RENAL FUNCTION IN THE PENGUIN (*SPHENISCUS DEMERSUS*) WITH SPECIAL REFERENCE TO THE RÔLE OF THE RENAL PORTAL SYSTEM AND RENAL PORTAL VALVES*

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ABSTRACT

The general morphology and direction of blood-flow in the renal portal complex, as well as the histochemistry, innervation and pharmacology of the renal portal valves were studied. The origin of the copious mucus present in the cloacal fluid was found to be in the collecting tubules and branches of the ureters.

By simulating closed renal portal valve conditions in one of two groups of birds an attempt was made to study the rôle of the renal portal blood system. Significantly higher water diuresis was recorded in the "closed valve" group. Increased amounts of potassium were secreted by the "open valve" group while sodium was reabsorbed in significantly higher amounts in the "closed valve" group. No evidence to support the hypothesis that the renal portal system promotes renal tubular secretion of uric acid was found, but the high concentration of urea recorded and the trend for urea values to be higher in the "closed valve" group, suggests that the renal portal system may be implicated in urea secretion.

INTRODUCTION

The kidneys of birds with a functional salt gland are not as efficient in excreting sodium chloride as the salt glands. The excretion of nitrogen, however, is exclusively controlled by the kidneys. Being exclusively carnivorous, the high protein diet of *S. demersus* must place a heavy burden on the kidneys. Although it is generally accepted that birds excrete uric acid as a water-conserving mechanism, the advantage of uric acid over urea in this respect still remains an open question (Sykes 1971). A functional renal portal system has been described in birds (Sperber 1948) and the renal portal valve seems to play a prominent rôle in this system. The physiological significance of this system, however, remains obscure although a rôle in the promotion of renal tubular secretion of uric acid has been suggested for it by Sykes (1971). To date all experiments on the function of this system have been carried out on the domestic fowl (Sperber 1948; Gordeuk and Grundy 1950; Rennick and Gandia 1954; Gilbert 1961; Akester 1964, 1967). This is unfortunate for if the renal portal system is in any way involved in osmoregulation, or kidney function, the fowl, with a non-functional salt gland (McLelland and Pickering 1969), may have been a poor choice as an experimental animal. *S. demersus* was therefore selected as a suitable experimental animal for a study of the rôle of the renal portal system in renal function. The effect of both open and closed renal portal valves upon blood-flow direction in the associated veins, glomerular filtration and tubular absorption and secretion was studied. In order to understand the function of the valves themselves a histochemical study of their innervation was carried out. The kidneys and ureters were also examined and compared with those of *Gallus* and the origin of the copious mucus in the excreta was traced.

* This study forms part of a thesis accepted for the degree of Master of Science at the Zoological Institute, University of Stellenbosch.

PROCEDURE

Blood-flow direction

Directions of blood-flow in the coccygeomesenteric and caudal renal portal veins of anaesthetised penguins were observed by the infusion of a fluorescent physiological saline solution under ultra-violet light. This solution contained 0,05% fluoruceinum (Simons and Michaels 1953) and was infused into the coccygeomesenteric vein. The effect of acetylcholine and adrenalin on the direction of the flow of the blood was observed by separate infusions of the same solutions, containing either 5 p.p.m. acetylcholine chloride or adrenalin.

The effect of adrenalin and acetylcholine on the renal portal valve

After having occluded all the veins that lead to and from the renal portal valve a slit was made in the posterior vena cava to expose the renal portal valve (Figures 1, 2 and 3). The valve

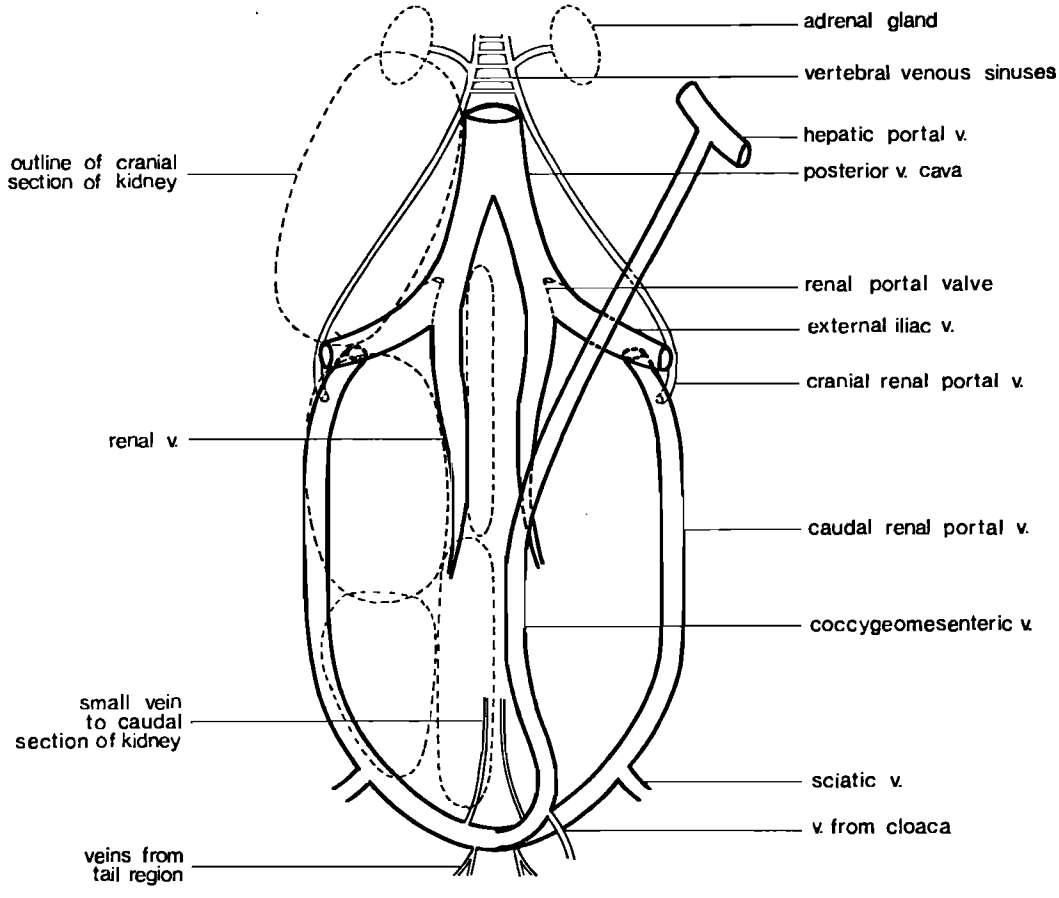

FIGURE 1
Diagrammatic representation of kidneys and associated veins (ventral view).

was then connected to a light lever arranged to write on a kymograph. Physiological saline solutions containing 5 p.p.m. adrenalin or acetylcholine were then alternately dripped onto the valve and the tonus of the valve was continuously recorded.

Morphology

Corrosion casts of the portal blood vessels were made in addition to a series of dissections on materials that had been injected with latex and resin (Figures 2 and 3). Some casts were made after applying a clamp to the external iliac vein in the region where the valve was situated. Another clamp on the coccygeomesenteric vein prevented the latex from flowing towards the hepatic portal vein (Figure 1).

Anatomy of the renal portal valve

A number of valves were dissected out, fixed in 10% formolsaline or alcohol and sectioned at 5, 8 and 10μ. Serial sections, cut at 12μ, were stained with Azocarmine and counter-stained with Azan. In addition, a number of histochemical techniques were used to show the presence of nerve cells and fibres, adrenergic, noradrenergic and cholinergic fibres. The techniques used to stain for nerve cells and fibres were: (i) Silver impregnation according to Golgi's rapid method (Mallory 1944 in Humason 1966); (ii) Fluorescent staining with Acridin Orange according to the method of Zeiger, Harders and Müller (1951) as described by Humason (1966).

The chromaffin reaction of Hillarp and Hökfeldt as described by Pearce (1960) was used to stain for adrenalin and noradrenalin. For acetylcholine esterases the tissue was stained according to the method of Adam and Czihak (1964).

Histology of the kidneys and ureters

Tissue samples of the ureters from near the cloaca and the cranial region of the kidney were fixed in 10% formolsaline, sectioned at 5, 8 and 12μ, and stained. Different sections of the kidneys were also sectioned at 5, 8 and 12μ, stained with Azocarmine and counter-stained with Azan after fixation in 10% formolsaline.

Tissue samples containing kidney as well as ureteral tissue were stained with Acridin Orange for mucin by the method of Hicks and Matthaei (1958) as described by Humason (1966). Two alternate methods for indicating mucin were also used, namely, Mayer's mucicarmine and Toluidine blue according to Humason (1966). Sections of the ureter from near the cloaca were stained in the same way.

The effect of open and closed renal portal valves upon tubular secretion and glomerular filtration

This experiment was carried out on birds which had been anaesthetized with an intraperitoneal injection of pento-barbitone sodium solution (Sagatal). A dosage of 0,5 ml/kg body mass took effect after 40–60 minutes and remained effective for six to twelve hours.

The ureters were then cannulated with plastic tubing by making a transverse incision immediately anterior to the cloaca. A sample of 1,5 ml of urine was first collected from the ureters to obtain base line values. The junction of the external iliac, caudal and cranial renal portal veins with the posterior vena cava was then located and the peritoneum over the kidneys and blood vessels in this region removed (Figure 1). Care was taken to prevent damage to the

kidneys as the posterior margin of the anterior section of the kidney frequently extended backwards over these blood vessels and had to be dissected free to obtain free access to the blood vessels. This dissection was performed bilaterally on all birds, irrespective of whether the blood vessels were occluded later or not. In this way any interacting effect of the surgery was obviated.

Twelve animals were used. In six of them the blood vessels housing renal portal valves were occluded bilaterally by applying clamps over the renal portal valves. Care was taken not to disturb any of the portal veins or to damage the ureter and renal veins that run median to this point and the external iliac artery that runs immediately dorsal to the veins. In the remaining six animals these blood vessels were not occluded and, on the basis of previous blood-flow studies using a fluorescent dye, it was assumed that the valves in these anaesthetized animals remained open.

Immediately after the above procedure, 100 ml physiological saline per kg body mass, containing 25 mg/kg body mass of inulin, was infused into the coccygeomesenteric vein. This was accomplished by preheating the saline-inulin solution to body temperature (40°C) in a flask that was raised above the point of infusion. Two pieces of polythene tubing were connected to the flask, one functioning as an air inlet. To the free end of the other tube a hypodermic needle was fitted. The needle was inserted into the coccygeomesenteric vein and the flow was regulated with a screw-type clamp on the tube to last 10 minutes. From the time infusion started, urine was collected from the cannulated ureters for five periods of 10 minutes each. The samples from each 10-minute period were kept and analysed separately but no distinction was made between samples drawn from the left and right ureters.

Analytical techniques

The urine samples were centrifuged at 12 000 g and the volume of uric acid was measured. The supernatant was then removed and frozen in sealed plastic vials for later analysis. Sodium and potassium concentrations were determined using standard flame photometric techniques (Instrumentation Laboratory, IL 343) and chloride concentrations by means of an automatic chloride titrator (Radiometer, CMT 10). Osmolalities were determined using an automatic high precision research osmometer (Advanced instruments, model 67 31RAS). Urea was determined enzymatically using the method of Richterich (1968) and an Eppendorf photometer model 1101 M, filter Hg 578 μm. Statistical analysis of the data consisted of testing for significance between treatment means by using Student's t test.

RESULTS AND DISCUSSION

Morphology

The morphology of the caudal section of the renal portal system in birds has long been known in spite of previous contradictory views on the existence of a portal system. Positive evidence for its existence was first supplied by Spanner (1925) and Sperber (1948). The existence of the cranial section was, however, only recently discovered by using infusions of radio-opaque materials and suitable X-ray techniques (Akester 1964, 1971).

Corrosion casts and a series of dissections on the renal portal complex of *S. demersus* showed the similarity of its gross anatomy to that of other birds as described by Sperber (1948),

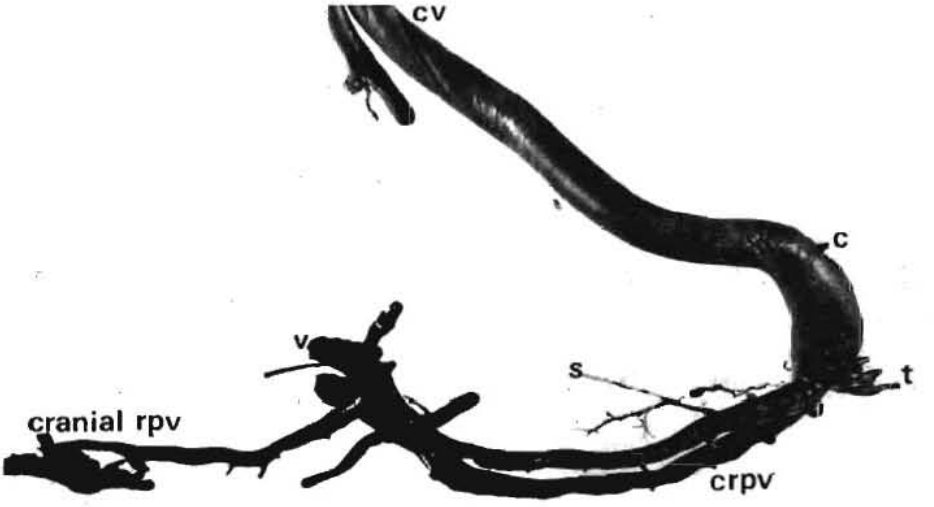

FIGURE 2
Cast of the renal portal system.
C = vein draining cloaca. CV = coccygeomesenteric vein. Cranial rpv = cranial renal portal vein. crpv = caudal renal portal vein. t = vein from coccyx and tail region. v = the point where the renal portal valve caused a break in the cast. s = small vein entering the caudal section of kidney. ($\times \frac{1}{2}$).

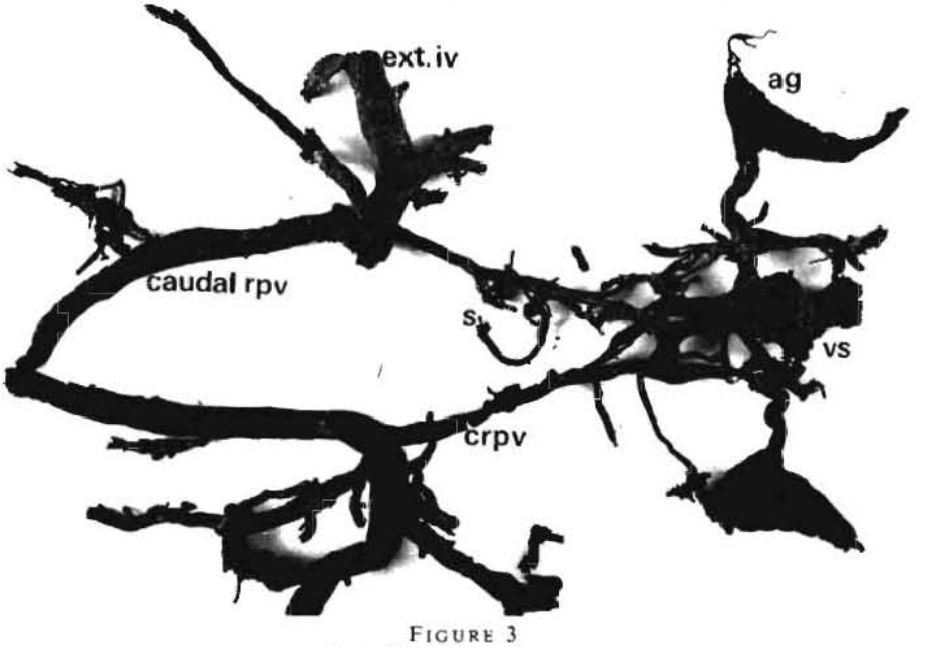

FIGURE 3
Cast of renal portal system.
ag = adrenal gland. ext. iv = external iliac vein. caudal rpv = caudal renal portal vein. crpv = cranial renal portal vein. s = small vein entering the cranial section of the kidney. vs = vertebral venous sinuses. ($\times \frac{1}{2}$).

[Figures 4 and 5 are on reverse of Figures 8 and 9].

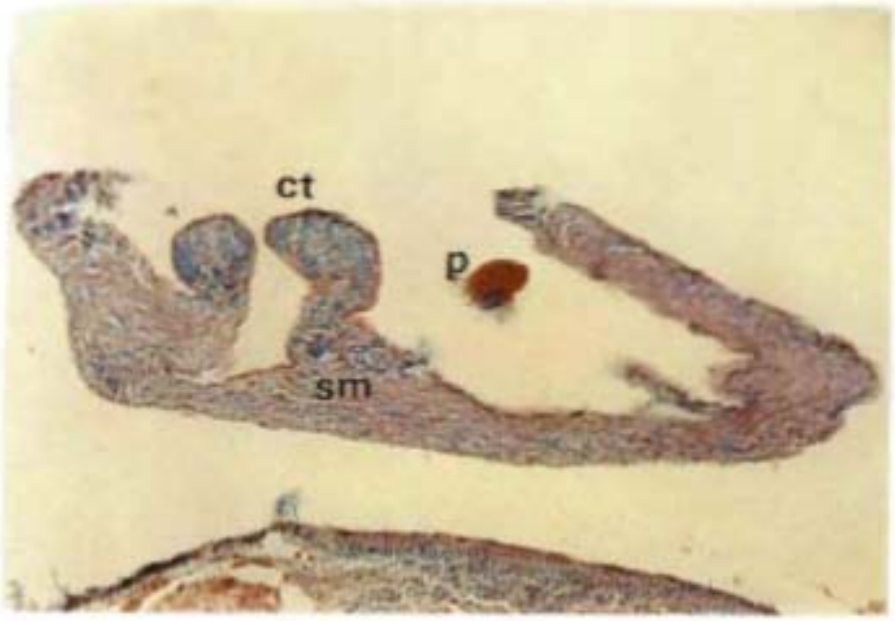

FIGURE 6
Section through aperture of renal portal valve. p = papillae. ct = connective tissue. sm = smooth muscle fibres. (X120)

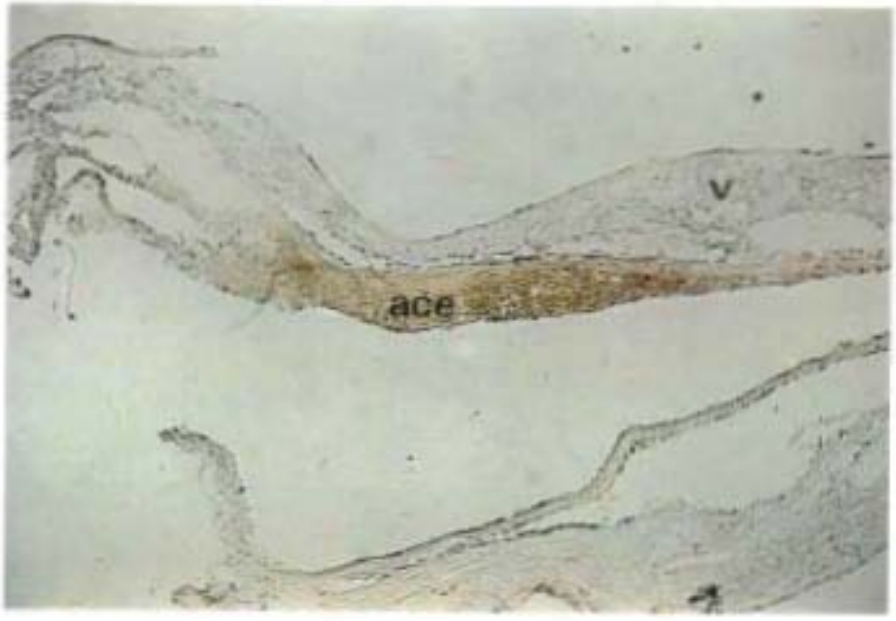

FIGURE 7
Positive reaction to acetylcholine esterases (ace) in the renal portal valve (v). (X180)

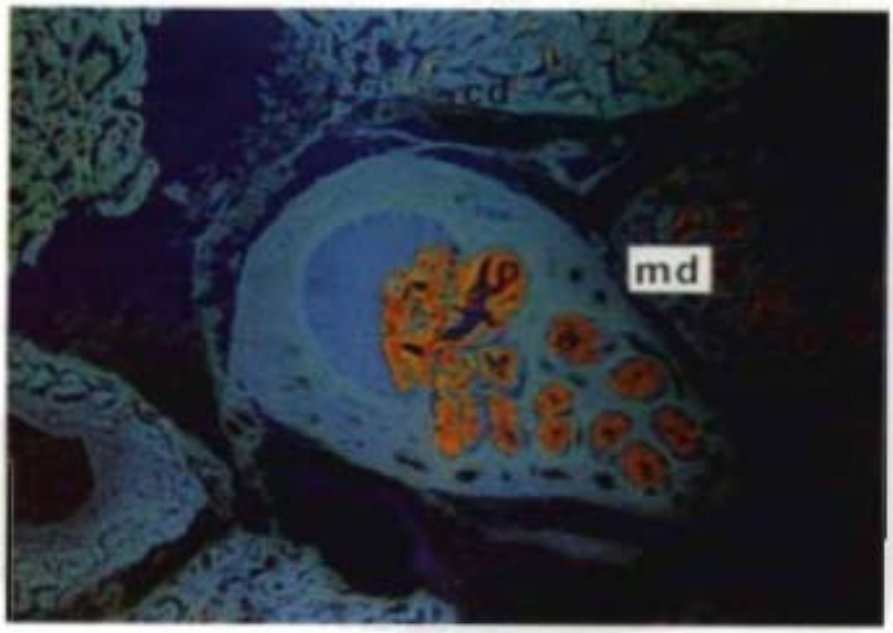

FIGURE 8
Positive fluorescence for mucus in the kidney and branches of the ureter. Intensity of fluorescence increases from the cortical collecting ducts (cd) through the medullary collecting ducts (md) to the secondary (s) and primary branches of the ureter. (Acridine orange, X250)

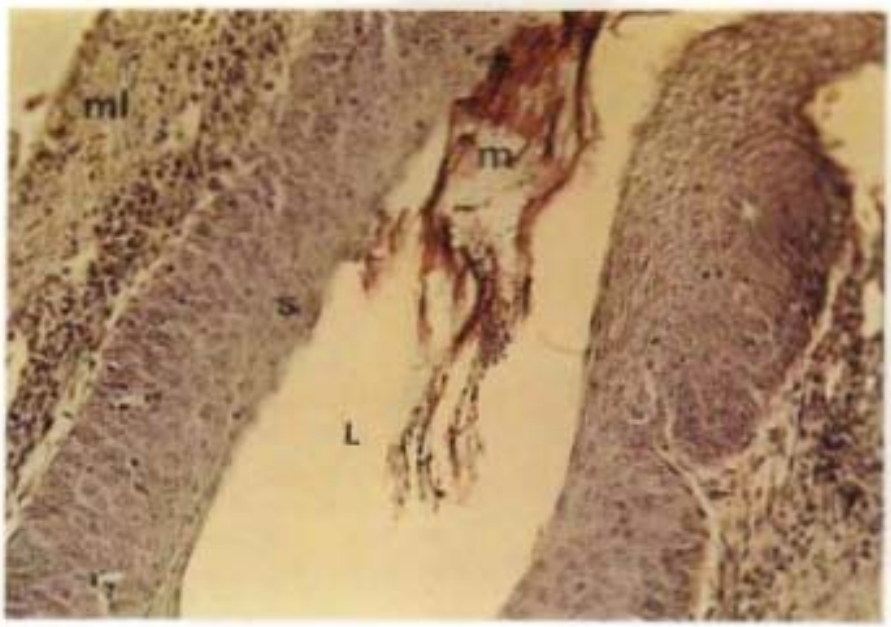

FIGURE 9
Cross section through the ureter. Positive reaction for mucus (m) in the lumen (l) only. s = submucosa, ml = muscle layers. (Toluodine blue, X400)

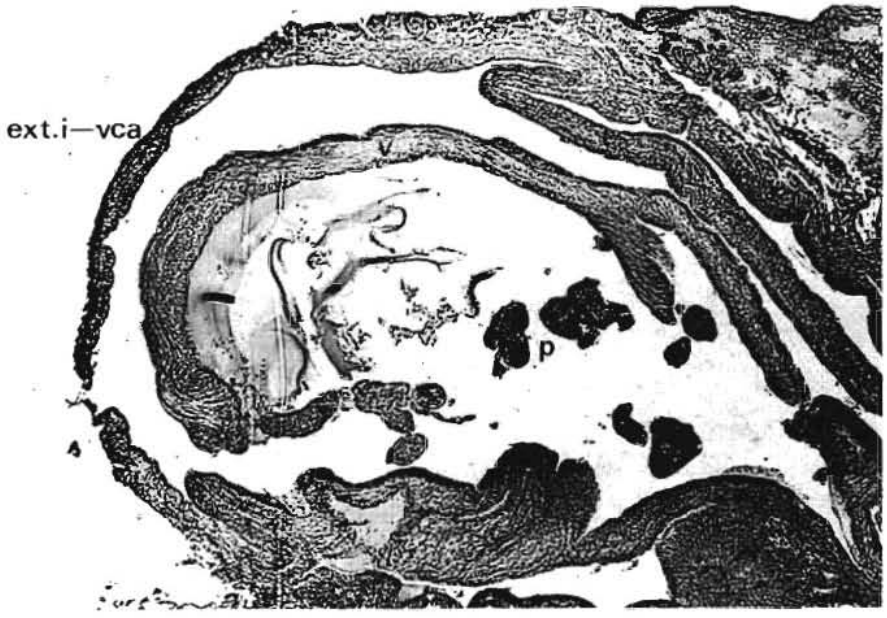

FIGURE 4
Section through aperture of renal portal valve and external iliac-vena cava anastomosis. ext. i-vca = external iliac-vena cava anastomosis. p = papillae. v = valve. (×80).

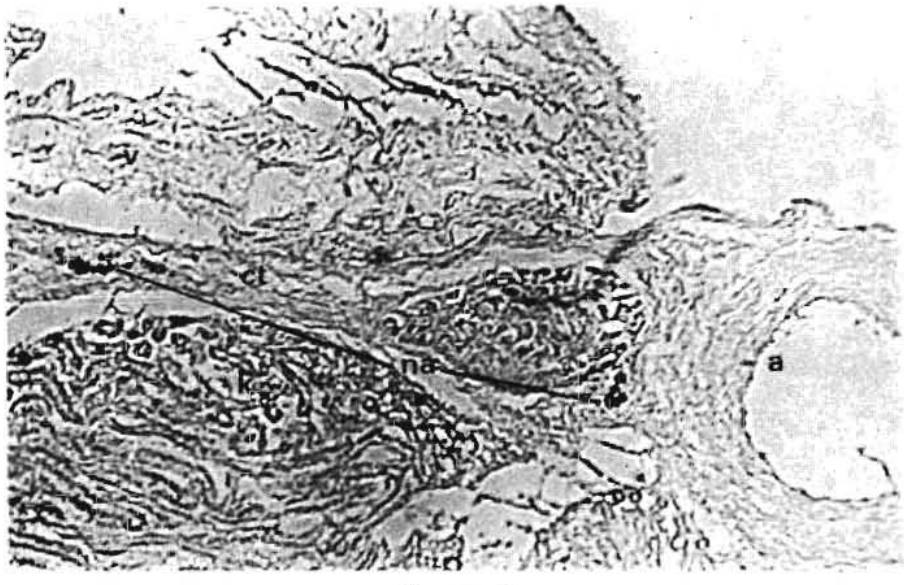

FIGURE 5
Positive reaction for noradrenalin (na) in the connective tissue (ct) surrounding the renal portal valve (v). k = kidney. a = femoral artery. (×200).

Spanner (1925) and Akester (1971) (Figures 1, 2, and 3). From the point where the caudal renal portal veins join, a large coccygeomesenteric vein runs forward, dorsal to the alimentary tract, and joins up with the hepatic portal system. The coccygeomesenteric vein receives only small tributaries from the posterior part of the alimentary canal, the vein that drains the cloaca being the most important (Figure 3). At the caudal margin of the posterior sections of the kidneys the coccygeomesenteric vein joins the venous arc formed by the caudal renal portal veins and these veins run ventral to the vertebral column and dorsal to the caudal and median sections of the kidneys to join up with the external iliac veins. The caudal renal portal veins receive, in addition to the large sciatic veins, a number of small veins from the coccyx and tail region (Figure 2). Only one afferent vein leaves each caudal renal portal vein and runs into the medio-ventral region of the caudal and median sections of the kidneys. Being very small this vein can convey very little blood to the kidney (Figures 1 and 3). Akester (1964, 1967, 1971) found that in *Gallus* several afferent veins enter the median and caudal sections of the kidney.

At the point where the caudal renal portal vein joins the external iliac vein, the small cranial renal portal vein branches off from the caudal renal portal vein (Figures 1, 2 and 3). The cranial renal portal vein joins the vertebral venous sinuses but it is not clear in which way the blood-flow in this vessel is directed. From this vein six to eight small veins run into the cranial region of the kidney (Figure 2). The portal blood supply to the cranial section of the kidney is better developed than the supply to the other sections. The veins draining the adrenal glands join the cranial renal portal veins and in *S. demersus* seem to be the main venous drainage in view of the extensive filling of the glands by latex in the corrosion casts (Figure 2). In contrast the main venous drainage of the adrenal glands in *Gallus* was found to be into the vena cava posterior (Goodchild 1969; Akester 1971).

The above-mentioned complex of veins is connected to the posterior vena cava by a median extension of the external iliac vein, the external iliac-vena cava anastomosis. This vein houses the renal portal valve. The position of the valve is such that when it is closed it prevents the blood from flowing from the portal and external iliac veins to the posterior vena cava. The blood is then partly shunted into the kidneys and eventually into the renal vein and vena cava or either partly into the vertebral venous sinuses via the renal portal vein, or partly to the liver via the coccygeomesenteric vein and hepatic portal system (Figure 1).

Morphology, histology and physiology of the renal portal valve

(i) *Morphology*

The renal portal valve is a unique structure in that it is the only valve in the blood vascular system, outside the heart, which contains innervated smooth muscle (Akester 1971).

Sperber (1948) investigated the renal portal valve of a penguin (species not identified) and described it as a membranous, funnel-like structure with one small aperture. Dissections on, and serial sections of, the valve of *S. demersus* showed it to be essentially the same.

The base of the valve is attached to the wall of the blood vessel (the external iliac-vena cava anastomosis) at the point where this vessel and the renal vein join to form the posterior vena cava (Figure 1). The valve is arranged with its long axis parallel to the flow of the blood in the

renal vein and posterior vena cava and as a result does not offer resistance to the flow of the blood in these two veins.

The valve of *S. demersus* therefore resembles the valve in *Gallus* (Akester 1967, 1971). It should be noted, however, that Figure 1 in Gilbert (1961) illustrating the valve in *Gallus* is misleading. Dissection of two fowls showed that the position of the valve in the vena cava external iliac anastomosis was incorrectly indicated by Gilbert. It is in fact more medially situated. Furthermore, the valve is illustrated as being an almost perfect cone, which it is not and the external iliac vein is incorrectly referred to as the femoral vein.

(ii) *Histology*

The renal portal valve in *S. demersus* consists of connective tissue and smooth muscle. In the basal region the circular arrangement of the smooth muscle cells is prominent and in places it is divided by connective tissue to form two separate bands and longitudinally arranged fibres are present on both sides of the circular muscle band. The valve thickens around the aperture and connective tissue is dominant while the papillae, arranged around the aperture, consist almost entirely of connective tissue (Figures 4 and 6). No large nerve trunks are present in, or enter, the valve in spite of the fact that the valve is extensively innervated by unmyelinated fibres, that showed clearly with fluorescent microscopy. This confirms some of the observations on the innervation of the valve in *Gallus* (Akester and Mann 1969) but is contradictory to what has been observed by Gilbert (1961), who found large nerve trunks entering the valve.

Noradrenergic fibres were positively identified in the connective tissue and vein walls in the immediate vicinity of the valve (Figure 5) but no positive staining reaction was observed in the valve itself. Histochemical staining of the valve for adrenergic fibres also gave negative results although nasal gland tissue, that was processed simultaneously, gave positive results. The presence of cholinergic fibres, however, was indicated by the positive reaction obtained for acetylcholinesterase staining (Figure 7). In view of the presence of cholenergic fibres and the positive pharmacological reaction of the valve to acetylcholine it would appear that the contraction of the valve is controlled by these fibres. Adrenergic fibres were found to be present in the valve of *Gallus* (Akester and Mann 1969) and the relaxation of the valve is under control of these fibres, although negative results for adrenergic fibres were obtained in the present investigation.

(iii) *Physiology*

The pharmacology of the renal portal valve of the turkey has been investigated *in vitro* by Rennick and Gandia (1954). I was able to confirm their results *in vivo* by exposing the valve and dripping saline solutions containing either 5 p.p.m. adrenalin or acetylcholine onto the valve alternatively. The valve contracted when acetylcholine was applied and was relaxed by adrenalin. In addition, saline fluoruceinum solutions containing either 5 p.p.m. adrenalin or acetylcholine were infused into the coccygeomesenteric vein under ultra-violet light. It was found that the normal direction of the blood-flow towards the kidneys was redirected towards the liver when an acetylcholine solution was infused but remained unchanged with the infusion of an adrenalin solution. The relaxing effect of infused adrenalin may implicate the circulating adrenal catecholamines in a regulatory rôle over the valve, either by potentiating the adrenergic effect of sympathetic neurons upon the smooth muscle of the valve, or by an antagonistic action on the cholinergic effect of the parasympathetic fibres innervating the smooth muscle of the valve.

The fluoruceinum-containing solutions were always infused into one of the small veins that join the coccygeomesenteric vein at right angles. The direction in which the fluorescent material was deflected could be traced without any difficulty and this technique ensured that the force with which the fluorescent solution entered the vein would not interfere with the direction of blood-flow in the vein.

Morphology and histology of the kidneys and ureters

The kidneys are divided into different regions which have been frequently but erroneously referred to as lobes (Sperber 1960; Sturkie 1954) if the definition of lobes and lobules of Ham (1965) is accepted. This has been pointed out by Goodchild and according to him the kidneys of the fowl should be divided into anterior, middle, and posterior divisions (Sillers 1971). The posterior division (region) of the kidneys of *S. demersus* consists of a number of small sections and no morphological boundary can be drawn between the middle and caudal regions. The cranial region is demarcated from the posterior section by the external iliac-vena cava anastomosis, but in one experimental animal, the cranial and middle divisions were continuous. It has been convenient to refer to the single large section, posterior to the external iliac-vena cava anastomosis, as the middle division, but it is clear that the division of the kidneys of birds into cranial, middle and caudal regions does not hold true in all cases.

As in the case of *Gallus*, the different regions of the kidney of *S. demersus* are lobulated, each lobe in turn consists of a large number of lobules. Within the lobules the cortical dominates the medullary tissue. In *Gallus* this was attributed to the small number of nephrons with medullary loops (Sillers 1971). The intralobular veins are large and this is rather surprising in view of the meagre portal blood supply to the kidneys, especially in the caudal division (Figure 3). The caudal portal blood supply to the kidneys of *S. demersus* seems to be less well developed in comparison to *Gallus* (Akester 1967).

The major part of the cortex is made up of intertwined nephric loops, mesangial cells are also present in the glomeruli. The juxta-medullary glomeruli are larger than the small cortical glomeruli which are arranged in a horseshoe arrangement around the central intra-medullary vein. The proximal convoluted tubules can readily be identified in sections stained with Toluidine blue by their brush border epithelium. In the medullary tract, the thin segments of the medullary loops are fewer in number than the thick segments. Cortical intermediate tubules are difficult to identify because, if the plane of sectioning through the tubules is tangential, proximal convoluted tubules may be mistaken for intermediate tubules. Nevertheless, the transition from proximal to distal convoluted tubules seems to be more gradual than in *Gallus*. The distal convoluted tubules are easily identified, as they are arranged in a peculiar fashion around the central veins. Five to eight tubules together form the sides of polygons which appear to be open spaces. This, however, could be an artifact caused by fixation. The collecting tubules are easy to identify by the intense orange-red fluorescence of the mucin in the tubules. The mucus probably acts as a transport agent for the expulsion of uric acid crystals. It increases in abundance towards the medulla, and the highest concentration of mucin is found in the primary and secondary branches of the ureters (Figure 8). In contrast to what has been found in *Struthio* (Louw *et al.* 1969) and in *Gallus* (Sillers 1971), the glandular epithelium of the ureters showed little sign of mucin secretion although mucin was always present in the lumen (Figure 9). The ureter walls contain outer circular and

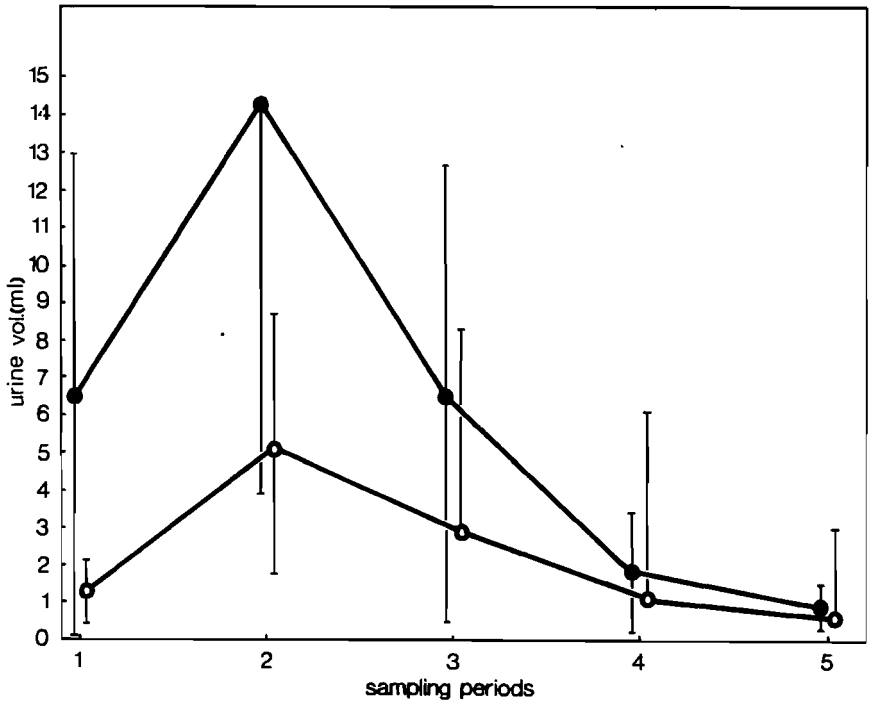

FIGURE 10
The effect of open and closed renal portal valves on urine volume.
○ "Open valve" group
● "Closed valve" group

inner longitudinal muscle fibres. The pulsating urine-flow from cannulated ureters (at ± 5 second intervals) can be attributed to the peristaltic contraction of the ureters, which was first observed in *Gallus* by Engelsman in 1869 (Sillers 1971).

It would appear therefore that the general histology of the kidneys and ureters of *S. demersus*, although differing in certain respects from *Gallus*, is typically avian. Moreover, none of the observations, apart from the copious mucin secretion, could be implicated in the efficient water balance of this species. This fact, in turn, emphasizes the importance of the nasal salt gland in the general water and electrolyte metabolism of *S. demersus*.

The effect of open and closed renal portal valves upon tubular secretion and glomerular filtration

Diuresis caused by waterloading in the fowl is thought to be brought about by inhibiting the secretion of anti-diuretic hormone (arginine vasotocin) by the neurohypophysis as well as by a higher glomerular filtration rate (Sykes 1971). In the present investigation the infusion of the previously described saline solution, containing inulin, into the coccygeomesenteric vein also resulted in diuresis (Figure 10). In one group of animals, the external iliac-vena cava anastomoses were bilaterally occluded to simulate conditions in the renal portal system when the renal portal valves are closed. In the second group the anastomoses were not occluded.

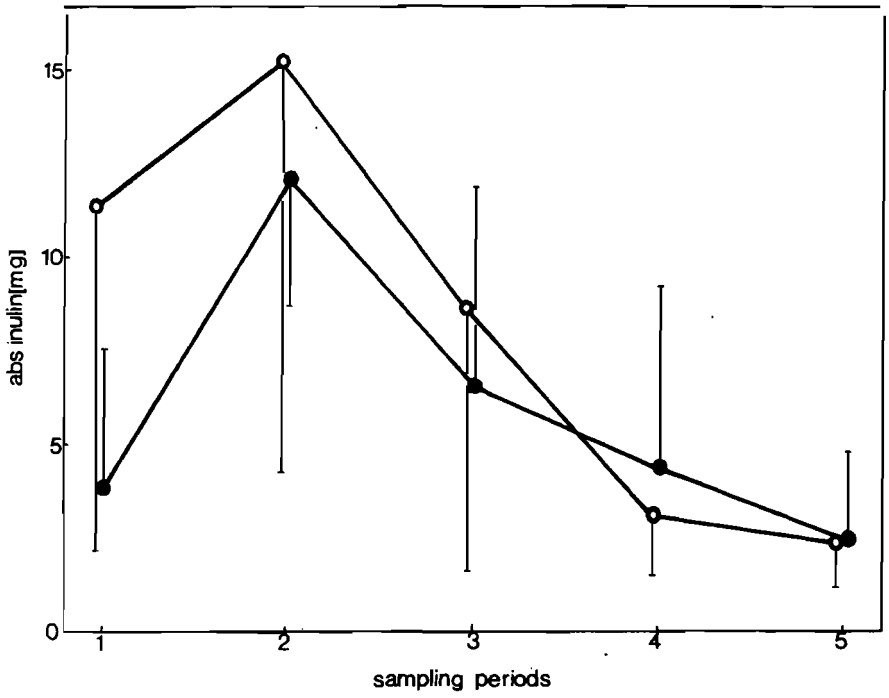

FIGURE 11

The effect of open and closed renal portal valves on the absolute inulin content of urine.

○ "Open valve" group
● "Closed valve" group

Significant differences were recorded ($P \leqslant 0{,}05$) in the urine volumes collected during the first two sampling periods. The mean volumes recorded in the group with the open valves were 6,4 ml during the first 10-minute period and 14,3 ml for the second 10-minute period whereas only 1,3 ml and 5,1 ml were collected respectively from the group in which the anastomoses were occluded (Figure 10). A similar difference was recorded in the inulin content of the urine (Figure 11) but the values from the two groups differed significantly only during the first 10-minute period ($P \leqslant 0{,}05$). This difference in inulin content, and therefore apparently also in filtration rate between the two groups, can probably best be explained as follows. First, inulin filtration in the "closed valve" group would have been delayed as inulin could only have reached the arterial circulation, and thus the glomeruli, by three possible routes; the first through the kidneys via the portal capillaries, renal vein and vena cava posterior, the second via the liver, and lastly there is a strong possibility that blood could have reached the arterial circulation via the cranial renal portal veins, the vertebral venous sinuses and external jugular vein as has been pointed out by Akester (1964, 1967) in *Gallus*. Any one, or any combination of these routes, would have caused a delay in the transport of the inulin to the glomeruli, while in the "open valve" group the inulin

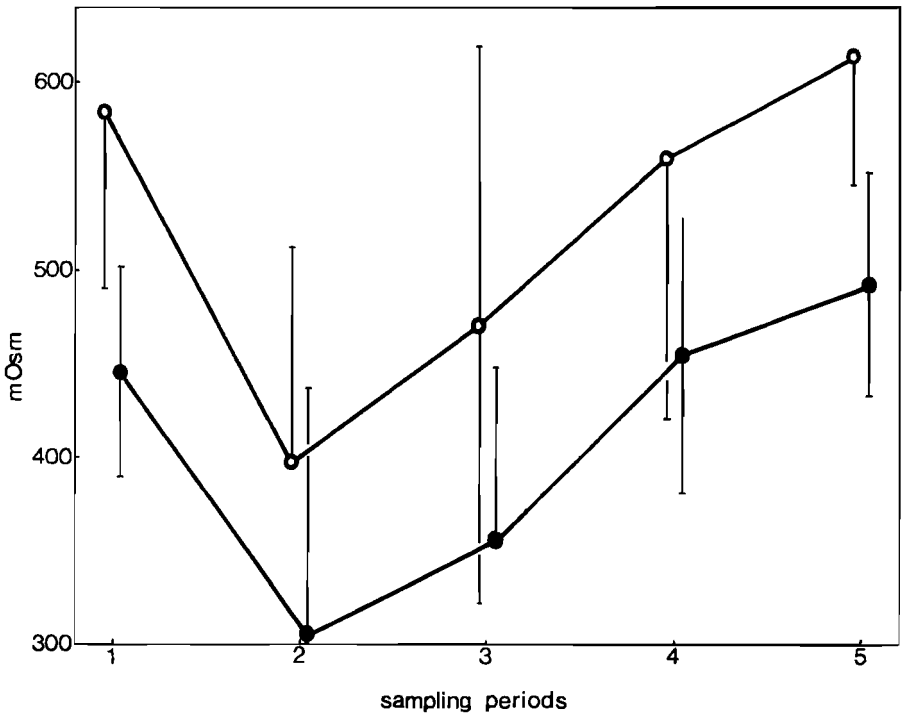

FIGURE 12
The effect of open and closed renal portal valves on the osmolality of urine.
○ "Closed valve" group
● "Open valve" group

would have entered the vena cava through the portal valve, and hence passed straight to the heart. This delay in the transport of the inulin to the glomeruli in the "closed valve" group could have resulted in less inulin being filtered during the first two periods of the experiment.

The second possibility is that the closing of the anastomoses could have caused an increased blood-flow through the afferent renal portal capillaries. In *Gallus*, the efferent glomerular arterioles open into the afferent portal capillaries (Sillers and Hindle 1969). This is most likely to be the same in *S. demersus* and an increased portal blood-flow would have caused an increase in pressure in the portal capillaries and subsequently a reduction in the flow of blood through the glomeruli. This in turn may have reduced the glomerular filtration rate and may partly explain the lower filtration rate in the "closed valve" group of animals. On the other hand, as filtration in the glomeruli is a purely physical process the higher blood pressure should partly have compensated for the decrease in blood-flow. For these reasons it is most likely that the difference in the inulin content of the urine between the two groups during the first periods of the experiment is a result of a lag effect caused by a more circuitous blood-flow, rather than a real difference in filtration rate (Figure 11).

The significantly lower ($P \leqslant 0{,}05$) volume of urine produced by the "closed valve" group

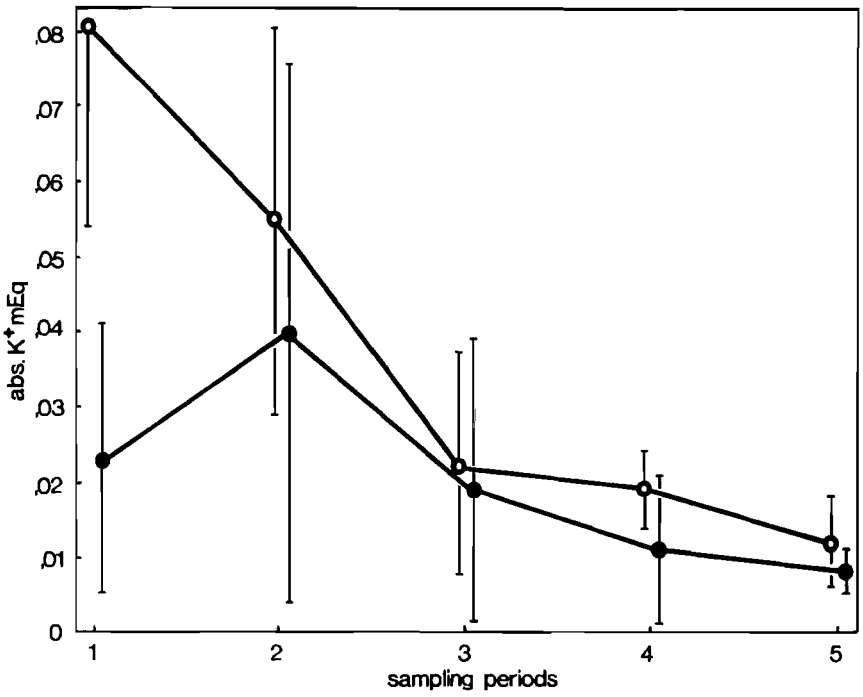

FIGURE 13
The effect of open and closed renal portal valves on the absolute potassium contents of urine.
○ "Open valve" group
● "Closed valve" group

during the second 10-minute period, 6,5 ml as opposed to 14,5 ml (Figure 10), can only be explained by a higher rate of water reabsorption in the "closed valve" group as during this time there was no significant difference in the glomerular filtration rate between the two groups. A higher reabsorption rate of water can be expected if the blood supply to collecting ducts and tubules is increased and this is what is to be expected when the portal system is in operation.

Osmolality

Reabsorption of water and the resulting concentration of urine appears to be more efficient in *S. demersus* than in most other birds. Osmoconcentrations of cloacal fluid (679,0 mOsm/l) as well as urine (651,0 mOsm/l) were found to be more than twice that of the plasma (298,0 mOsm/l, Oelofsen 1973). These values were for hydrated birds and are higher than the highest values obtained for cloacal fluid in dehydrated fowls (500 mOsm/l; Skadhauge 1967) and those for the road-runner (484,5 mOsm/l; Ohmart 1972). They are slightly less than the values for cloacal fluid obtained from dehydrated ostriches (800,0 mOsm/l; Louw *et al.* 1969). In view of the above it would be very interesting to establish the maximum concentrating ability of the kidneys of *S. demersus* when the animals are in a state of dehydration.

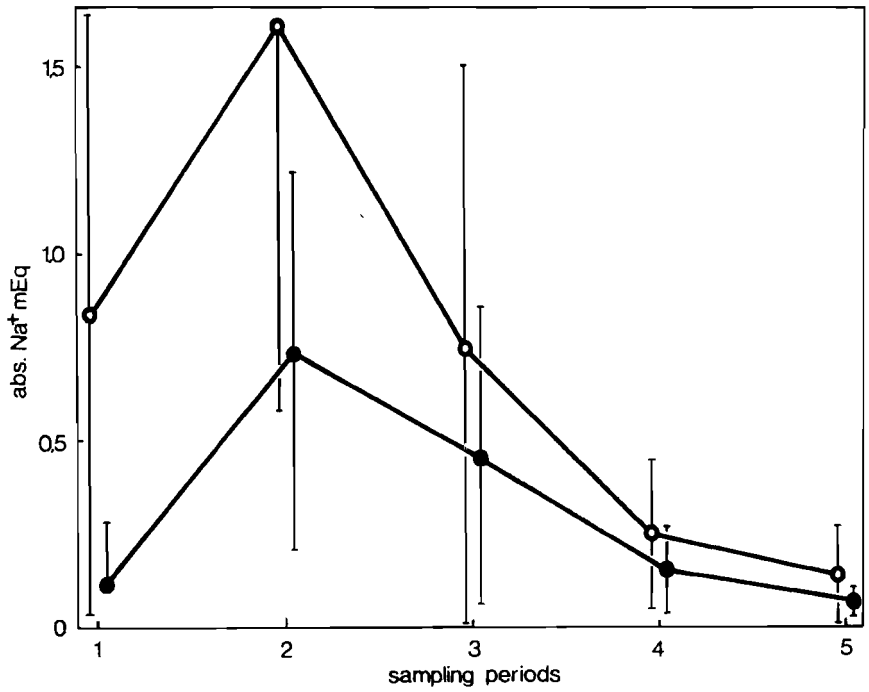

FIGURE 14

The effect of open and closed renal portal valves on the absolute sodium contents of urine.

○ "Open valve" group
● "Closed valve" group

Electrolytes

Net urinary excretion rates of potassium, sodium and chloride are shown in Figures 13, 14 and 15. Excretion rates for these ions follow a similar pattern to that shown by urine volume and inulin. However, sodium was excreted in significantly ($P \leqslant 0{,}05$) smaller amounts during the first two 10-minute periods of sampling in the "closed valve" group (Figure 14) whereas chloride values (Figure 15) in the two groups differed significantly ($P \leqslant 0{,}05$) only during the first sampling period. This difference in the excretion of sodium between the two groups could only have resulted from the reabsorption of sodium in the "closed valve" group as there was no difference in the filtration rate in the two groups of animals. The reabsorption of sodium follows the trend in urine volume closely and therefore water reabsorption as well. This is to be expected, as the reabsorption of water in the kidney tubules is dependent on reabsorption of sodium ions.

Potassium was secreted in significantly ($P \leqslant 0{,}01$) greater amounts by the kidney tubule in the "open valve" group. Although the filtration rate during the second 10-minute period was greater than during the first period (Figure 11), the absolute excretion rate of potassium was greater during the first period. Because the absolute excretion rate of potassium in the "closed valve" group follows the inulin excretion closely, the increased potassium excretion in the "open

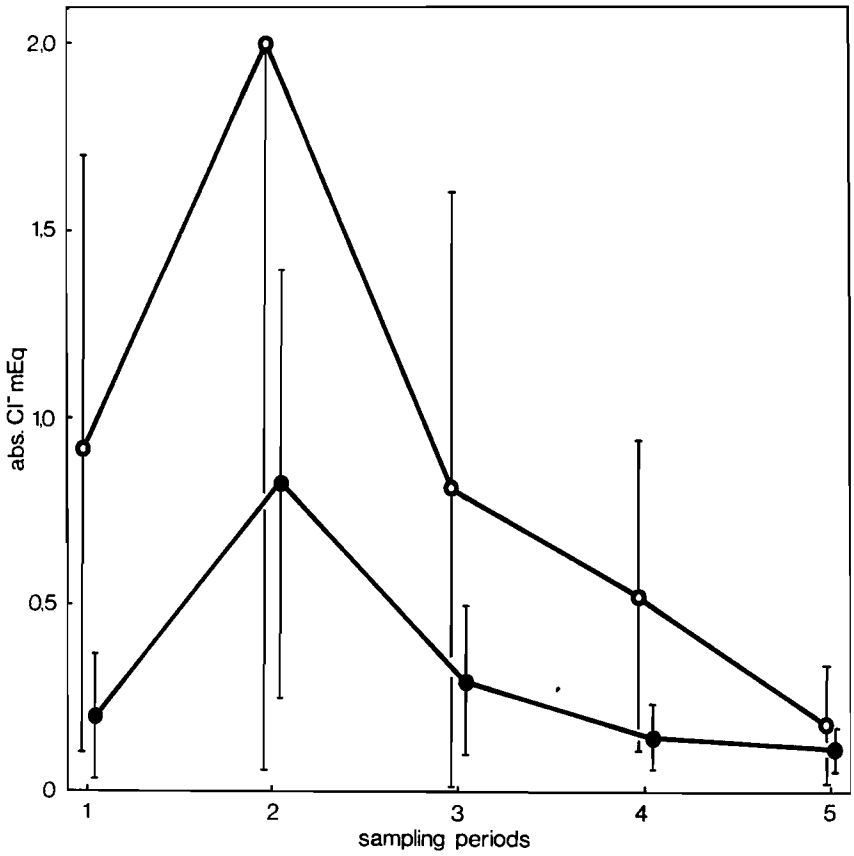

FIGURE 15

The effect of open and closed renal portal valves on the absolute chloride contents of urine.
○ "Open valve" group
● "Closed valve" group

valve" group must have resulted from tubular secretion because inulin clearance during the same period was not increased. This finding, however, is contradictory to what has been found in *Gallus* when a potassium sulphate solution was infused into the renal portal system. Potassium was excreted by the kidney on the infused side, that is the side in which the renal portal system was functioning (Rennick and Gandia 1954; Sykes 1971).

Uric acid and urea

Urine osmolality in both groups was greatest when urine flow was at its lowest and *vice versa* (Figure 12). Urine osmolality in the "closed valve" group was significantly greater ($P \leqslant 0{,}05$) during all periods, except for the second period and, as urine flow was reduced in the "closed valve" group, this was to be expected. It has been suggested that the main function of the

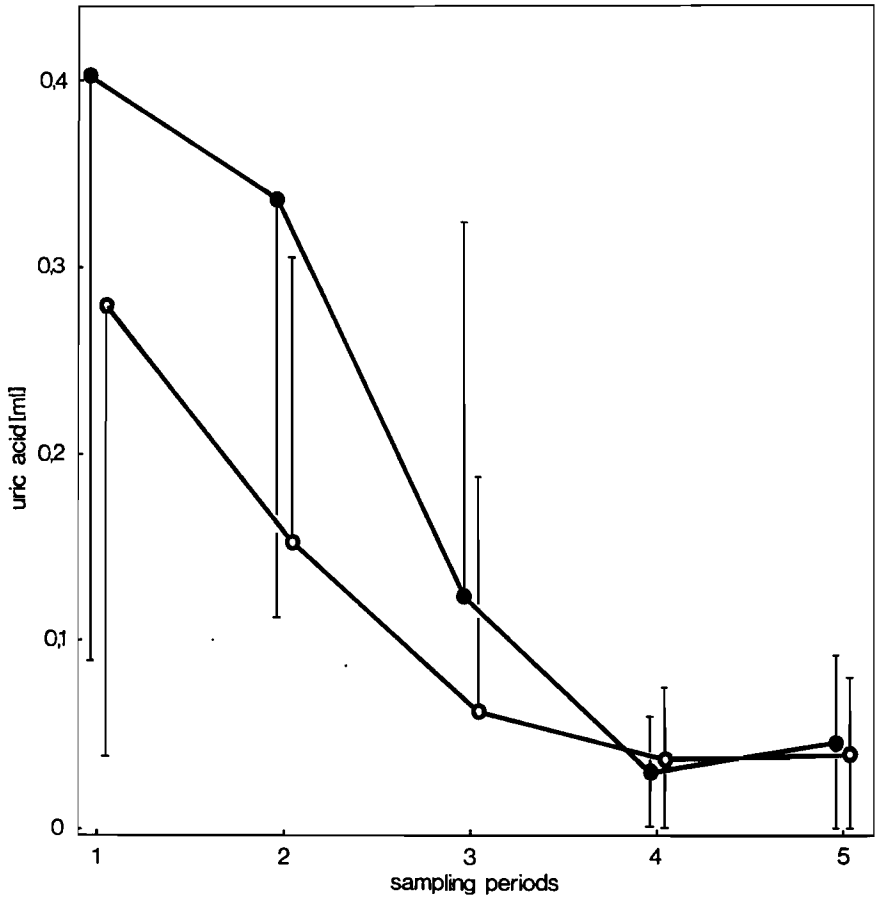

FIGURE 16
The effect of open and closed renal portal valves on the uric acid volume in urine.
● "Open valve" group
○ "Closed valve" group

renal portal system is to promote the tubular secretion of uric acid (Sykes 1971). In this experiment there were, however, no significant differences in undissolved uric acid content of the urine between the two groups of animals (Figure 16). The initial high values in uric acid content were probably due to a flushing effect of water diuresis upon the collecting tubules and ducts and not due to an increase in tubular secretion of uric acid. The uric acid/inulin ratio during the first 10-minute period was higher in the "closed valve" group (1:74) than in the "open valve" group (1:35). This trend was, however, reversed during the second period; open 1:21, closed 1:13. In the last three periods the values reached a plateau and were exactly equal during the last two periods.

Although only statistically significant at $P \leqslant 0{,}10$, there was a strong tendency for urea values

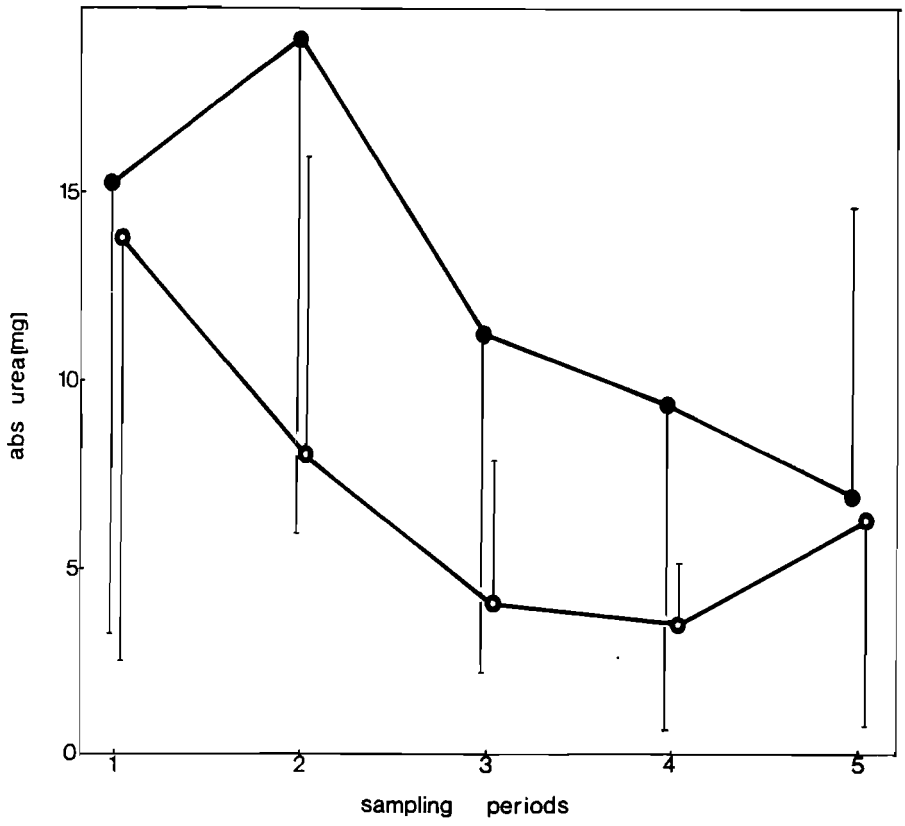

FIGURE 17

The effect of open and closed renal portal valves on the absolute urea content of urine.

○ "Open valve" group

● "Closed valve" group

to be higher in the "closed valve" group (Figure 17) and this is in direct contrast to the tendencies shown by all other urinary components that were determined (potassium, sodium, chloride and uric acid). The amount of urea excreted was only 5–6 times less than the uric acid excreted, if only the last two 10-minute periods, when the uric acid values reached a plateau, are considered. Birds generally excrete from 20–60 times more uric acid than urea (Campbell 1970) with the exception of the ostrich which excretes a considerable amount of urea (2–3 mg/ml; Schütte 1973) but in the present experiment uric acid only exceeded the urea by ± 20 times during the first 10-minute period, when the diuretic "flush" effect on the uric acid was at its greatest. It therefore seems probable that the values for the last two periods are more representative of the true ratio of urea to uric acid excreted.

The large amount of urea (maximum of ± 15 mg/ml) excreted during the first 10-minute period ("closed valve" group) and the trend for urea values in the "closed valve" group to be

higher than in the "open valve" group, in spite of a higher glomerular filtration rate in the "open valve" group, indicate the possible existence of a tubular secretory pathway for urea, although no positive evidence for such a mechanism has yet been found in *Gallus* (Sykes 1971).

The high degree of variation in individual values, particularly in the case of electrolytes, was probably due to two factors. First, inherent variation of these ions in the urine of birds is notoriously high as pointed out by Sykes (1971) and secondly, the assumption that the renal portal valve in the "open valve" group was open throughout the collection period, may not always have been justified. For example, Akester (1967) has shown that the renal portal valve in *Gallus* was closed for 25% of the time while the birds were anaesthetized. Another factor that probably influenced the results was the unphysiological conditions created by excessive water-loading of the experimental animals. The latter procedure was, however, essential to obtain a measurable flow rate over a relatively short experimental period.

SUMMARY AND CONCLUSIONS

In final summary therefore it is clear that, under the conditions of this experiment, individual variation and small sample size precluded any definite conclusions. Nevertheless, statistically significant differences in glomerular filtration rates, osmolality and the excretion rate of sodium and potassium were recorded between the "open" and "closed" valve treatment groups. The higher urea values ($P \leqslant 0{,}10$) in the "closed valve" group and the large amount of urea excreted by these birds makes it tempting to speculate on the existence of a tubular secretory pathway for urea in *S. demersus*. The high concentration of urea may account in part for the high osmolality of the urine, especially during the last two sampling periods (Figure 12). It has been argued that the precipitation of uric acid crystals in the kidney tubules of birds, by blocking and impairing the flow of glomerular filtration, would prevent an improvement in the maximum concentrating ability of the avian kidney (Schmidt-Nielsen, Borut, Lee and Crawford 1963). The presence of large amounts of mucus, however, in the urine of some birds like *S. demersus* and the ostrich (Louw *et al.* 1969) can replace water as a transport agent for the uric acid crystals and this fact contradicts the above argument to some extent. It is therefore theoretically possible for these birds to concentrate their urine to a higher degree and it would be of no disadvantage if a larger amount of urea was excreted as in *Struthio* (Schütte 1973) and particularly in the case of a carnivorous bird such as *S. demersus* with a high intake of digestible protein.

It has been suggested that the renal portal system plays a rôle in the promotion of tubular secretion of uric acid (Sykes 1971). The higher ($P \leqslant 0{,}10$) urea values, however, in the "closed valve" group and the relatively large amount of urea excreted by *S. demersus* makes it tempting to speculate on the existence of a tubular secretory pathway for urea in this animal. This suggests a rôle in the promotion of urea secretion, rather than for uric acid secretion, for the renal portal system. Before any concrete conclusions can, however, be made, further detailed research is required.

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