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# EXCRETION IN THE CEPHALOPOD, *OCTOPUS DOFLEINI*

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Although excretory processes in vertebrate animals have been studied extensively during the past fifty years, comparatively few investigations have been carried out on invertebrates. The cephalopod molluscs represent a group which is suited to experimental study due to the large size that has been attained by some of the forms, their relatively high and constant level of metabolic activity and the presence of excretory structures which are accessible and well defined. For these reasons a study was initiated on the large octopus of Puget Sound, *Octopus dofleini martini* (Pickford, 1964).

Previous studies of the excretory system of the octopus have been limited for the most part to morphological investigations. The majority of these have been reviewed by Turchini (1923). The early physiological experiments conducted by Solger (1881), Kovalevsky (1889) and Cuénot (1899) consisted of injections of dye solutions with subsequent observations of the areas of localization. These authors reported dye accumulation in the renal appendages, the branchial hearts and the branchial heart appendages. From these results they considered these to be sites of excretion in the octopus.

In 1906 a brief physiological investigation of excretion in the octopus was made by Gompel & Henri. They injected various substances into the blood stream and followed their changes in concentration in the urine. From the results obtained, they concluded that secretion in the octopus seems to occur in the same fashion as in the dog.

Mayer & Rathery (1907) carried out the most detailed experiments reported on the excretory system of the octopus. These authors were concerned primarily with the problem of determining the function of the constituent elements in the complicated vertebrate kidney. They noted a similarity of the epithelium of the renal appendage of the octopus to that of the convoluted tubule of the mammal and began a study of excretion in the octopus in the hope that they would make some progress in interpreting the cellular activity of the epithelium of the vertebrate kidney.

After having shown that the molecular concentration of sea water, of blood and urine in the octopus are the same, the three liquids freezing at $-2{\cdot}24°$ C., they altered the concentration of the blood by the injection of glucose, sodium chloride or urea. In a short time urine was formed in greater than normal volume and was more concentrated in crystalloids. After a period of time the normal concentration of the blood was re-established. They concluded that the elimination of injected substances did not arise from a filtration mechanism because the urinary liquid was more strongly concentrated than the blood. Nor did they think that it arose by osmosis or diffusion

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since the differences in concentration of blood and urine were maintained. They concluded that the cells of the renal epithelium were capable of secreting into the urine whatever molecular species was present in the blood in abnormally high amount.

The authors proposed finally that the organs present in the renal sac were capable of reabsorption. When they injected glucose or urea into the renal sacs and ligated the urinary papillae, the concentration of the injected substance remained high in the urine for a few hours but was reduced to a mere trace 24 hr. after the beginning of the experiment. They did not attempt to identify the specific structures concerned in the reabsorption and did not propose a mechanism by which this was accomplished.

Bruni (1937) reported a somewhat similar type of study employing the injection of dyes intramuscularly. He followed the changes in concentration of dye in the urine, but not in the blood. He reported that different dyes are eliminated at different rates and that they are capable of inducing different degrees of polyuria. He concluded that the coloured materials were eliminated by a process of filtration.

Although important contributions have been made by these investigators, some of their conclusions are contradictory. In addition, the techniques employed did not permit evaluation of the dynamics of the processes going on in the animal. It has been the purpose of this investigation to try to establish a few of the mechanisms functioning in urine formation in the octopus under conditions as physiological as possible. The technique adopted was to follow for extended periods of time the concentration of specific agents in samples of blood, urine and pericardial fluid taken serially before, during and after the administration of metabolic poisons.

## MATERIALS AND METHODS

The animal used in this investigation was the large octopus of Puget Sound, *Octopus dofleini martini* (Pickford, 1964). The animals were obtained from two sources, intertidal dens found on the shores of islands of the San Juan archipelago and from the shallow waters of Salmon Beach near Tacoma, Washington. The animals kept well in the laboratory at a temperature between 10 and 13° C. and were fed 1–2 kg. specimens of *Cancer magister* or several small fish two or three times a week. The majority of the animals used in the experiments weighed from 10–12 kg., the range being from 8·4 to 21·7 kg.

To facilitate an understanding of the operative procedure, the general organization of the excretory system will be described briefly. According to Turchini (1923), a number of structures are considered to be important in excretion. These are special groups of cells in the liver and intestine, the renal appendages, the branchial hearts and the branchial heart appendages. The special cells of the intestine and liver whose activity has been considered by Mayer & Rathery (1907) will not be discussed here since they do not contribute waste products to the urine.

The renal appendages are seen in the two large, transparent, independent renal sacs which lie on the postero-ventral surface of the visceral mass (Fig. 1). These appendages hang free from blood vessels and contain spaces which communicate with the lumen of the blood vessels. Each appendage is irregular in form, deeply furrowed and covered by a layer of columnar epithelium which separates the blood on the inside of the appendage from the urine on the outside. The appendages and blood

vessels are independently contractile and the pulsating motion of the appendages can be seen through the wall of the sac. The urine accumulates in the renal sacs and is expelled through the urinary papillae which are situated on the anterior aspect of the sacs.

The supply of blood to the renal appendages is primarily venous. Part of it is from the large anterior vena cava which runs along the ventral median line to approximately the level of the lower margin of the liver. At this point the vessel bifurcates and its branches, the lateral vena cavae, pass laterally to the branchial hearts. Emptying into the lateral vena cavae are the abdominal veins which return blood from the ventral sinus which is closely associated with the digestive system. The lateral vena cavae and the abdominal veins pass through the wall of the renal sac well before their anastomoses and have the characteristic renal appendages hanging from their walls into the sac.

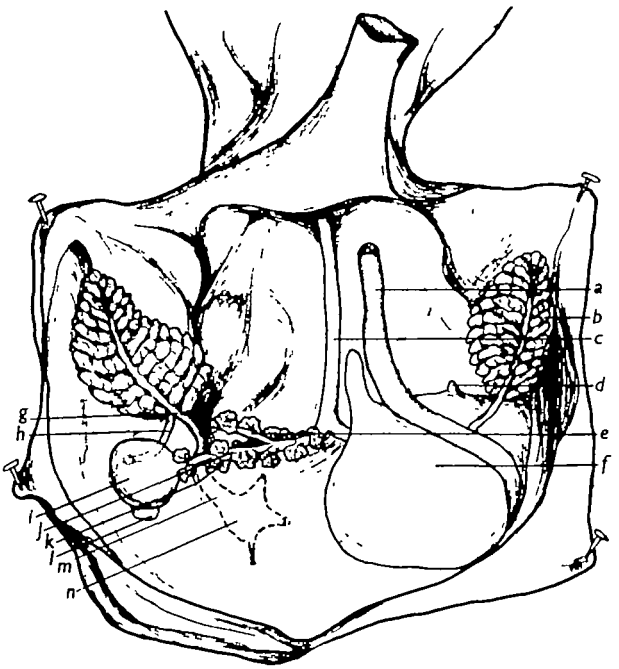


Fig. 1. Excretory and related circulatory system of *Octopus dofleini martini*, ventral view. Right renal sac is removed to show contained structures. *a*, Gonoduct; *b*, gill; *c*, anterior vena cava; *d*, urinary papilla; *e*, abdominal vein; *f*, renal sac; *g*, efferent branchial vein; *h*, afferent branchial vein; *i*, branchial heart; *j*, lateral vena cava; *k*, renal appendages; *l*, branchial heart appendage; *m*, auricle; *n*, ventricle.

The branchial hearts lie lateral to the renal sacs. These glandular-muscular organs are purplish in colour and have a small cavity which ramifies throughout the wall giving it a somewhat spongy consistency. The thick walls contain cells which are considered to be secretory in nature.

The small white branchial heart appendage is attached to the branchial heart by a stalk. This organ contains a cavity continuous with that of the branchial heart. The cavity subdivides into branches which distribute blood throughout the organ. The exterior surface is somewhat irregular as a result of numerous invaginations

The branchial hearts receive blood directly from the vessels on which the renal appendages are attached. A portion of the blood undoubtedly is forced through the channels of their spongy tissue as well as into the branchial heart appendages. Most of the blood is pumped immediately to the gills through the afferent branchial veins and away from them by the efferent branchial veins. The efferent branchial veins conduct the blood towards the main heart to be distributed throughout the body.

In comparison with other molluscs the pericardial cavity of cephalopods is much reduced and in octopods encloses only the branchial heart appendage. The pericardial cavity on each side communicates with the renal cavity by a reno-pericardial canal which is lined by cilia. These cilia beat in such a way as to direct the contents of the canal toward the renal sac. The canal opens into the renal sac by a ciliated funnel near the urinary papilla.

The pericardial cavity also communicates with the gonadial cavity by means of the aquiferous canal. A valve is present at the opening of this canal into the pericardial cavity which permits flow of fluid only away from the gonadial cavity. The relationships among these cavities can be seen in Fig. 2.

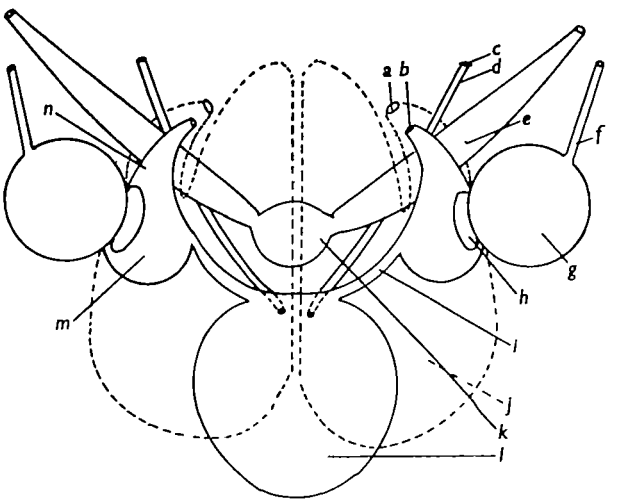


Fig. 2. Diagram showing the relationships among the circulatory system, derivatives of the coelom and the excretory system of the octopus. Adapted from Turchini, 1923. *a*, Urinary pore; *b*, reno-pericardial canal opening into the renal sac; *c*, gonopore; *d*, gonoduct; *e*, auricle; *f*, afferent branchial vein; *g*, branchial heart; *h*, branchial heart appendage; *i*, aquiferous canal; *j*, renal sac; *k*, ventricle; *l*, gonadial coelom; *m*, pericardial cavity; *n*, reno-pericardial canal.

The animals were prepared for study the evening before the day on which an experiment was to be performed. An octopus was placed in a tub containing a known volume of water. Ethyl alcohol, used for anaesthesia, was added gradually to the sea water until the concentration was approximately 2·5 %. When the mantle was relaxed sufficiently, which normally took between 15 and 30 min. from the initial addition of alcohol, hands were inserted into the mantle opening and the structure to be catheterized was brought to the exterior. First the renal sacs were catheterized by inserting rubber catheters through the renal pores into the sac. The catheters (4·15 mm. O.D., 2·5 mm. I.D.) which were multiply perforated for approximately 20 cm. of their

length were tied in place at the urinary papillae. Next the circulatory system was cannulated with polyethylene tubes (O.D. 2·0 mm.). In the early experiments the branchial heart was cannulated directly. In later experiments both the anterior vena cava and the afferent branchial vein were cannulated. This operation permitted the infusing of test material into the afferent branchial vein and sampling of blood at the anterior vena cava. In those experiments where pericardial fluid was obtained, the reno-pericardial canal was catheterized near the funnel and then tied off by a suture between the catheter and the funnel.

At the close of the operation the animal was taken out of the tub and replaced in circulating sea water. Shortly after the animal had resumed its normal respiration and righted itself, the body was placed in a net bag or skirt which was tied at the constriction between the arms and the body proper. This precaution was necessary to prevent the animal from pulling out the cannulae with the tentacles and so bleeding to death. The animal then was allowed to recover from the operation until the next morning at which time the experiment was begun.

In preparation for the injection and sampling procedures long polyethylene tubes of small capacity (O.D. 2·0 mm.) were connected to the catheters in the renal sacs and blood vessels and the net bag was removed. The animal was free to move about in the aquarium and appeared normal. Injections could be made into the circulatory system and samples withdrawn from the vein and renal sacs. At the end of an experiment the animal was again anaesthetized, the tubes were removed, and the wounds closed with purse-string sutures. The animals lived very well in spite of the operation, would take food, and were often used in subsequent experiments after a week or two had elapsed.

The sampling and injection procedure which was used for the majority of the experiments was the following. After normal blood and urine samples had been taken, the test solution was infused gradually into the circulatory system until the blood concentration was brought to a desired level. Additional test solution was infused very slowly into the circulatory system throughout the experiment in order to maintain the blood concentration. Blood samples were taken generally at $\frac{1}{2}$ hr. intervals for the duration of the experiment. To compare with each blood sample a representative urine sample was collected for equal periods of time, generally 15 min., before and after the time of the blood sample. When metabolic poisons were administered they were infused with the test substances. It was hoped that this precaution would reduce the possibility of injury to the tissues by local high concentrations of the poisons.

The normal blood is blue in colour due to the presence of large amounts of haemocyanin. The amount of protein in the blood averaged 10·2% for ten different animals, the range being from 8·2 to 11·6%. The analytical method used was that of Ballentine & Gregg (1947). The blood cells are few in number. Generally the initial drops of blood taken from the catheter were cloudy as the result of the collection of cells in the catheter overnight. This sort of loose plug must help to limit bleeding which is controlled ordinarily by vasoconstriction as no blood clotting mechanism is present.

The initial urine samples were somewhat cloudy on account of the presence of mucus, various granules and mesozoans. As the experiments proceeded the urine clarified. The complete emptying of the renal sacs was difficult to establish because

of the interior structure of the renal sac. Each sac is incompletely divided into three compartments and filled with numerous renal appendages which further increases the irregularity of the interior of the cavity. Rinsing out such a structure proved to be so time consuming as to interfere with the subsequent sampling period. Generally the urine samples were obtained by draining the fluid out of the renal sacs under slight negative pressure during the last 5 min. of the sampling period.

During the course of the experimentation the excretion of the following substances was tested; inulin, glucose, *p*-amino hippuric acid, phenolsulphonphthalein and urea. The deproteinization of the blood and urine in all cases was made by the zinc sulphate technique (Somogyi, 1930). A Beckman DU spectrophotometer was used for all the colorimetric analyses. Standard procedures were followed in a majority of the cases. Glucose was assayed using Dreywood's anthrone reagent (Morris, 1948) and the glucostat method (Teller, 1956). For urea the method of Archibald (1945) was used. Goldring & Chasis's method (1944) was employed for the determination of *p*-amino hippuric acid. For inulin the method of Harrison (1942) was used as well as the anthrone method (Young & Raisz, 1952).

The analysis of phenol red presented some complications due to the masking of the colour by the haemocyanin of the blood and to the lower pH of the urine which changed the colour of the phenol red. Haemocyanin was reduced by the addition of a 0·6% solution of sodium thiosulphate in sea water to give a 1:3 dilution of the blood. In addition, the pH of the sample was adjusted by the addition of 0·5 N-NaOH in small quantities. This did not cause denaturation of the proteins and the optical density of the blood samples could be determined without further preparation. It was necessary to add normal blood to the standards which were then reduced and the pH adjusted in the same manner as for the samples.

The protein content of the urine was so low that the amount of dye bound was negligible, but was yet sufficient to produce a turbid solution when the pH was adjusted by the addition of a basic solution. Consequently, the solutions were deproteinized and the pH was adjusted before the optical density was determined. In all cases the concentration of the substance was determined by reference to a standard curve of concentration versus optical density, based upon dilutions of the solutions injected into the animal.

## RESULTS

### (1) *Filtration*

In an investigation of excretion it is important to know whether urine formation involves filtration of the blood. The polysaccharide inulin has been found to satisfy adequately the criteria proposed for the excretion of a substance by filtration alone (Smith, 1951). The experiments described below were performed to ascertain how inulin is excreted in the octopus.

When inulin is infused into the circulatory system of the octopus, it is excreted slowly. The details of the initial injection, the subsequent rate of infusion and the addition of poisons for each experiment are given in Table 1. The results of a typical experiment are presented in the upper graph of Fig. 3. It may be noted that the blood concentration was brought quickly to a certain level and maintained there quite consistently throughout the experiment. The urine concentration of inulin reached

Table 1. *Summary of the experimental procedures*

| Expt. no. | Weight (kg.) | Material injected (mg) | | Injection rate (mg./kg./hr.) | Blood concentration (mg. %) | Drug time and dosage (mg./kg.) | Urine flow (ml./hr. per 10 kg.) | Pericardial fluid flow (ml./hr. per 10 kg.) |
|---|---|---|---|---|---|---|---|---|
| 1 | 10·4 | Inulin | 60 | 4·3 | (Fig. 1) | Benemid | R. 13·9 | — |
| | | PSP | 8 | 0·6 | 1 | 312–525 min. | L. 18·8 | — |
| | | PAH | 24 | 1·7 | 2·3 | 11·5 | | |
| 2 | 11·7 | Inulin | 100 | 0·9 | 5 | DNP | R. 4·4 | — |
| | | PSP | 12 | 0·1 | 0·9 | 300 min. | L. 7·2 | — |
| | | PAH | 50 | 0·5 | 0 6 | 5·2 | | |
| 3 | 11·0 | Inulin | 75 | 5·1 | 14 | Benemid | R. 4·1 | — |
| | | PSP | 15 | 1·0 | 2·7 | 260 min. | L. 5·2 | — |
| | | PAH | 75 | 5·1 | 7·9 | 1·8 | | |
| 4 | 9·6 | Inulin | 35 | 2·1 | 5 | DNP | R. 7·8 | — |
| | | PSP | 7 | 0·4 | 1·4 | 260–510 min. | L. — | — |
| | | PAH | 35 | 2·1 | 1·2 | 7·3 | | |
| 5 | 13·1 | Inulin | 50 | 3·8 | 7 | | R. 4·4 | — |
| | | | | | | | L. 8·5 | — |
| 6 | 10·0 | Inulin | 50 | 5·0 | 10 | Benemid | R. 12·6 | — |
| | | PSP | 5 | 0·5 | 0·8 | 310–445 min. | L. 10·9 | — |
| | | PAH | 20 | 2·0 | 1·5 | 5·0 | | |
| 7 | 13·8 | Inulin | 50 | 3·6 | 12 | Benemid | R. 6·1 | — |
| | | PSP | 7 | 0·5 | 1·3 | 270–490 min. | L. 16·2 | — |
| | | PAH | 30 | 2·2 | 2·7 | 9·3 | | |
| 8 | 10·2 | Inulin | 160 | 7·8 | 15–30 | Phlorizin | R. 7 6 | — |
| | | Glucose | 400 | 19·6 | (Fig. 7) | 330–630 min. | L. 27·1 | — |
| | | | | | | 13·5 | | |
| 9 | 9·7 | Inulin | 171 | 8·7 | 20–45 | — | R. 5·5 | — |
| | | | | | | | L. 46·5 | — |
| 10 | 13·6 | Inulin | 150 | 7·4 | (Fig. 6) | — | R. 23·4 | R. — |
| | | PAH | 30 | 1·2 | (Fig. 19) | — | L. — | L. 18·7 |
| 11 | 10·0 | Inulin | 140 | 7·2 | 23 | — | R. 17·5 | R. — |
| | | PAH | 42 | 3·2 | 3 | — | L. — | L. 13·3 |
| 12 | 14·3 | Inulin | 225 | 9·5 | 20 | — | R. — | R. 11·6 |
| | | PAH | 45 | 1·9 | 1·4 | — | L. — | L. — |
| 13 | 14·0 | Inulin | 150 | 5·0 | 17 | — | R. | R. 11.0 |
| | | PAH | 30 | 1·0 | 1 | — | L. — | L. — |
| 14 | — | Inulin | 200 | — | 2 3 | — | R. — | R. 7·2* |
| | | PAH | 38 | — | 1·5 | — | L. 16·1† | L. — |
| 15 | 21·7 | Glucose | | 19, 85 | (Fig. 8) | — | R. 11·8 | R — |
| | | | | | | | L. — | L. 7·4 |
| 16 | — | — | | — | (Fig. 9) | — | R. 1·0† | R. — |
| | | | | | | | L. — | L. 3·2* |
| 17 | 9·7 | — | | — | (Fig. 10) | Phlorizin | R. — | R. 7·5 |
| | | | | | | 245–450 min. | L. — | L. 8 9 |
| | | | | | | 10·3 | | |
| 18 | 12·0 | PSP | 5 | 0·3 | 0·4 | — | R. 10·2 | — |
| | | | | | | | L. 6·3 | — |
| 19 | 8·4 | PAH | 40 | 3·6 | 3·6 | — | R. 27·1 | — |
| | | Urea | 120 | 10·9 | 64 | — | L. 5·1 | — |
| 20 | 17·7 | PSP | 10 | 0·3 | 0·7 | — | R. 17·2 | — |
| | | | | | | | L. 20·1 | — |
| 21 | 18·6 | PSP | 5 | 0·4 | (Fig. 18) | DNP | R. 14·5 | R. — |
| | | | | | | 390–930 min. | L. — | L. 7·3 |
| | | | | | | 10 | | |
| 22 | — | PAH | 20 | (20 mg./hr.) | 1·5 | — | R. 22·5† | R. — |
| | | | | | | | L. — | L. 7·5* |
| 23 | 10 | PAH | 20 | 2·0 | 0 3 | DNP | R. 44·0 | R. — |
| | | | | | | 510–885 min. | L. — | L 1·0 |
| | | | | | | 15 | | |
| 24 | — | Urea | 2000 | (800 mg./hr.) | 22 | — | R. 15·0† | — |
| | | | | | | | L. 2·5† | — |
| 25 | 8·4 | Urea | 1200 | 675 | 34 | — | R. — | — |
| | | | | | | | L. 3·0 | — |

* Pericardial flow in ml./hr. for the animal.
† Urine flow in ml./hr. for the animal.
R, right renal sac; L, left renal sac.

the blood concentration only after a lag period of approximately 1½ hr. Subsequently the urine concentration was the same as the blood concentration within the limits of the experimental methods used.

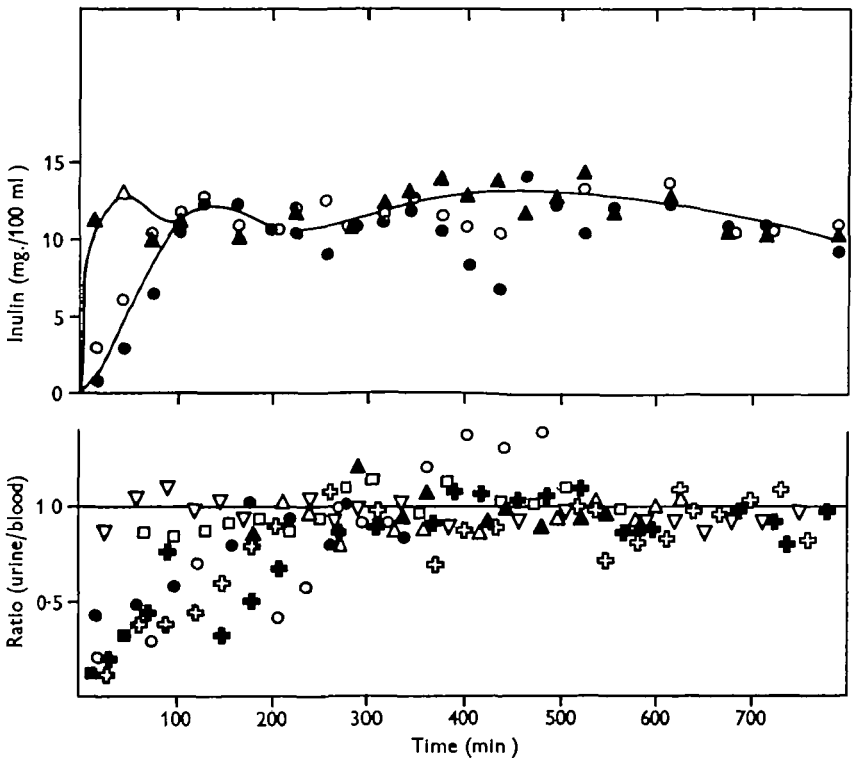


Fig. 3. Upper graph: the relationships of the concentrations of inulin in the blood, right renal sac urine and left renal sac urine during a typical experiment. Expt. 1: ▲, blood inulin; ●, right renal sac urine; ○, left renal sac urine. Benemid given from 315 to 525 min. The total dosage of benemid given was 11·5 mg. per kg. Lower graph: the ratio of the concentration of inulin in the urine to that of the blood for each sampling period during perfusion experiments. The approximate concentration of inulin in the blood and the time and dosage of drugs are shown in Table 1. Expt. 2: ○, average of right and left renal sac urine; Expt. 3: ●, average of right and left renal sac urine; Expt. 4: △, right renal sac urine; Expt. 5: ▲, average of right and left renal sac urine; Expt. 6: □, average of right and left renal sac urine; Expt. 7: ✣, average of right and left renal sac urine; Expt. 8: +, left renal sac urine; Expt. 9: ▽, left renal sac urine.

In order to summarize quickly all of the experiments performed with inulin, there are plotted in the lower graph of Fig. 3 the ratios of the urine concentration to blood concentration against time for eight separate animals. After the initial lag period, which differs from animal to animal, the values of the ratios distribute about a value of 1. In general the administration of metabolic poisons did not appear to affect the ratios. In Expt. 2 dinitrophenol seemed to produce an increase in the ratio. However, in this experiment the concentrations in blood and urine were so low that experimental errors became unduly important. Consequently, the significance of this increase in ratio is questioned.

Since in the vertebrate the relationship of clearance to blood concentration is a critical test for judging whether a substance is filtered, clearances were calculated for

the experiments on inulin. Clearance is defined as UV/B where U equals the concentration of inulin in the urine, B equals the concentration of inulin in the blood and V equals the volume of urine per unit time. When the clearances obtained from each renal sac for a sampling period are plotted against blood concentration the scatter of the data is such that no clear relationship emerges. The parameter which is the source of the variability is the rate of urine flow. From Fig. 4 it is clearly apparent that in the octopus the inulin clearance values vary directly with, and can be considered equivalent to, the rates of urine flow. As the urine/blood inulin ratios remained at 1 even at the lowest rates of urine flow, no significant amount of reabsorption of water occurs. In the octopus, then, a more useful criterion for determining whether inulin is filtered is to plot the urine/blood inulin ratio against blood concentration. As seen in Fig. 5 the ratio appears to be independent of the blood concentration and it is concluded that inulin is excreted by filtration.

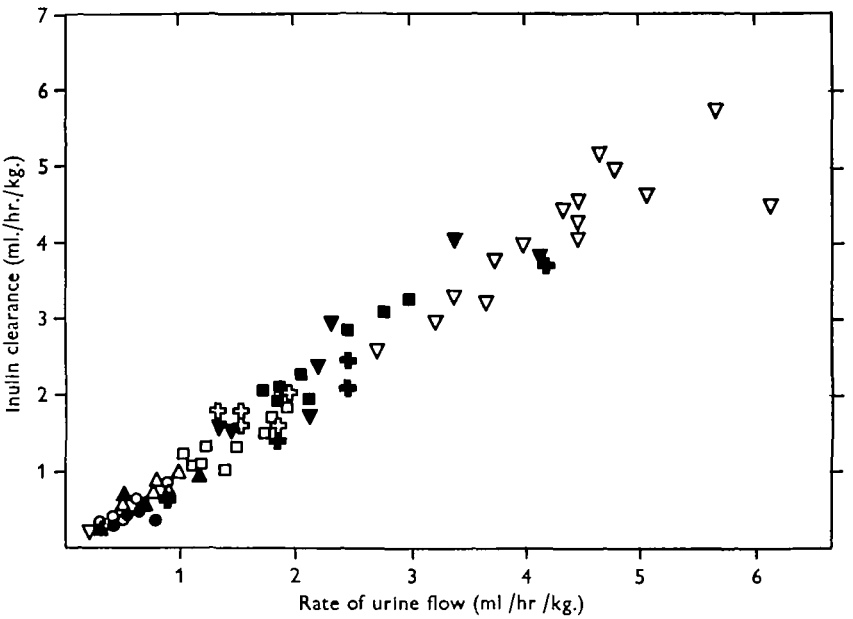


Fig. 4. The relationship of the clearance obtained from each renal sac at a sampling period to the rates of urine flow at that sampling period. Expt. 1: ■, right and left renal sac urine; Expt. 2: ○, right and left renal sac urine; Expt. 3: ●, right and left renal sac urine; Expt. 4: △, right renal sac urine; Expt. 5: ▲, right and left renal sac urine; Expt. 6: □, right and left renal sac urine; Expt. 7: ✣, right and left renal sac urine; Expt. 8: +, right and left renal sac urine; Expt. 9: ▽, right and left renal sac urine; Expt. 10: ▼, right renal sac urine.

The question which arose immediately after the results with inulin were obtained was where filtration took place. To determine whether the pericardium is involved, experiments were performed with one reno-pericardial canal catheterized and then ligated between the catheter and the funnel to prevent flow of fluid from the pericardial cavity into the renal sac. The other reno-pericardial canal–renal sac relationship was not altered. It became immediately obvious after the initiation of the experiment that no urine could be collected from the renal sac isolated from the pericardium, although the production of urine from the unaltered side was normal. To simulate

normal conditions, a volume of sea water comparable to that of the volume of urine usually produced was injected into the 'isolated' renal sac. In the upper part of Fig. 6 are shown the results from such an experiment. The pericardial fluid collected from the left reno-pericardial canal, the right kidney urine and the blood, all contained inulin in approximately the same concentration. The sea water injected into the left renal sac was essentially free from inulin. Additional experiments showed that these same results were obtained with the right reno-pericardial canal catheterized and the right renal sac 'isolated' instead of the left and when the blood concentration of inulin was varied from 2 to 30 mg./100 ml.

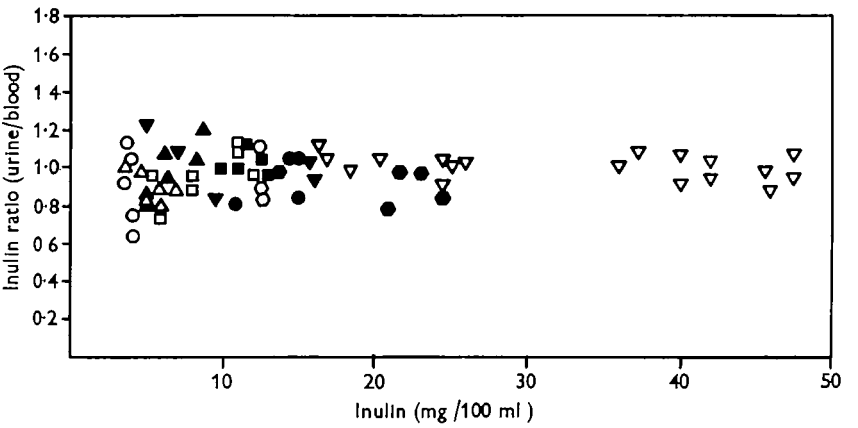


Fig. 5. The relationship of the urine/blood inulin ratios to the concentration of inulin in the blood. Expt. 1: ■, right and left renal sac urine; Expt. 2: ○, right and left renal sac urine; Expt. 3: ●, right and left renal sac urine; Expt. 4: △, right renal sac urine; Expt. 5: ▲, right and left renal sac urine; Expt. 6: □, right and left renal sac urine; Expt. 7: ⬡, right and left renal sac urine; Expt. 8: ⬢, right and left renal sac urine; Expt. 9: ▽, right and left renal sac urine; Expt. 10: ▼, right renal sac urine.

In the lower part of Fig. 6 is a bar chart showing the volumes of fluid collected. The volume of pericardial fluid collected from the left side varied during the sampling period but was of the same order of magnitude as that of the urine usually produced on that side in other animals (cf. Table 1). The volume of urine produced on the right side as well as the volume of sea water recovered from the left renal sac varied also. The sea water injected into the left renal sac was difficult to recover quantitatively. Except for samples 1 and 2 where 5 ml. had been injected, 10 ml. was injected at each sampling period. However, the amount which could be recovered after each 30 min. period ranged from 2 to 23 ml. The largest amount of fluid was collected at 225 min. At this period approximately 10 ml. in excess of the total amount injected up to that time was collected from the left renal sac. This additional volume may have been present in the sac from the beginning of the experiment and expelled only after an extensive contraction of the mantle. During the 500 min. of the experiment a total of 150 ml. was injected into, and 152 ml. collected from, the left renal sac. In subsequent experiments with similar catheterization the total volume of fluid collected from the 'isolated' renal sac varied only by a few millilitres from that injected.

From these results it is concluded that the pericardial fluid is a filtrate of the blood and flows by way of the reno-pericardial canal into the renal sac. Stoppage of this

flow results in the cessation of urine flow. The absence of inulin in the sea water injected into the 'isolated' renal sac reaffirms the conclusion that inulin is excreted by filtration and only in the branchial heart appendage.

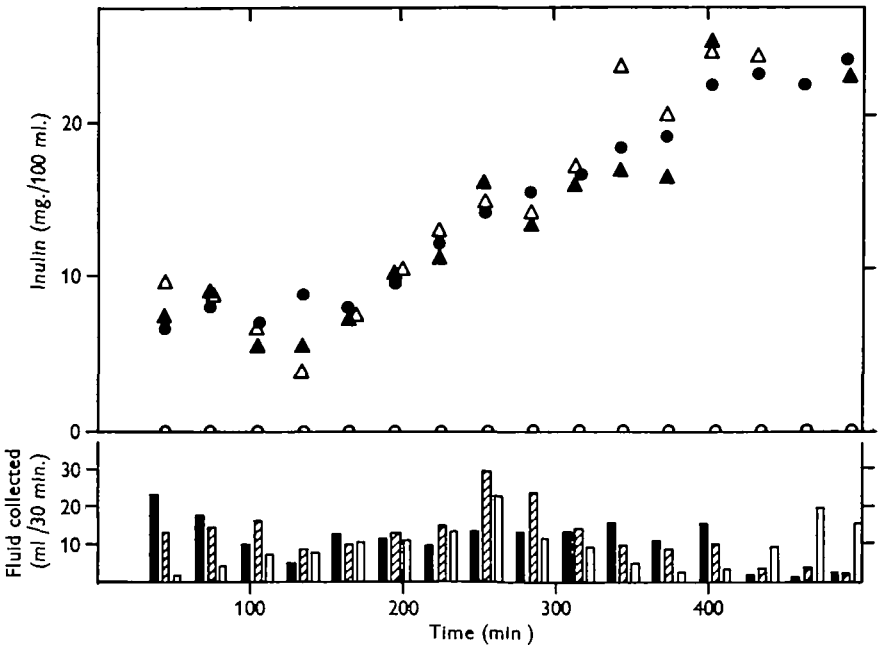


Fig. 6. Upper graph: the relationships of the concentrations of inulin in the blood, pericardial fluid and right and left renal sac urine during a typical experiment. Expt. 10: ▲, blood; △, left pericardial fluid; ●, right renal sac urine; ○, left renal sac urine (sea water). Lower graph: the volume of fluid collected per 30 min. for each sampling period during the experiment. Expt. 10: ■, left pericardial fluid; ▨, right renal sac urine; □, left renal sac urine (sea water).

### (2) *Reabsorption*

Filtration as the initial process in urine formation is likely to result in the loss of useful constituents of the blood. Glucose is an example of an important metabolic constituent of most animals which has been demonstrated to be filtered and subsequently reabsorbed in a number of different species (Smith, 1951). For this reason, preliminary analyses were made to ascertain the amount of carbohydrate present in normal samples of octopus blood, pericardial fluid and urine. The anthrone reagent (Morris, 1948) used in these determinations is not specific for glucose. However, since there was little or no reaction to the reagent if the samples were boiled with concentrated NaOH which degrades simple sugars (Little, 1949), the carbohydrate present was assumed to be glucose primarily and later analyses indicated this to be the case.

The glucose concentrations in operated but otherwise untreated animals are presented in Table 2. Although there was a wide range of concentration in the blood, pericardial fluid and urine, the relationships among the fluids within an animal were always the same: blood > pericardial fluid > urine. In those animals where urine and pericardial fluid were collected it should be noted that the samples were from

opposite sides. When either urines or pericardial fluids were collected from both sides simultaneously, the glucose concentration from the two sides was not always the same. Consequently, the concentration difference between the pericardial fluid and the urine from opposite sides may be the result of circulatory differences between the operated and unoperated side or of catheterization of the reno-pericardial canal before its termination.

When glucose was infused into the circulatory system the sugar concentration in the body fluids increased. Fig. 7 displays the results of an experiment involving

Table 2. *Glucose concentrations in the normal body fluids*

| Blood (mg /100 ml.) | Pericardial fluid (ml./100 ml.) | Right renal sac urine (mg./100 ml.) | Left renal sac urine (mg./100 ml.) |
|---|---|---|---|
| 74 | — | — | 3 |
| 61 | — | 5 | 4 |
| 28 | — | 1 | 2 |
| 25 | — | 3 | 8 |
| 25 | — | < 1 | < 1 |
| 22 | — | 7 | 6 |
| 18 | — | < 1 | < 1 |
| 15 | — | < 1 | 4 |
| 15 | — | 4 | 3 |
| 92 | 66 | 1 | 'Isolated' |
| 69 | 48 | 31 | 'Isolated' |
| 62 | 35 | 'Isolated' | 15 |
| 52 | 16 | 3 | 'Isolated' |
| 25 | 13 | 'Isolated' | 2 |

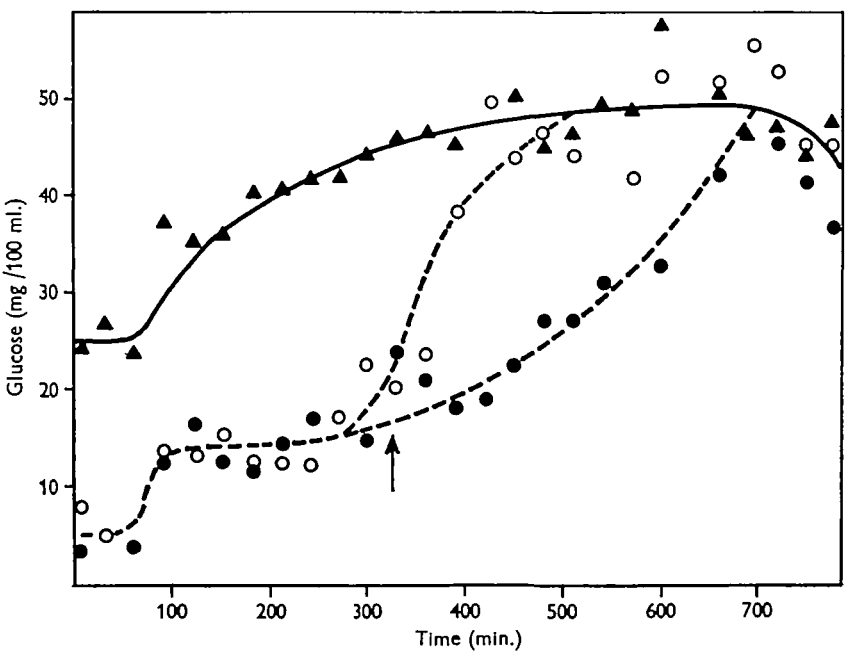


Fig. 7. The relationships, during a glucose perfusion experiment, of the concentration of glucose in the blood and in right and left renal sac urines before and after the administration of phlorizin. Phlorizin given between 330 and 630 min. Total dosage 13·5 mg./kg. Expt. 8: ▲, blood; ●, right renal sac urine; ○, left renal sac urine.

changes in the sugar concentration of the blood and of the urine from the right and left kidney and shows the effect of phlorizin. After the administration of this metabolic poison the concentration in the urine increased to approximately that in the blood. The same effect was observed with varying amounts of exogenous glucose infusion. Additional experiments without glucose infusion showed that phlorizin administration also resulted in an increase in the concentration of endogenous glucose in the urine to the level of that in the blood. In general the over-all effect of the poison was the same. However, the reaction was usually more rapid in one kidney than the other. Furthermore, greater variability in the results was noted after the administration of the poison. Since it was possible by prolonged administration of phlorizin at high dosage to cause the death of the animal, this variability may have been due to the adverse effect of the poison on the general metabolism of the animal.

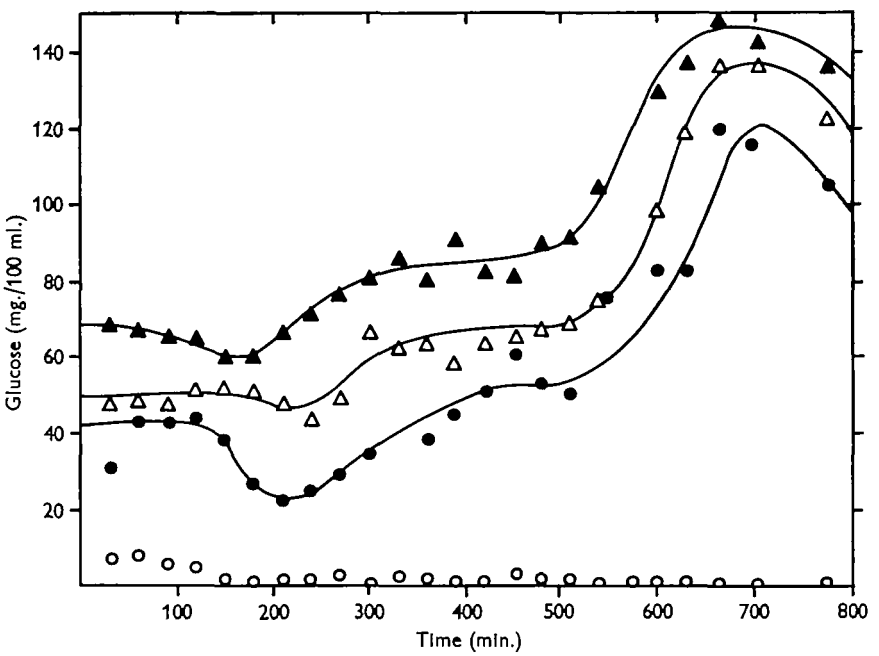


Fig. 8. The relationships, during a glucose perfusion experiment, of the concentrations of glucose in the blood, pericardial fluid and urine. Expt. 15: ▲, blood; △, left pericardial fluid; ●, right renal sac urine; ○, left renal sac urine (sea water). Glucose perfusion rate of 19 mg./hr./kg. between 180 and 510 min. Glucose perfusion rate of 85 mg./hr./kg. between 510 and 705 min. All perfusion terminated at 705 min.

The relationships between the blood, pericardial fluid and urine before and during the infusion of glucose are shown in Fig. 8. The pericardial fluid collected from the ligated left reno-pericardial canal was intermediate in concentration between the blood and the urine from the right renal sac. The sea water injected into the left renal sac was essentially devoid of glucose. The rise of glucose in the blood with concomitant rise in pericardial fluid and urine results in a gradual increase in the pericardial fluid/blood and urine/blood ratios. The values of the ratios are approaching 1 asymptotically which implies a saturation of the reabsorption mechanism.

The glucose concentration in the pericardial fluid is indicative of reabsorption from the filtrate before it reaches the renal sac. The concentration in the pericardial fluid depended in part on the time during which the filtrate was in contact with the tissues. In the experiment represented in Fig. 9 most pericardial fluid samples were taken by continuous drainage of fluid from the duct as it was produced. An average of 0·45 mg. glucose was reabsorbed per 30 min. during this time. If the flow from the reno-pericardial canal was stopped and the fluid accumulated in the canal and cavity during the sample period, a lower concentration of glucose was present than if the fluid drained continuously. In the experiment under consideration the pericardial fluid samples at 110, 170 and 230 min. were not drained continuously. Because of the lag period much change in concentration was not evident for 30 min. After that time there was a decrease in concentration of glucose in the pericardial fluid due to an increased rate of sugar reabsorption which reached about 0·75 mg./30 min.

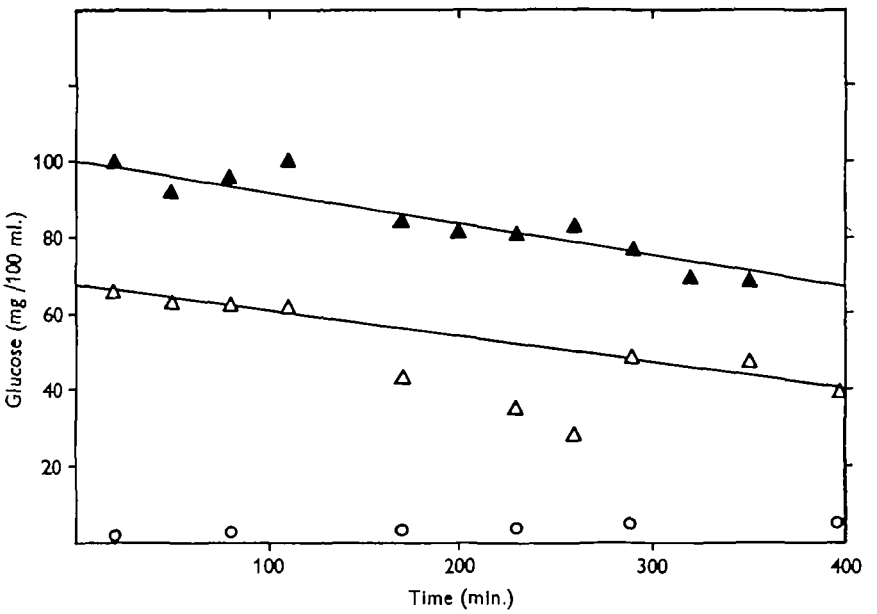


Fig. 9. The relationships of the concentrations of glucose in the norma blood, pericardial fluid and urine with the pericardial fluid either drained continuously or accumulated in the pericardial cavity and reno-pericardial canal. Samples at 110, 170 and 230 min. were not drained continuously. Expt. 16: ▲, blood; △, left pericardial fluid; ○, right renal sac urine.

The effect of phlorizin on the glucose concentration in the pericardial fluid is shown in Fig. 10. In this experiment the pericardial fluid was accumulated in the canals and cavities until the last five minutes of the sampling period when it was drained. After phlorizin was administered the concentration of glucose in both pericardial fluids increased to the level of that in the blood.

These results indicate that reabsorption of glucose takes place from the blood filtrate. The inhibition of this reabsorption by phlorizin results in an increase of glucose concentration in the pericardial fluid and the urine to the level of that in the blood.

### (3) *Secretion*

Secretion as an excretory process in the octopus has been emphasized from early histological and cytological studies of the kidney. These investigations have been reviewed by Turchini (1923). The histological evidence for secretion has been confirmed by the work of experimentalists who found that the urine was more concentrated in certain substances than the blood (cf. introduction). In order to extend further the knowledge of secretion by the renal organs, the excretion of phenolsulphonphthalein (PSP), *p*-amino hippuric acid (PAH) and urea was studied. As the pattern of excretion of PSP and PAH is very similar they will be considered together.

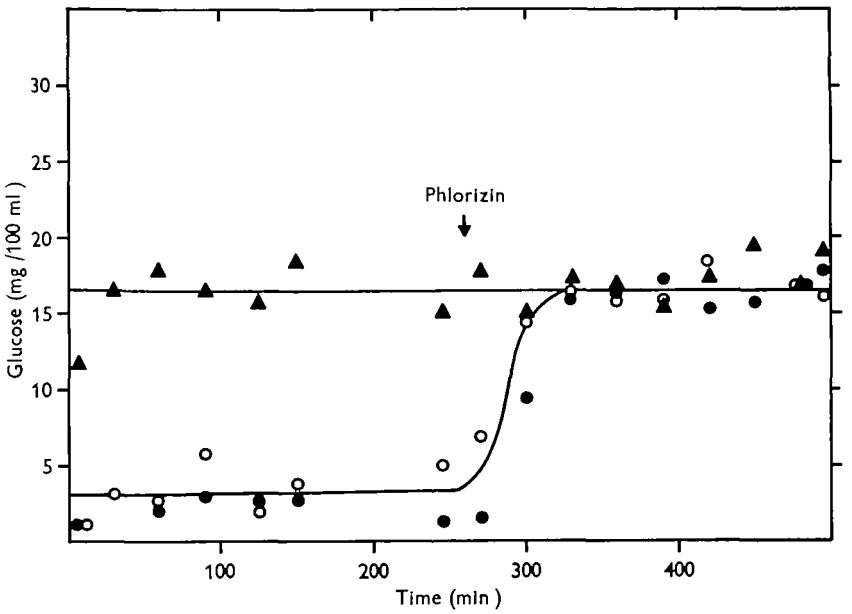


Fig. 10. The relationships of the normal concentration of glucose in the blood and pericardial fluid before and after the administration of phlorizin. Expt. 17: ▲, blood; ●, right pericardial fluid; ○, left pericardial fluid. Phlorizin given from 245 to 450 min. at a dosage of 10·3 mg./kg.

When PSP and PAH were infused into the blood stream the concentration in the urine rapidly exceeded that in the blood and continued to rise until a steady state was established. As was typical with most animals, the urine obtained from the left and right renal sacs was not of the same concentration. This may have been due to the partial interference with the circulatory pathway due to the operation or may have been the result of circulatory or histological differences. Additional experiments with PSP and PAH showed that qualitatively the animals responded in the same way to these substances but differed in the urine/blood (U/B) ratios at equilibrium.

The ability to concentrate PSP and PAH was decreased after the administration of 2,4-dinitrophenol (DNP). This result is evident in Expts. 2 and 4 where inulin, PSP and PAH were infused simultaneously. In Fig. 11 are presented the results with PSP. The dose used in Expt. 2 was less than that in Expt. 4. It may be noted that the smaller dose was insufficient to inhibit completely the secretion of PSP by the

kidney and, indeed, some recovery from the effect of the single dose can be seen. In Expt. 4 the concentration of PSP in the urine was reduced to approximately that in the blood. In Fig. 12 are the results with PAH. With this substance the higher dosage of DNP in Expt. 4 did not result in a U/B ratio of 1 as it did for PSP. Throughout the periods of action of DNP the urine continued to be formed at the rate characteristic of that animal during the first part of the experiment. The simultaneous U/B ratios for inulin in these experiments can be seen in Fig. 3.

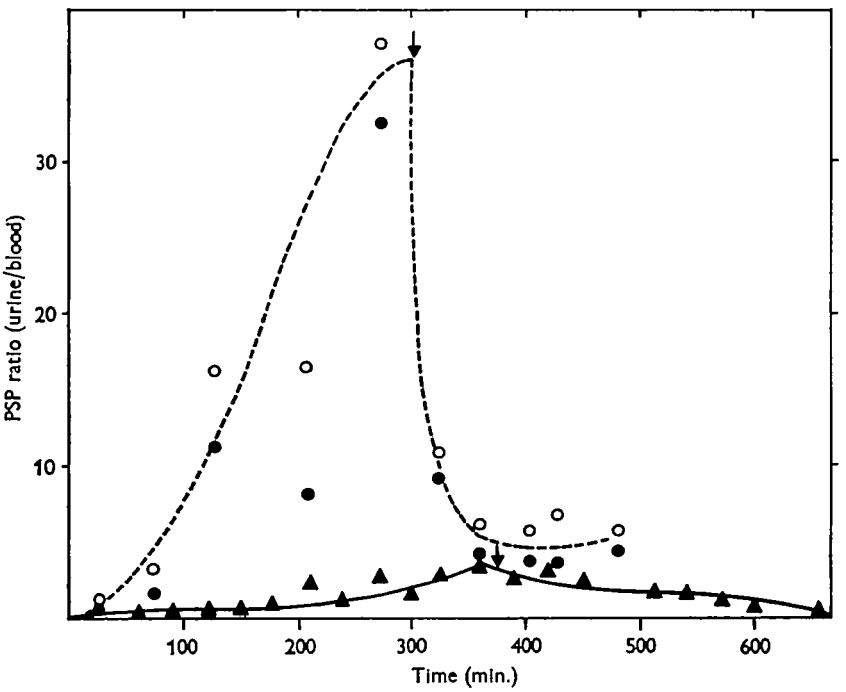


Fig. 11. The urine/blood ratios of PSP concentrations for each sampling period during perfusion experiments before and after DNP administration. Expt. 2: ●, right renal sac urine; ○, left renal sac urine; DNP given at 300 min. Expt. 4: ▲, right renal sac urine; DNP given from 360 to 510 min.

The effect of benemid on secretion was more difficult to demonstrate. This substance had to be injected in low concentration by reason of the reaction of the animal to the drug. Frequently the respiratory movements stopped and it became difficult to obtain blood samples. In Fig. 13 are shown the results of an experiment with PSP where 11·5 mg./kg. of benemid was given. In the upper part of the figure it can be seen that the secretion of PSP continued and in fact the concentration in the urine actually increased. In this experiment PAH was infused simultaneously and the same effect was observed with PAH. However, as seen in the lower part of the figure, there was a decrease in the rate of urine produced. Consequently despite the continued output of PSP there was a drop in the total excretion of PSP per unit time. In Figs. 14 and 15 the rates of excretion for PSP and PAH respectively are plotted against time for three experiments. This treatment of the data reveals the reduction in excretion rate as well as the duration of the action of the benemid.

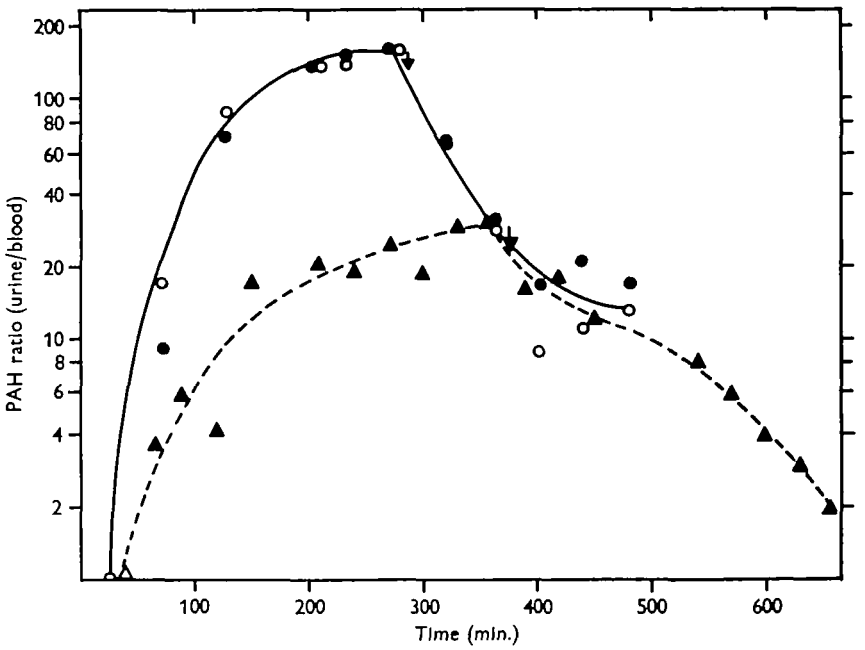


Fig. 12. The urine/blood ratios of PAH concentrations for each sampling period during perfusion experiments before and after DNP administration. Expt. 2: ●, right renal sac urine; ○, left renal sac urine; DNP given at 300 min. Expt. 4: ▲, right renal sac urine; DNP given from 360 to 510 min.

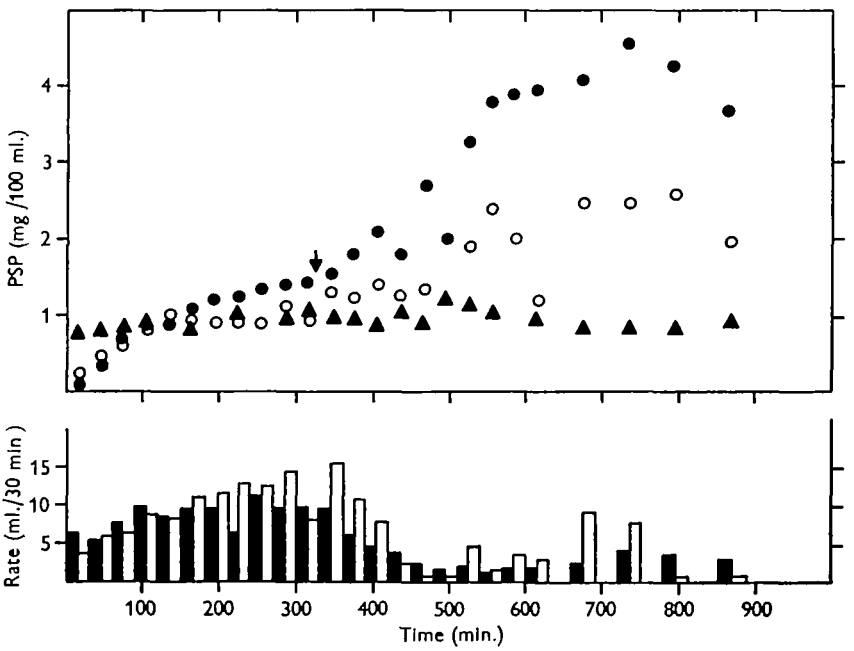


Fig. 13. Upper graph: the relationships of the concentrations of PSP in the blood and urine before and after benemid administration. Expt. 1: ▲, blood; ●, right renal sac urine; ○, left renal sac urine. Benemid given from 315 to 525 min. Lower graph: the volume of urine produced per 30 min. for each sampling period before and after benemid administration. Expt. 1: ■, right renal sac urine; □, left renal sac urine.

The clearance of PSP and PAH is dependent on their concentrations in the blood. In the vertebrate it is customary to demonstrate this phenomenon by plotting the PSP/inulin or PAH/inulin clearance ratio against blood concentration. However, it was shown earlier that the inulin U/B ratio in the octopus is 1. Consequently the clearance ratio parameter is essentially the U/B ratio for PSP.

$$\frac{U_{PSP}}{B_{PSP}}\text{Vol} \div \frac{U_{In}}{B_{In}}\text{Vol} = \frac{U_{PSP}}{B_{PSP}}$$

As shown in Fig. 16, the U/B ratio for PSP decreases with increasing blood concentration. Fig. 17 shows the same phenomenon for PAH. This effect probably represents a saturation of the transport mechanism at the higher concentration.

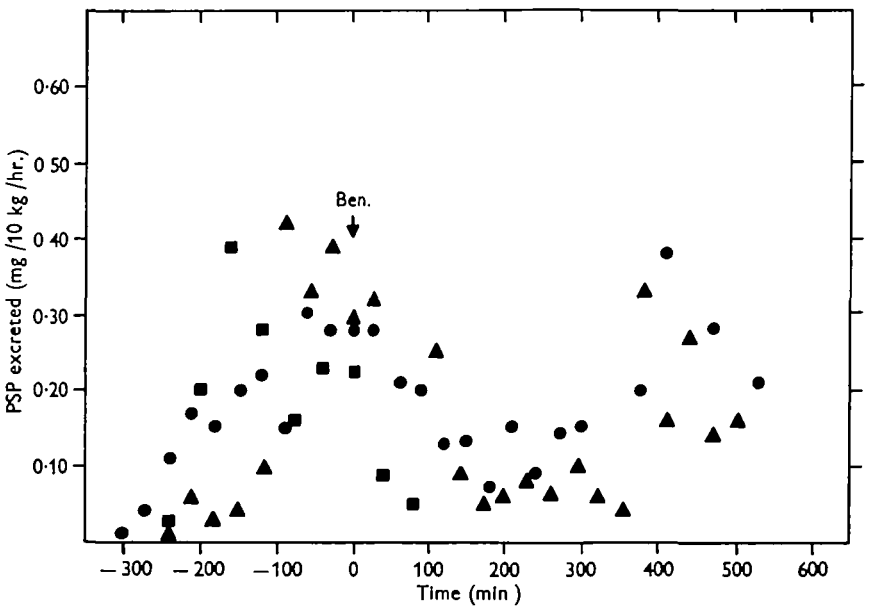


Fig. 14. The rate of PSP excretion in mg./hr./10 kg. before and after the administration of benemid. Expt. 1: ●, right renal sac urine; Expt. 3: ■, right renal sac urine; Expt. 7: ▲, left renal sac urine.

The results of an experiment involving the simultaneous collection of blood, urine and pericardial fluid for PSP are given in Fig. 18. In this animal the left reno-pericardial canal was catheterized and the left renal sac was isolated from the pericardium and then injected with sea water. It is interesting to note that PSP was definitely concentrated in the fluid from both the right and the left renal sacs. This is in contrast to the results with inulin and glucose where these materials were present only in the sac which was in communication with the pericardium. The same effect was obtained with PAH and is shown in Fig. 19 for a different animal submitted to the same operation. The renal appendages, then, secrete these substances into the fluid present in the sac even though it is not the normal blood filtrate.

The concentration of PSP in the pericardial fluid was in general lower than in the blood. As seen in Fig. 18, except when PSP blood perfusion was terminated, it was

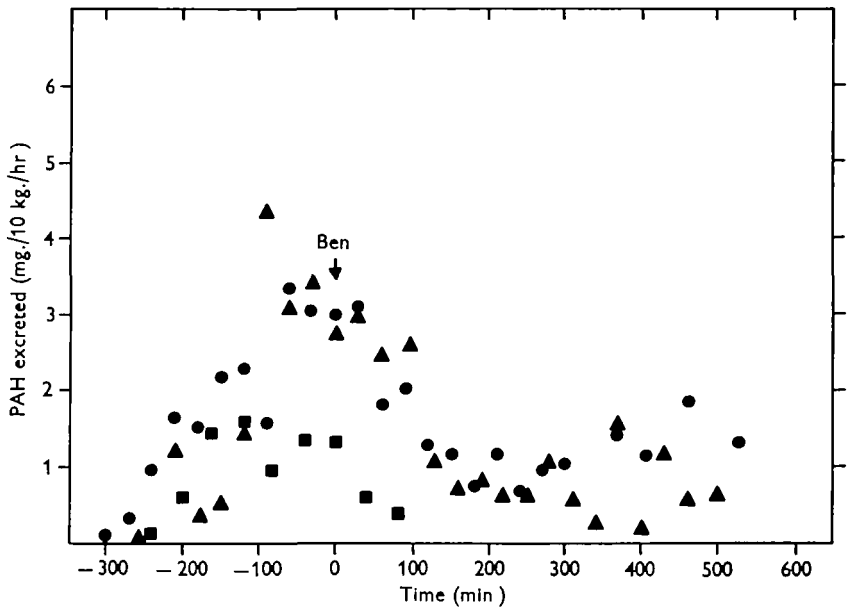


Fig. 15. The rate of PAH excretion in mg./hr./10 kg. before and after the administration of benemid. Expt. 1: ●, right renal sac urine; Expt. 3: ■, right renal sac urine; Expt. 7: ▲, left renal sac urine.

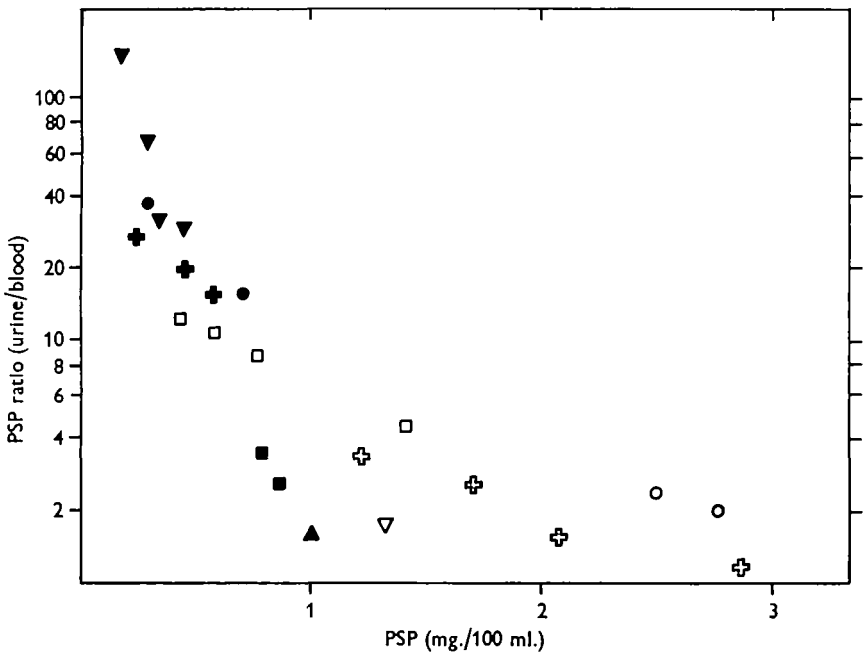


Fig. 16. The urine/blood ratios of PSP concentration plotted against the PSP concentration of the blood. Expt. 1: ▲, right renal sac urine; Expt. 2: ●, left renal sac urine; Expt. 3: ○, right renal sac urine; Expt. 4: ✣, right renal sac urine; Expt. 6: ■, left renal sac urine; Expt. 7: ▽, left renal sac urine; Expt. 18: +, left renal sac urine; Expt. 20: □, right renal sac urine; Expt. 21: ▼, right renal sac urine.

on the average about one half that in the blood. As the concentration of inulin in the pericardial fluid was approximately the same as in the blood, this lower concentration may represent binding of the PSP to the blood protein. With PAH the concentration in the pericardial fluid was also lower than in the blood. In Fig. 20 the pericardial fluid/blood ratios corrected for the simultaneous inulin ratios are plotted against time for five experiments.

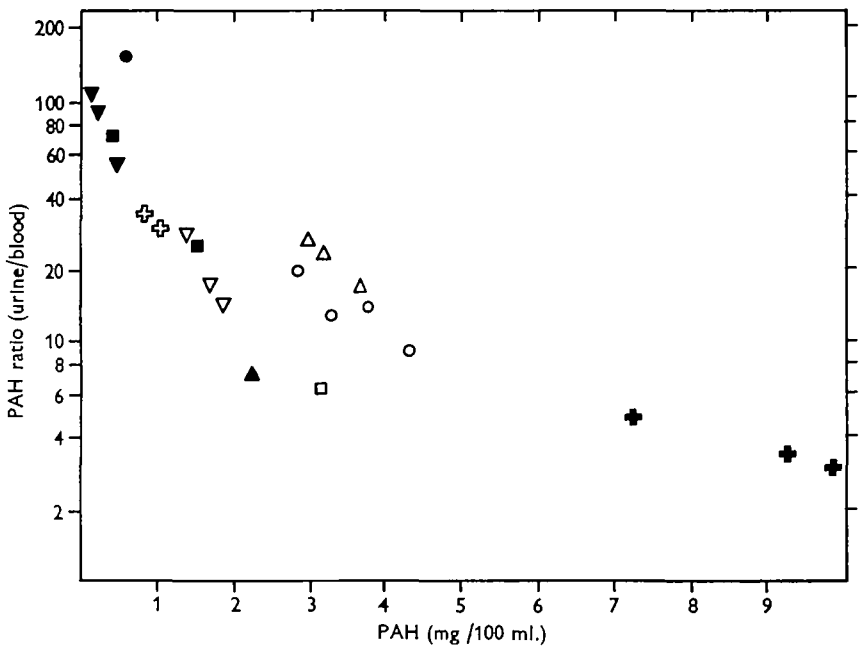


Fig. 17. The urine/blood ratios of PAH concentration plotted against the PAH concentration of the blood. Expt. 1: ▲, right renal sac urine; Expt. 2: ○, left renal sac urine; Expt. 3: +, right renal sac urine; Expt. 4: ✣, right renal sac urine; Expt. 6: ■, left renal sac urine; Expt. 7: □, left renal sac urine; Expt. 11: △, right renal sac urine; Expt. 19: ●, left renal sac urine; Expt. 22: ▽, right renal sac urine; Expt. 23: ▼, right renal sac urine.

The average maximal rates of excretion of PSP and PAH for the experiments in which these substances were infused independently and simultaneously are given in Table 3. For PSP the maximum excreted in a renal sac was approximately 1 mg./hr./10 kg. at blood concentrations of 0·4, 0·7 and 0·9 mg./100 ml. and for PAH it was 10 mg. at blood concentrations of 0·3, 0·6, 3·2 and 3·6 mg./100 ml. These rates may not represent absolute maxima but can be used in an estimation of renal blood flow. The minimum amount of blood necessary to supply these quantities of PSP and PAH to a renal sac at the lowest blood concentration are 250 and 3300 ml./hr./10 kg., respectively. In contrast to this the average filtration rate was low, 13 ml./hr./10 kg. per renal sac. At low blood concentrations these substances are excreted almost entirely by secretion.

Generally lower rates of excretion resulted when PSP and PAH were infused simultaneously. This may be indicative of competition of the two substances for the same transporting system. However, before this is established conclusively more data are necessary.

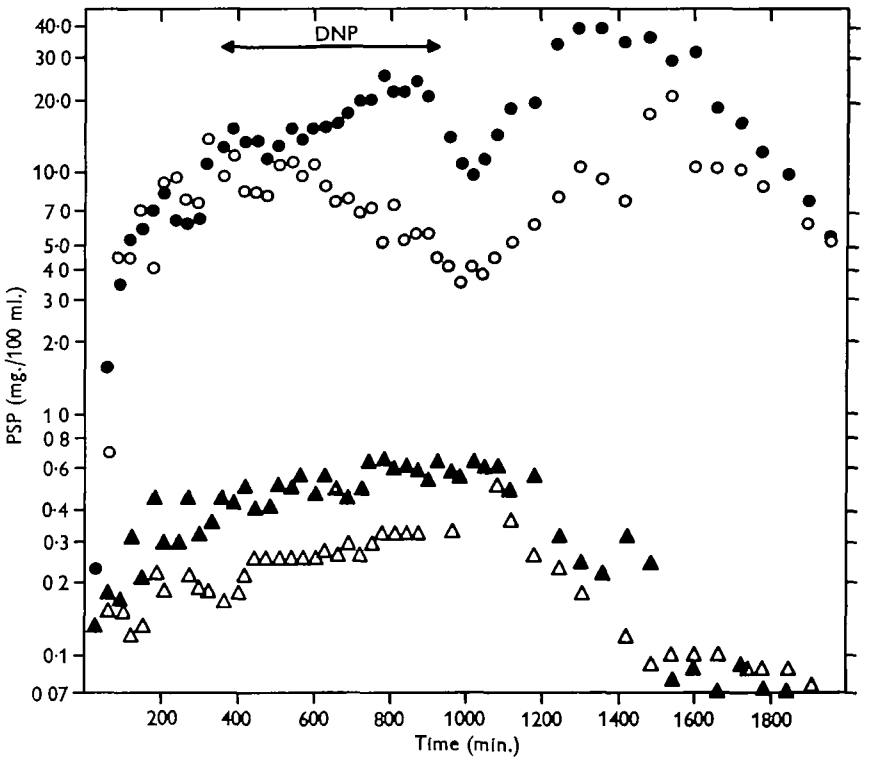


Fig. 18. The relationships of the concentrations of PSP in the blood, pericardial fluid and urines during a typical experiment. Expt. 21: ▲, blood; △, pericardial fluid; ●, right renal sac urine; ○, left renal sac urine (sea water). DNP given from 360 to 930 min.

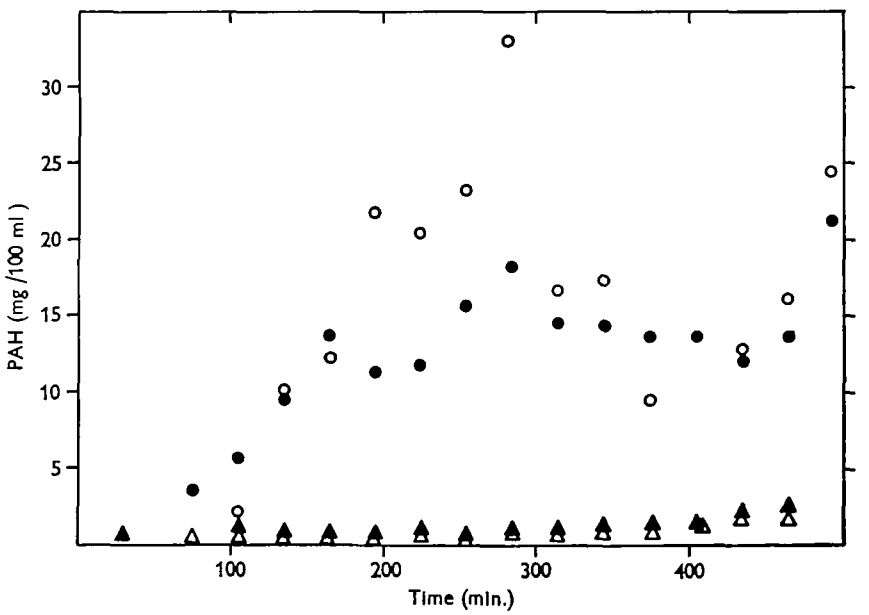


Fig. 19. The relationships of the concentrations of PAH in the blood, pericardial fluid and urines during a typical experiment. Expt. 10: ▲, blood; △, pericardial fluid; ●, right renal sac urine; ○, left renal sac urine (sea water).

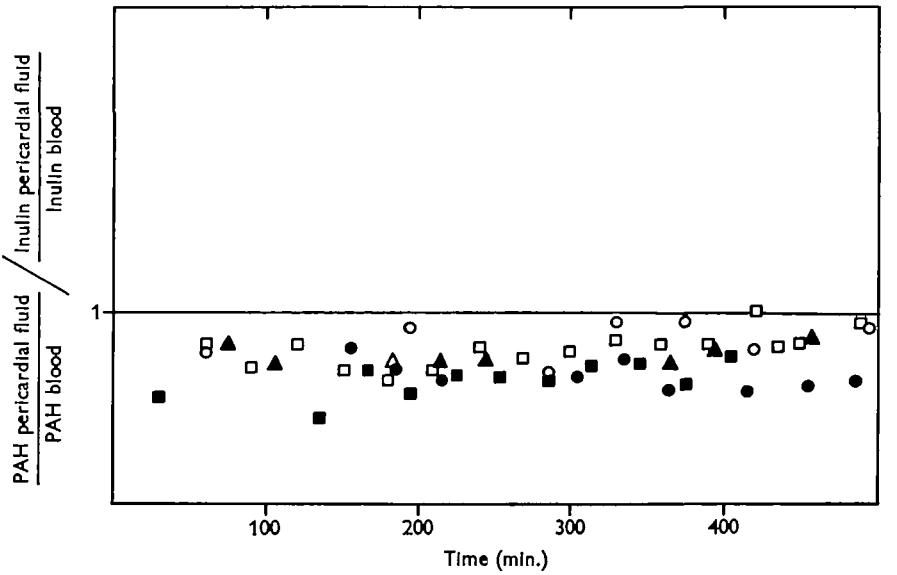


Fig. 20. The pericardial fluid/blood ratios of PAH concentration corrected for the simultaneous inulin ratios for each sampling period during the experiments. Expt. 10: ■, left pericardial fluid; Expt. 12: ○, right pericardial fluid; Expt. 13: ●, right pericardial fluid; Expt. 14: ▲, right pericardial fluid; Expt. 22: □, right pericardial fluid.

Table 3. *Average maximal rates of excretion of* p-*amino hippuric acid and phenolsulphonphthalein*

| | PAH | | PSP | |
|---|---|---|---|---|
| Expt. number | Right renal sac (mg./hr./10 kg.) | Left renal sac (mg./hr./10 kg.) | Right renal sac (mg./hr./10 kg.) | Left renal sac (mg./hr./10 kg ) |
| 1 | 3·1 | 2·2 | 0·28 | 0·28 |
| 2 | 5·8 | 6·9 | 0·92 | 0·62 |
| 3 | 1·3 | 1·5 | 0·26 | 0·23 |
| 4 | 2·3 | — | 0·34 | — |
| 6 | 2·7 | 4·0 | 0·16 | 0·22 |
| 7 | 3·2 | 1·2 | 0·11 | 0·37 |
| 10 | 4·0 | 3·5 | — | — |
| 11 | 11·6 | 3·2 | — | — |
| 13 | 3·7 | — | — | — |
| 18 | — | — | 0·76 | 0·52 |
| 19 | 10·8 | 2·7 | — | — |
| 20 | — | — | 1·04 | 0·73 |
| 21 | — | — | 0·81 | 1·08 |
| 23 | 8·0 | 11·2 | — | — |

In order to determine whether the renal organs of the octopus were able to concentrate a naturally occurring nitrogenous compound, some preliminary experiments were made in which urea was infused into the circulatory system. The data obtained from these experiments is summarized in Fig. 21 where the U/B ratios for urea are plotted against time. It may be seen that some degree of concentration was obtained though of a different order of magnitude to that for PSP or PAH.

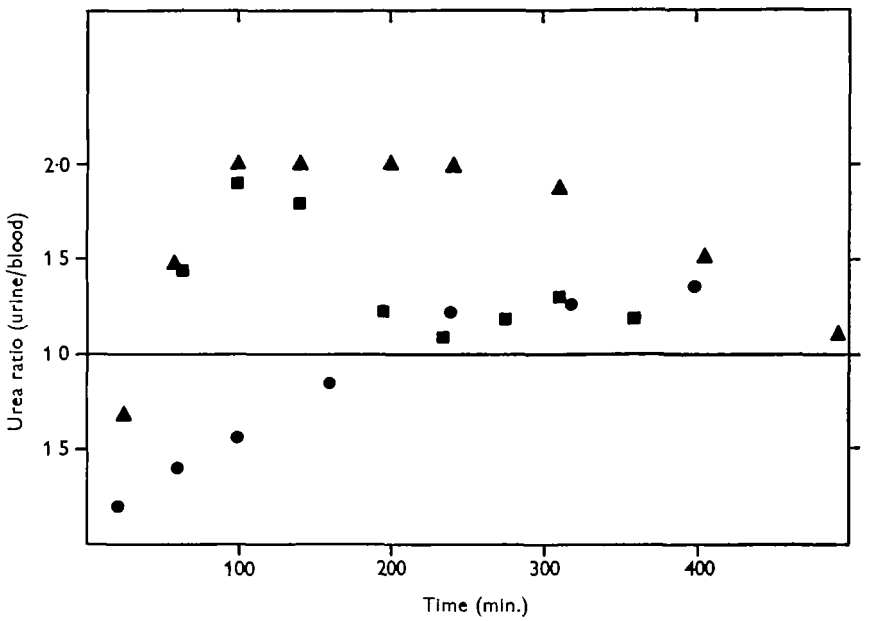


Fig. 21. The urine/blood ratios of urea concentration for each sampling period during the experiments. Expt. 19: ■, right renal sac urine; Expt. 24: ●, right renal sac urine; Expt. 25: ▲, left renal sac urine.

## DISCUSSION

A number of different mechanisms have been proposed by earlier investigators for the formation of urine in the octopus. Turchini (1923) has reviewed substantial evidence for secretion. Mayer & Rathery (1907) presented evidence for a process of reabsorption. These latter authors concluded from their results that filtration did not take place. Re-examination of their data prompts the questioning of this interpretation. It is not shown clearly in any instance that filtration could not have taken place in their experiments, though admittedly only accompanying an active secretory process. Bruni (1937) concluded that a series of dyes was eliminated by a process of filtration. From the results reported here it is concluded that all three processes are involved in urine formation, the first step being a filtration of the blood with a subsequent reabsorption from the filtrate and a secretion into it.

The evidence for filtration is provided by the results with inulin. Upon infusion into the circulatory system, inulin appeared, after a lag period, in approximately the same concentration in the urine as in blood. The fact of its presence in the urine is presumptive evidence for a filtration mechanism since in the investigation of vertebrate kidneys the great preponderance of data supports the excretion of this substance by filtration alone. In particular, the kidney of the aglomerular fish, which forms urine by secretory processes only, does not excrete inulin. In 1941 Maluf reported that inulin was secreted by the green gland of the crayfish. So unusual a finding led Forster & Zia-Wohlrath (1941) to conduct experiments on the lobster, *Homarus americanus*. In contradiction of the results of Maluf, the results of these investigators indicated that in this crustacean inulin was excreted by filtration. Burger (1957) extended the work of Forster & Zia-Wohlrath (1941) and reported

inulin U/B ratios of 1·0 to 1·1. Riegel & Kirschner (1960) repeated the work of Maluf and although the inulin U/B ratios were always greater than 1 they concluded this to be the result of filtration followed by reabsorption and not of secretion.

Aside from the mere presence of inulin in the urine the fact that the concentration in the blood and urine reaches the same level is very satisfactory evidence of filtration. There is very little likelihood that the excretory organs would secrete inulin in such a constant relationship to its blood concentration under the various experimental conditions used.

The study of the clearance of inulin provides further evidence for filtration. Unlike materials which are secreted, the U/B ratio is independent of the blood concentration and implies that the material is eliminated by a passive metabolic process.

The most convincing evidence for filtration is given with the results of experiments involving collection of pericardial fluid. The pericardial fluid, the blood and the urine from the renal sac in communication with the pericardium contained inulin at the same concentration. The absence of inulin or filtrate in the renal sac 'isolated' from the pericardium indicates that the pericardium is the source of the filtrate. Here the anatomical separation of the filtration and secretion processes shows unequivocally that inulin is excreted by filtration.

It is of interest at this point to consider next something of the dynamics of the process of filtration. From the results presented it is concluded that the filtrate is formed within the pericardial cavity. This as a possible site of filtrate formation has been proposed previously (Grobben, 1891; Strohl, 1913–14; Hoffman, 1927; Picken, 1937; Harrison, 1961). For filtration to occur a surface across which filtration may take place is necessary as well as a source of pressure. The surface in the octopus may be in the wall of the branchial heart appendage. According to Marceau (1905) the terminations of the branched blood cavity of the appendages are covered by a single layer of epithelium which is closely associated with the layer of epithelium invaginated from the external surface of the branchial heart appendage. It is perhaps at this site of interdigitation that filtration occurs. The pressure necessary for filtration may be supplied by the contractions of the branchial heart. Pressure measurements in the afferent branchial vessel and branchial heart ranged from 25 to 50 cm. of water systolic and about 15 diastolic (Johansen & Martin, 1962). Although no direct measurements were made in the branchial heart appendage, it is likely that sufficient pressure for filtration would exist here.

The rate at which urine could be collected varied from the right and left renal sac and during experimentation. The average volume of fluid collected from a renal sac was 13 ml./hr./10 kg., the range being from 1 to 45 ml. In a series of animals with only the renal sacs catheterized daily collections were made. Here, too, there were differences in rates from side to side and with time. Although no other physiological evidence has been found to support it, there may exist a method of regulating the rate of filtration by changing the supply of blood to the appendages. Marceau (1905) describes in the branchial heart appendages striated muscle fibres which surround certain blood vessels. He proposes that they may modify the circulation in the appendage.

The process of reabsorption from the filtrate was established in the experiments performed on the excretion of glucose. The concentration of glucose in normal samples

of blood was greater than that in the pericardial fluid which in turn was greater than that in the urine. Infusion of glucose resulted in an increase in the glucose concentration in all the fluids. When large amounts of exogenous glucose were infused, the body fluid to blood ratios approached one asymptotically, a result which is characteristic of the saturation of a reabsorption mechanism.

When the metabolic poison, phlorizin, was administered the glucose concentration in the urine and in the pericardial fluid increased to that in the blood. Similar blockage of glucose reabsorption has been reported for vertebrate kidneys (Smith, 1951) and among the invertebrates for the lobster (Burger, 1957) and crayfish (Riegel & Kirschner, 1960). According to Lotspeich (1960) experimental results support the theory that phlorizin blocks sugar transport by binding carrier sites in the cell membrane.

The complete blockage of glucose reabsorption by phlorizin before the filtrate reaches the renal sac implies that reabsorption may take place entirely in the pericardial cavity and reno-pericardial canal. In some preliminary experiments to determine whether glucose reabsorption took place in the renal sacs also, glucose was injected into the 'isolated' renal sacs and phlorizin was administered. Conclusive evidence of glucose reabsorption was not obtained. Mayer & Rathery (1907) reported glucose reabsorption from the renal sacs but their results are questionable because of their technique. Until experiments are carried out where an indifferent substance such as inulin is injected simultaneously with the glucose, no definite decision can be made about reabsorption from the renal sacs. Be that as it may, since reabsorption does take place in the pericardial system, the separation of the reabsorption and secretion processes suggest interesting histological and cytochemical investigations.

The process of secretion into the blood filtrate was demonstrated by the results with PSP, PAH and urea. A high degree of concentration may be achieved by the secretory cells of the renal organs of the octopus. Both in the vertebrate and in the octopus PAH is transported very actively. The highest degree in the octopus was a urine concentration of 155 times that of the blood, while in man a concentration in the urine of about 260 times that of the blood may be attained (Smith, 1951). In the lobster (Burger, 1957) and the abalone (Harrison, 1961) the highest U/B ratio reported was approximately 5. It will be noted that the renal organs of the octopus compare well with the vertebrate kidney.

More insight into renal function may be gained by a comparison of clearance ratios. The highest clearance ratio of PAH to inulin in man is 5·0–5·5. In the octopus the comparable value is about 150. In man when the correction for filtration and reabsorption provided by an inulin clearance is made, the clearance ratio is relatively low. In the octopus the value is relatively high since secretion appears to be a dominant factor and filtration a subordinate one.

It is noteworthy that secretion of PAH and PSP in both the vertebrates and the octopus is inhibited by the same agent, 2,4-dinitrophenol. The general action attributed to DNP is the uncoupling of the energy source from the energy-requiring system. According to a recent theory DNP uncouples oxidative phosphylation by interfering with the formation of high-energy intermediates (Eisenhardt & Rosenthal, 1964). Secretion in both cases does not occur in the absence of the energy source and suggests that the transporting mechanisms may be similar.

Another agent, benemid, produced an effect on excretion somewhat different from the one observed in the vertebrate. In the dog benemid depresses to a great extent the tubular secretion of PAH at the same time leaving filtration and tubular reabsorption unchanged (Beyer *et al.* 1951). In the octopus benemid depresses the total amount of PAH and PSP secreted per unit time, but the maximal concentration of benemid it was possible to obtain in the octopus did not prevent the concentration of these substances. The other physiological effects of the drug, perhaps on the circulatory system, were so severe that urine flow almost ceased.

In the vertebrate, Smith (1951) proposes that PSP and PAH are secreted by the same transporting mechanism and is able to demonstrate competition between the two substances. The evidence for competition in the octopus is suggestive but not conclusive and needs substantiation.

From the average maximal excretion rates of PAH a minimal renal blood flow (for a single renal sac) of 3300 ml./hr./10 kg. was estimated. The blood volume in the octopus is reported to average 5·8% of the body weight (Martin, Harrison, Huston & Stewart, 1958). According to the estimate of blood flow, a 10 kg. octopus with a blood volume of 580 ml. would circulate a minimum of 55 ml./min. of blood through each renal sac. The actual rate must be greatly in excess of this, however, if one takes the following into consideration. The sampling procedure used was to take blood samples from the anterior vena cava which delivers blood to the renal sec. The pericardial fluid was taken from blood filtered after it had passed the renal sacs. Assuming that no binding of PAH occurs the approximate ratio of 0·7 for the PAH concentration, pericardial fluid to blood, may be taken as resulting from an extraction of 30% of the PAH. This being the case the 55 ml./min. would represent only 30% of the renal flow and the actual rate would be more like 180 ml./min./10 kg. The comparable value for man is approximately 100 ml./min./10 kg. It must be remembered that almost the total blood flow passes through the renal appendages in returning to the heart. Chapman & Martin (1957) have shown that the minute volume in an 18 kg. octopus may reach 320 ml./min., an amount which supports the conclusions reached about the blood flow through the kidney.

One of the forms of nitrogenous wastes excreted by the octopus is urea. Rather a small fraction of the total urinary nitrogen is excreted in this form so perhaps it is not surprising that urea was not concentrated to a very great extent.

From the results reported here and from information cited from other investigations, a theory of urine formation in the octopus is proposed. The basic step in urine formation is a filtration of the blood. This appears to take place across the wall of the branchial heart appendage into the pericardial cavity under pressure resulting from contractions of the branchial heart. The filtrate formed passes along the reno-pericardial canal through the reno-pericardial funnel into the renal sac. As the fluid is *en route* to the renal sac important metabolic constituents, specifically glucose, may be reabsorbed from the filtrate. In the renal sac both naturally occurring and foreign substances are secreted actively into the filtrate. The urine is expelled through the urinary papillae into the sea water by strong contractions of the mantle.

SUMMARY

1. Experiments have been performed to determine some of the processes involved in urine formation in the octopus. The concentration of specific substances was followed in serial samples of the blood, pericardial fluid and urine for extended periods of time before and after the administration of metabolic poisons.

2. The results with inulin indicate that it is filtered since its concentration is approximately the same in the blood, pericardial fluid and urine. It is proposed that filtration of the blood occurs within the pericardial cavity.

3. The results with glucose indicate that it is reabsorbed from the filtrate in the pericardial cavity and reno-pericardial canal. Phlorizin administration increased the glucose concentration in the pericardial fluid and urine to the level of that in the blood.

4. The filtrate flows by way of the reno-pericardial canal into the renal sac. The results with phenolsulphonphthalein (PSP), para-amino hippuric acid (PAH) and urea indicate that active secretion into the filtrate takes place in the renal sacs. PSP, PAH and urea were in higher concentration in the urine than in the blood and pericardial fluid. The secretion of PAH and PSP was inhibited by DNP and benemid.

5. The theory of urine formation proposed is the following. Filtration of the blood occurs across the wall of the branchial heart appendage into the pericardial cavity. The filtrate formed passes by way of the reno-pericardial canal into the renal sacs. Reabsorption occurs *en route* to the renal sacs. Active secretion into the filtrate occurs in the renal sacs from where the filtrate is expelled to the outside as urine.

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