, the arterial pressure was raised from about 120 to 195 mm Hg was so large that no certain change in plasma flow occurred. It is true that in these experiments the possibility of reflex vasoconstriction and of adrenaline release was not excluded, but in view of the findings of Selkurt, Hall & Spencer (1949) and of Brull & Louis-Bar (1950) in the anaesthetized dog, findings to which we have already alluded, it would seem improbable that such factors were operating to any prominent degree. And seeing that the change in filtrate flow also was minimal in the presence of the big rise in arterial pressure, an increased viscosity of the blood in the vasa efferentia could not have been playing a significant role in the increased resistance to blood flow. Our few and experimentally unanalyzed observations on carotid occlusion and on temporary compression of the renal artery loop are, then, consistent with the view that the preglomerular vessels are responsible for the change in resistance of the vascular bed with change in arterial infusion pressure, and that this resistance phenomenon is an intrinsic renal response. Working with the isolated kidney Winton (1932) has shown that whereas the increased viscosity of the blood leaving the glomerulus plays, in this preparation, a substantial role in the increasing resistance of the vascular bed at increasing arterial pressures, it does not play a dominant one. He concludes that the other factor (probably localized in or about the glomerular or preglomerular vessels, since the resistance to glomerular filtration increases with arterial pressure in the same general way as does that to blood flow (Winton 1952, 1953)), 'must be some anatomical, mechanical affair rather than an active physiological vasoconstriction' (Winton 1952) seeing that 'if poisons are used to eliminate vasoconstriction, the increased resistance to blood flow with pressure is, substantially speaking, unchanged'. Winton believes, therefore, that physiological vasoconstriction can play no part whatever in the phenomenon. While one hesitates to deny that such mechanically induced increase in resistance is operative in the living animal, one must recognize that the properties of the kidney perfused in the isolated state differ significantly from those of the kidney in a physiological environment. Thus with the isolated kidney, increase in arterial pressure always produces an increase in blood flow, and this increase is a substantial one, e.g. for rises in pressure from 120 to between 160 and 175 mm Hg it is of the order of 25 % (Winton 1951, figs. 18, 19 and 21). Similarly the glomerular filtration rate shows a marked increase over such pressure ranges, e.g. with a rise in perfusion pressure from 120 to 190 mm Hg there was an increase of some 60 % in an experiment by Eggleton, Pappenheimer & Winton (1940, fig. 2) and of 75 % when in another experiment the pressure was raised from 120 to 180 mm. Hg (Winton 1952, fig. 22). Moreover, the isolated perfused kidney exhibits an intrarenal pressure of some 10 mm Hg (Winton, 1931, 1933); and the gross disparity in responsiveness between the isolated kidney and the kidney *in vivo* to at least one agent is shown by the following facts. In the living dog a marked inhibition of urine flow during an established water diuresis is given by the intravenous injection of post-pituitary extract in a dose of the order of 1 mU, whereas in a heart-lung double-kidney preparation with

a circulating blood volume of about one litre, a dose of about 500 mU is needed to produce a relatively comparable degree of inhibition of the watery diuresis there exhibited. It may be that in the living kidney as contrasted with the isolated perfused kidney, the apparently greater increase in resistance of the preglomerular vessels with increasing blood pressure is mediated by an intrinsic physiological process which disappears or fails to operate under isolated conditions, so that whereas in the living animal the factors underlying the resistance phenomenon are exclusively or predominantly physiological, in the isolated kidney they are exclusively or predominantly mechanical. In both instances the underlying events are unknown. With respect to the phenomenon of recovery of kidney size during maintained compression of a renal artery loop, we have already emphasized the need in its interpretation of measuring and following the course of the arterial pressure peripheral to the clamp: this should be done after removal of the contralateral kidney so that the course of urine flow can be accurately followed at the same time.

Finally, with respect to the rhythmic changes in plasma and filtrate flows which appeared in the animal made hypertensive by partial obstruction of the renal artery of the sole remaining kidney, both pre- and post-glomerular vessels were apparently constricted during the periods of marked reduction in these flows seeing that during the immediately succeeding periods of vascular relaxation the filtration fraction usually fell. Were the gross changes in vascular resistance being produced by parallel variation in the output of a substance responsible for the rise in arterial pressure, one would have expected that this pressure would have varied inversely with the plasma and filtrate flows. We were unable to detect any such correlation: the variation in pressure was small, and even if such correlation were present it would not explain the excessive increases in plasma flow that followed the periods of marked reduction (table 7). Furthermore, the gross variations in plasma and filtrate flows largely disappeared after denervation of the kidney, although the arterial blood pressure was then as high as before. And when, after a period of diminishing hypertension, the arterial pressure was raised to almost its previously high level by dividing the collateral blood supply (see Verney & Vogt 1938), the plasma and filtrate flows were almost as stable thereafter as before the constricting clip was applied to the artery. The observations suggest that the innervation of the kidney was in some way associated with the intense reactivity of the renal vasculature exhibited by the animal when it first became hypertensive, and they perhaps have some bearing on the limitation that the renal nerves impose on the degree of relaxation that the preglomerular vessels undergo after water or saline administration. More experiments are clearly needed in order to determine whether the gross variations that we have observed in plasma and filtrate flows are indeed an invariable accompaniment of experimental renal hypertension of severe degree in the dog. If this proves to be so, we suggest that the only way of deciding whether the phenomenon is or is not intrinsic to the kidney will be to obstruct both renal arteries in an animal in which provision has been made for the separate collection of urine from each kidney, and thereafter to see whether the variations in the plasma and filtrate flows of the one kidney synchronize with those of the other.

Mr A. Hogwood has given technical assistance throughout this work, and his efficient help is gratefully acknowledged.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Note added in proof (9 *December* 1954). With regard to the factors that underlie the increasing resistance of the renal vascular bed with increasing arterial pressure (pp. 393–394), L. Brull and D. Louis-Bar have recently produced evidence (1954, *Arch. int. Physiol.* **62**, 140) that the phenomenon is absent with perfused transplanted kidneys when their vasculature has been exposed to ganglioplegic or local anaesthetic drugs. The authors conclude that 'Le dispositif qui tend à maintenir constante au-dessus d'une certaine pression—de l'ordre de 90 à 100 mm.—la circulation rénale, n'est donc pas de nature anatomique, c'est une régulation nerveuse'.

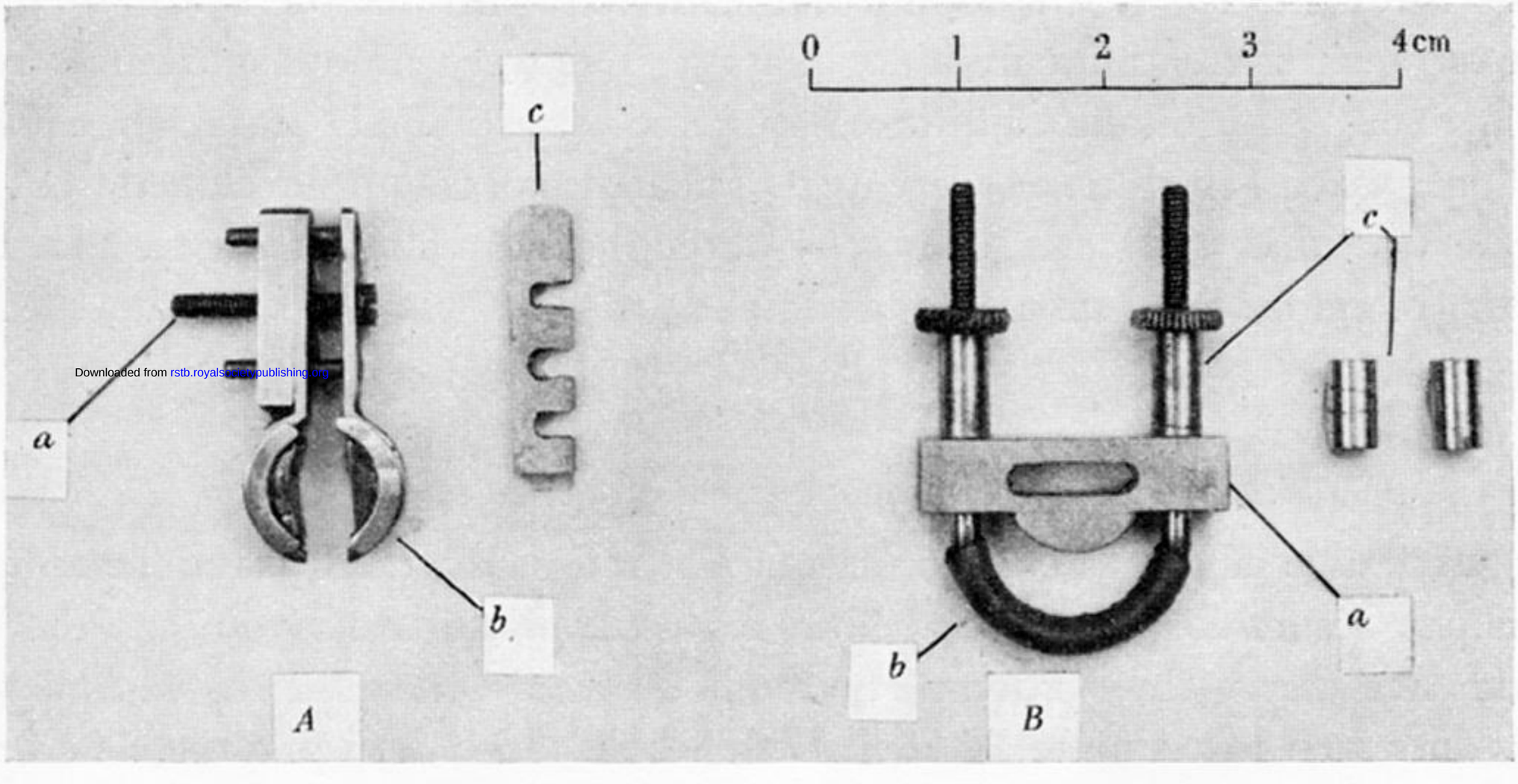
References

Bratton, A. C. & Marshall, E. K. Jr. 1939 *J. Biol. Chem.* **128**, 537.
Brodie, T. G. & Cullis, W. C. 1906 *J. Physiol.* **34**, 224.
Brull, L. 1950 *Arch. int. Physiol.* **58**, 321.
Brull, L. & Louis-Bar, D. 1950 *Arch. int. Physiol.* **58**, 329.
Cowan, S. L., Verney, E. B. & Vogt, M. 1938 Communication to Physiological Society, 12 March. See Verney, E. B. (1946*a*).
Eggleton, M. G., Pappenheimer, J. R. & Winton, F. R. 1940 *J. Physiol.* **98**, 336.
Folin, O. & Wu, H. 1919 *J. Biol. Chem.* **38**, 81.
Haldane, J. S. & Priestley, J. G. 1916 *J. Physiol.* **50**, 296.
Handovsky, H. & Samaan, A. 1937 *J. Physiol.* **89**, 14.
Hart, P. D'Arcy & Verney, E. B. 1934 *Clin. Sci.* **1**, 367.
Hartmann, H., Ørskov, S. L. & Rein, H. 1936 *Pflüg. Arch. ges. Physiol.* **238**, 239.
Janssen, S. & Rein, H. 1928 *Arch. exp. Path. Pharmak.* **128**; *Verh. dtsch. pharm. Ges.* p. 107.
Klisiecki, A., Pickford, M., Rothschild, P. & Verney, E. B. 1931 *J. Physiol.* **72**, 26*P*.
Klisiecki, A., Pickford, M., Rothschild, P. & Verney, E. B. 1933 *Proc. Roy. Soc.* B, **112**, 496.
Levy, S. E., Robinson, C. S. & Blalock, A. 1938 *Amer. J. Physiol.* **123**, 383.
Lockett, M. F., O'Connor, W. J. & Verney, E. B. 1942 *Quart. J. Exp. Physiol.* **31**, 333.
O'Connor, W. J. & Verney, E. B. 1942 *Quart. J. Exp. Physiol.* **31**, 393.
O'Connor, W. J. & Verney, E. B. 1945 *Quart. J. Exp. Physiol.* **33**, 77.
Peters, J. H. 1942 *J. Biol. Chem.* **146**, 179.
Phillips, R. A., Dole, V. P., Hamilton, P. B., Emerson, K. Jr., Archibald, R. M. Jr. & Van Slyke, D. D. 1946 *Amer. J. Physiol.* **145**, 314.
Priestley, J. G. 1916 *J. Physiol.* **50**, 304.
Rein, H. 1928 *Z. Biol.* **87**, 394.
Rein, H. 1929*a* *Z. Biol.* **89**, 195.
Rein, H. 1929*b* *Handb. biol. Arb Meth. Abt* (5) **8**, 693.
Rein, H. 1931 *Ergebn. Physiol.* **32**, 28.
Rhoads, C. P. 1934 *Amer. J. Physiol.* **109**, 324.
Rydin, H. & Verney, E. B. 1938 *Quart. J. Exp. Physiol.* **27**, 343.
Selkurt, E. E. 1946 *Amer. J. Physiol.* **147**, 537.
Selkurt, E. E., Hall, P. W. & Spencer, M. P. 1949 *Amer. J. Physiol.* **159**, 369.
Sellwood, R. V. & Verney, E. B. 1955 *J. Anat., Lond.* **89**, (In the Press).
Shannon, J. A. 1936 *Amer. J. Physiol.* **117**, 206.
Shannon, J. A. 1942 *J. Exp. Med.* **76**, 387.
Smith, H. W., Finkelstein, N., Aliminosa, A., Crawford, B. & Graber, M. 1945 *J. Clin. Invest.* **24**, 388.
Theobald, G. W. & Verney, E. B. 1935 *Quart J. Exp. Physiol.* **25**, 341.
Unna, K. 1935 *Pflüg. Arch. ges. Physiol.* **235**, 515.
Van Leersum, E. C. 1911 *Pflüg. Arch. ges. Physiol.* **142**, 377.
Verney, E. B. 1929 *Lancet*, **216**, 539.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Verney, E. B. 1946*a* *Lancet*, **251**, 739.
Verney, E. B. 1946*b* Demonstration to Physiological Society, 11 May.
Verney, E. B. 1947 *Proc. Roy. Soc.* B, **135**, 25.
Verney, E. B. & Vogt, M. 1938 *Quart. J. Exp. Physiol.* **28**, 253.
Walker, A. M., Schmidt, C. F., Elsom, K. A. & Johnson, C. G. 1937 *Amer. J. Physiol.* **118**, 95.
Winton, F. R. 1931 *J. Physiol.* **71**, 381.
Winton, F. R. 1932 *Trans. XIV Congresso Internaz. di Fisiol.* p. 264.
Winton, F. R. 1933 *J. Physiol.* **78**, 9*P*.
Winton, F. R. 1952 *Third Conference on Renal Function* (*Oct.* 1951), *sponsored by the Josiah Macy, Jr. Foundation, New York.*
Winton, F. R. 1953 *Harvey Lectures*, 1951–52, p. 21. New York: Academic Press Inc.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES



IGURE 1. Two types of clamp that have been used to produce in the living animal temporary compression of the renal artery as it lies in a loop of skin. *A* is the type used in the experiments reported here. On the inside of each jaw is a mobile distance piece *b*. The jaws are closed on to the arterial loop by means of the screw *a*. A suitable distance plate *c* is slipped between the uprights of the clamp, the centre slot accommodating the screw *a*, and the other two the metal guides above and below. When the screw is tightened a fixed and reproducible degree of compression is thus produced. It has been found convenient to have a series of distance plates *c* of thickness 0·50, 0·75, 0·95, 1·25 and 1·5 mm.

The second type of clamp (*B*) consists of a brass U covered below by a thin rubber tube *b*. The ends of the brass limbs are threaded for an equal distance. The U is passed under the loop and the brass plate *a* slipped on to the limbs of the U. This is followed by a pair of distance tubes *c* selected from a paired series of varying length. The nuts are then tightened, thus compressing the loop between *a* and *b*.