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SOME EXCRETORY PROCESSES IN THE ABALONE, *HALIOTIS RUFESCENS**

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INTRODUCTION

Most of the investigations of the excretory system of the abalone reported in the literature during the past 100 years have been morphological in nature (Haller, 1886; von Jhering, 1877; Wegmann, 1884; von Erlanger, 1892; Pelseneer, 1896; Fleure, 1902, 1904; Totzauer, 1902, 1905; Spillman, 1905; Palmer, 1907; Crofts, 1929). The only physiological experiments conducted (Kowalevsky, 1889; Cuénot, 1899) employed the method of physiological injection. This technique involves the injection of certain dyes into the body spaces with subsequent observations of their distribution in the animals. The results of Cuénot and Kowalevsky confirmed the morphological studies as far as the identification of the organs involved in the excretion of foreign substances, but did not provide information about the dynamics of the formation of the excretory products. As experiments had not been performed on the abalone using the modern techniques developed in the study of the vertebrate kidney (cf. Smith, 1951), an investigation was made on the large abalone, *Haliotis rufescens*, in an attempt to establish some of the mechanisms involved in urine formation.

MATERIAL AND METHODS

The animals used in the experiments were obtained in the shore area below the limits of the low tide and were maintained at approximately 12–14 °C. in aquaria provided with fresh-running sea water. The animals were supplied with a diet of brown algae, usually *Macrocystis*, and survived well under these conditions.

A brief morphological account of the excretory system is appropriate since the descriptions in the literature differ in some of the details of the general organization. The renal organs of *Haliotis rufenscens* are closely associated with the reproductive system and the pericardial cavity. According to Goodrich (1945), this is characteristic of molluscs in general and is the result of the development of these structures as derivatives of the coelom. The two renal organs are of unequal size, of different colour and shape and of different organization. The right organ is very extensive, consisting of numerous lobes which project anteriorly and posteriorly. It is brownish in colour and internally exhibits a spongy appearance. The sex products from the single gonad empty into the right renal organ and are conducted to the outside through the right renal pore. The left organ, also called the papillary sac, is small, oval in form, whitish in

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colour and the internal walls are covered with masses of papillae. The renal organs open externally into the mantle cavity by symmetrical slit-like apertures on either side of the base of the rectum. Both kidneys communicate with the pericardium by means of reno-pericardial canals. In the pericardium are found two atria and a single ventricle which is traversed by the intestine.

The animals were prepared for experimentation in the following manner. They were removed from the aquaria and sufficient shell surrounding the respiratory openings was chipped away, without visible injury to the animal, to expose the slit in the mantle dorsal to the ctenidia. The opening in the shell was extended posteriorly to expose the dorsal surface of the pericardium. Then, the anterior aorta, the right efferent branchial vessel, and in some animals the pericardium, were catheterized with thin plastic tubes (O.D. 1·0 mm.). This was done by lifting with small forceps the tissue dorsal to the structure to be catheterized, cutting a small opening, inserting the tube, and then ligating it in place. The process of catheterization of the kidneys presented more of a problem as there was insufficient tissue on the dorsal aspect of the excretory pore to tie the catheters in place so that a leak-proof system could be obtained. This difficulty was overcome in part by the use of plastic tube (O.D. 2·0 mm.) the diameter of which had been increased externally a short distance from the tip by a bulb of bees-wax. The catheter was inserted with slight force into the kidney cavity and then a stitch was taken with a curved surgical needle down through that part of the mantle which lies immediately laterad of the right excretory pore, around both the catheters at their entrance into the kidney, making sure to include the tissue of the excretory pores, and then up through the mantle tissue laterad of the left excretory pore. The stitch was tied tightly enough to anchor the catheters by the bulb against the mantle, but loosely enough to prevent occlusion of the blood vessels in that vicinity. Extreme care had to be taken in handling the animals to avoid injuring a major circulatory vessel, for otherwise an uncontrollable haemmorrhage took place which rendered the animal useless for experimentation. After the operation the animals were weighed and returned to the aquaria until the following day.

At the onset of an experiment thin plastic tubes were connected to the catheters previously placed in the animal. This permitted the taking of samples without removal of the animals from the sea water. Then, normal samples of the body fluids to be examined were obtained for use in the preparation of blanks and standards.

An experiment was initiated by the slow introduction of test materials into the anterior aorta by means of a syringe or under a slight positive pressure of gravity. The infusion was continued until the blood concentration was brought to the desired level and then the rate was decreased for the remainder of the experiment to a level which was thought to be sufficient to maintain the blood concentration. Serial samples of blood and urine were taken for the duration of the experiment. The volume of blood removed for each sample was from 0·2 to 0·4 ml. The urine and pericardial fluid samples were obtained by collecting all the fluid which drained from the catheters during the interval between blood samples. Immediately after the samples were taken they were centrifuged to remove the sex products and other materials from the urine, and the cells from the blood.

During the course of the experimentation, the excretion of the following substances was tested: inulin, *p*-amino hippuric acid, phenolsulphonphthalein and glucose. Inulin

was determined by the anthrone method (Young & Raisz, 1952) and the resorcinol-thiourea method of Roe, Epstein & Goldstein (1949). For the analysis of this substance and all others which require a protein-free filtrate, the proteins were removed by the Somogyi method (1930).

The analysis of *p*-amino hippuric acid was carried out by the method of Goldring & Chasis (1944). The concentration of glucose was determined according to the method of Mokrasch (1954) and phenolsulphonphthalein by colorimetric comparison with standards of known concentration which had been prepared simultaneously. For all the analyses the Beckman DU Spectrophotometer was used to measure the light absorption of the sample at the wavelength specified by the method.

RESULTS

In an investigation of excretory methods it is useful to know whether filtration is an important process in urine formation. If it can be established that some substance is excreted by filtration alone, then if the relative concentration of another substance is sufficiently greater than the reference substance, it can be concluded that it is secreted actively into the urine. If the relative concentration of another substance is lower than the reference substance it would probably indicate that it is being reabsorbed. Inulin was used to test for the possibility of a filtration process for it has been found to satisfy quite well the criteria proposed for the excretion of a substance by filtration alone (Smith, 1951). Phenolsulphonphthalein and *p*-amino hippuric acid were used to test for a secretion process and glucose for a reabsorption process. The test materials were dissolved in sea water.

The normal blood samples obtained were cloudy in appearance and generally had sufficient hemocyanin in them to render them a pale blue. The normal urine collected from the left kidney was a clear, colourless fluid. That collected from the right often contained mucus and brownish granules initially, but as the flow of urine continued it clarified. Frequently eggs or sperm were present in the right kidney urine. The pericardial fluid was clear and colourless like the urine from the left kidney.

The amount of fluid which was collected from each catheter during a sampling period fluctuated during the course of an experiment. Also, the rate of flow from the kidneys and pericardium varied from experiment to experiment. In Table 1 are given the average rates of flow of the fluids for the experiments reported. There does not appear to be any consistent pattern of flow from the organs. Sometimes it was greater from the right organ than the left, and vice versa. Almost always the rate of flow from the pericardium was lower than from the kidneys. This may be the result of using the smaller diameter catheter in this cavity.

It can be noted from Table 1 that a considerable volume of fluid was lost from the animals during the course of an experiment. A part of this volume was replaced by the infusion of the test materials. The amount of sea water introduced this way was from 2 to 4 ml. per hour.

Filtration

When inulin was infused into the circulatory system of the abalone the blood concentration increased rapidly initially and then levelled off or increased more slowly as the rate of infusion was lowered. Usually within 60 min. after the beginning of an

experiment the concentration of inulin in the urine from both kidneys and in the pericardial fluid reached that of the blood and then remained approximately at the concentration level of the blood until the termination of the experiment. In the upper portion of Fig. 1 are presented the analytical results of a typical experiment. The results of four additional experiments are presented in the lower portion of Fig. 1 where the ratio of the body fluid to urine concentration was calculated for each sampling period and plotted against time. The ratios were distributed about a value of one which is the ratio obtained when the concentrations are identical. This relationship was maintained with blood inulin concentrations ranging from 4 to 70 mg. per 100 ml. These results indicate that the urine originates as a filtrate of the blood.

Table 1. *Summary of the experimental procedures*

Exp. no.	Wet weight minus shell (g.)	Sex	Material injected	Rate of injection (mg./kg./hr.)	Blood level (mg%)	Urine flow (ml./kg./hr.)	Pericardial fluid flow (ml./kg./hr.)
1	720	Male	Inulin Glucose	7 14	Fig. 1 Fig. 4	R. 18·9 L. 0·7	1·5
2	962	Male	Inulin	21	23	R. 2·8 L. 3·5	—
3	1025	Female	Inulin PAH	10 2·4	50–70 2·2	R. 6·0 L. 13·4	—
4	746	Male	Inulin	4	4	R. 12·4 L. 0·5	1·5
5	1025	—	Inulin PSP PAH	10 0·9 1·0	30 0·3 0·8	R. 6·0 L. 6·1	—
6	393	Male	PSP	0·7	0·4	R. 12·3 L. —	4·8
7	1195	Male	PSP	0·3	0·2	R. 4·4 L. 1·2	0·5
8	834	Female	PSP PAH	0·7 2·4	0·1 1·4	R. 2·3 L. 3·4	—
9	824	Female	PAH	1·8	0·8	R. 2·4 L. 0·7	2·7
10	1290	Female	Glucose	4·1	10·0	R. 5·3 L. 2·3	2·2
11	816	Female	Glucose	6·1	18	R. 10·1 L. 1·9	1·3
12	689	Female	T-1824		Pericardial infusion, Fig. 5		
13	627	—	T-1824, inulin, glucose, PAH		Pericardial infusion, Fig. 6		

R, right; L, left.

Secretion

If filtration occurs in the process of urine formation in an animal it is commonly the case that secretion takes place into this filtrate. Morphologists identified the kidneys from their glandular tissue and were convinced that secretion was an important part of urine formation. To determine the extent to which the kidneys were capable of active

secretion, phenolsulphonphthalein (PSP) and *p*-amino hippuric acid (PAH) were infused into the circulatory system. The results obtained with PSP are presented in Fig. 2. The concentration level of PSP remained essentially the same in the blood, pericardial fluid and left kidney urine. However, the level in the right kidney urine was much greater than the other fluids and in one experiment the level reached almost 200 times that in the other fluids.

The results of experiments using PAH are presented in Fig. 3. It can be seen that PAH was also secreted actively by the right kidney. The ratios obtained, however, were of a lower order of magnitude than those with PSP. The left kidney urine, pericardial fluid and blood were approximately of the same concentration. Thus there is no evidence for the active secretion by the left kidney of either PSP or PAH, substances which typically are concentrated by the vertebrate kidney.

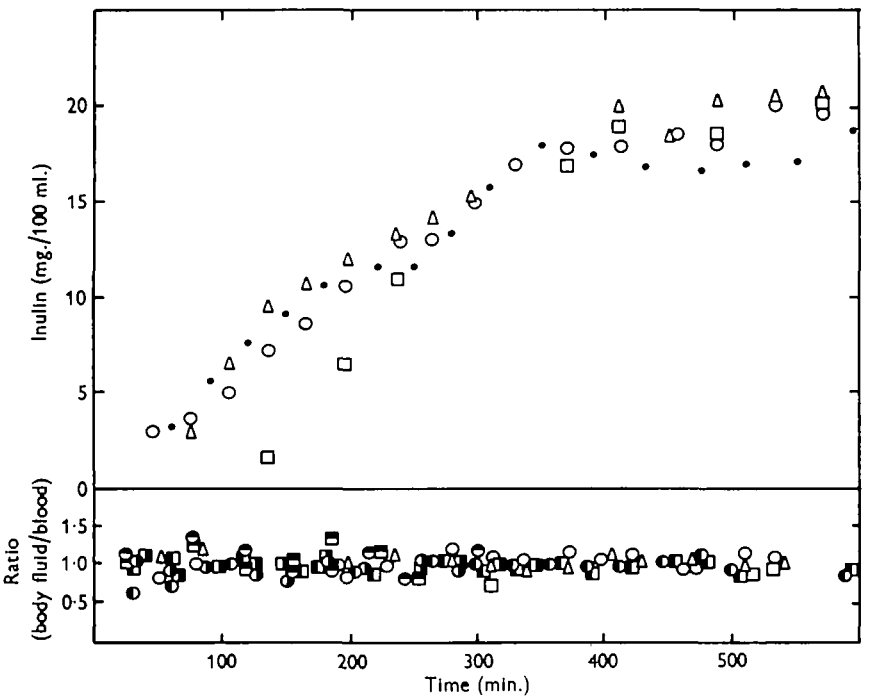

Fig. 1. Upper graph. The relationships of the concentrations of inulin in the blood, pericardial fluid and right and left kidney urines during a typical experiment. Expt. 1: ●, blood inulin; △, pericardial fluid inulin; ○, right kidney urine inulin; □, left kidney urine inulin. Lower graph. The ratio of the concentration of inulin in the body fluid to that of the blood for each sampling period during perfusion experiments in which the blood inulin concentrations varied as shown in Table 1. Expt. 2: ◓, right kidney urine; ◙, left kidney urine. Expt. 3: ◑, right kidney urine; ◧, left kidney urine. Expt. 4: △, pericardial fluid; ○, right kidney urine; □, left kidney urine. Expt. 5: ◐, right kidney urine; ◧, left kidney urine.

With some animals the levels of PSP and PAH in the right kidney urine were approximately the same as in the other fluids. This was due possibly to the animals being in poor physiological condition or perhaps to a restriction of the flow of blood by the stitch holding the catheters in place. In any case the fact that the concentration was the same as that of the blood confirms that PSP and PAH, like inulin, appear in the

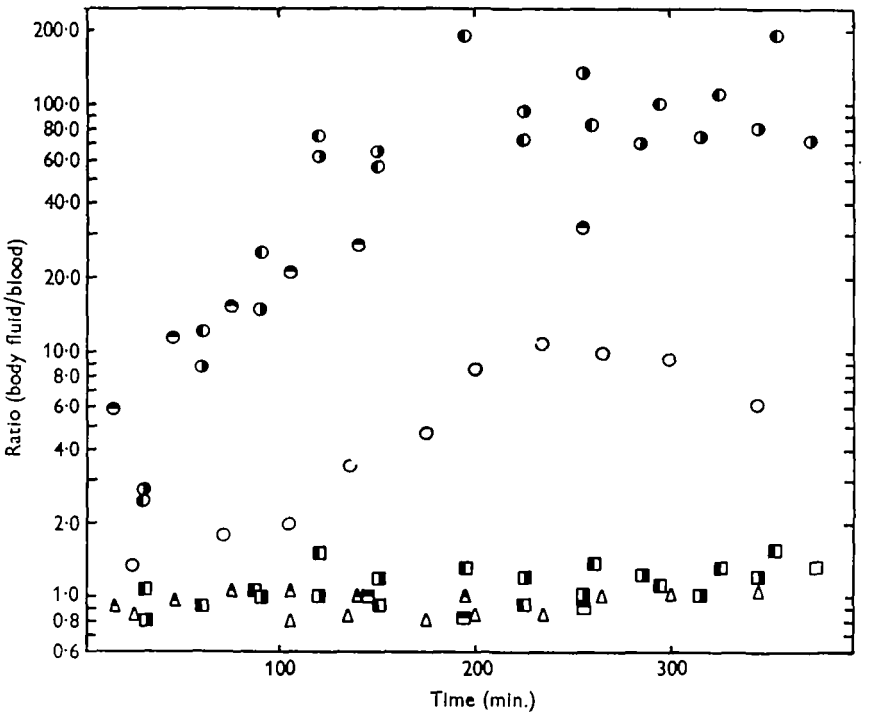

Fig. 2. The ratio of the concentration of phenolsulphonphthalein in the body fluid to that of the blood for each sampling period during perfusion experiments. Expt. 5: ◐, right kidney urine; ◧, left kidney urine. Expt. 6: △, pericardial fluid; ○, right kidney urine. Expt. 7: ▲, pericardial fluid; ◓, right kidney urine; ▬, left kidney urine. Expt. 8: ◑, right kidney urine; ◨, left kidney urine.

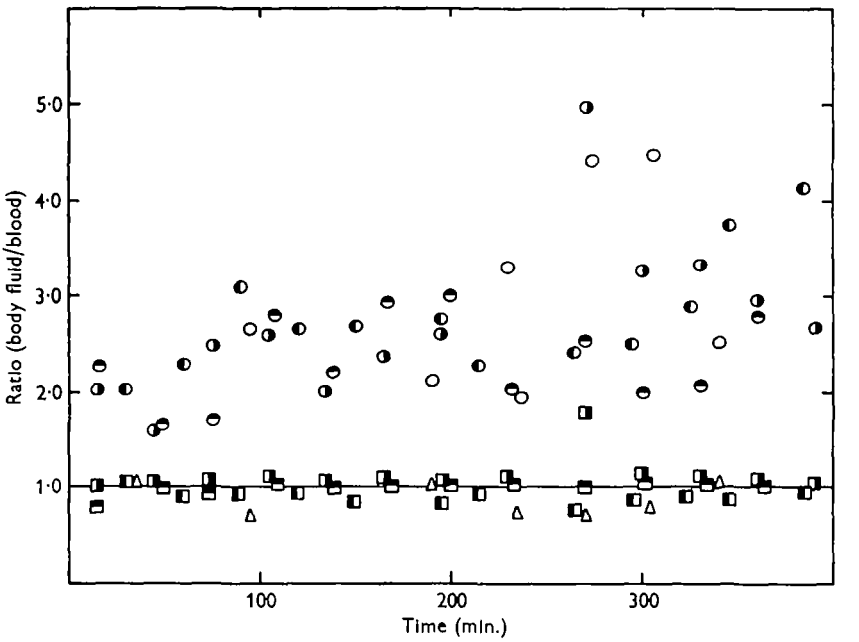

Fig. 3. The ratio of the concentration of *p*-amino hippuric acid in the body fluid to that of the blood for each sampling period during perfusion experiments. Expt. 3: ◓, right kidney urine; ▬, left kidney urine. Expt. 5: ◐, right kidney urine; ◧, left kidney urine. Expt. 8: ◑, right kidney urine; ◨, left kidney urine. Expt. 9: △, pericardial fluid; ○, right kidney urine.

urine through a filtration of the blood. These results imply further that the additional amounts of these substances in the right kidney urine of the other animals were secreted by the right kidney.

Reabsorption

A few experiments were performed to determine whether a glucose reabsorption mechanism was present. The possibility of such a mechanism was indicated from the distribution in the normal body fluids of the reducing carbohydrate level. The results are presented in Table 2. Glucose standards were used in these analyses. In a given animal the concentration was always highest in the blood and lowest in the left kidney urine.

Table 2. *Carbohydrate level of the normal body fluids*

Blood (mg./100 ml.)	Pericardial fluid (mg./100 ml.)	Right kidney urine (mg./100 ml.)	Left kidney urine (mg./100 ml.)
14·4	—	6·8	—
—	10·6	—	3·9
11·8	—	9·2	1·8
9·8	8·5	8·2	3·0
8·4	7·8	4·0	2·7

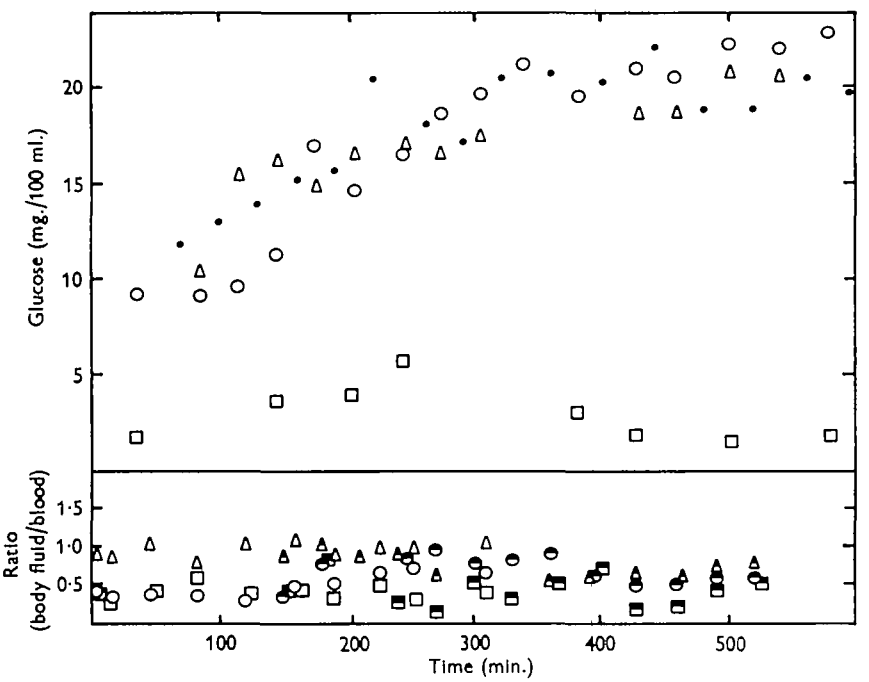

Fig. 4. Upper graph. The relationships during a glucose perfusion experiment of the concentration of reducing carbohydrate in the blood, pericardial fluid and right and left kidney urines. Expt. 1: ●, blood; △, pericardial fluid; ○, right kidney urine; □, left kidney urine. Lower graph. The ratio of the concentration of reducing carbohydrate in the body fluid to that of the blood for each sampling period during glucose perfusion experiments. Expt. 10: △, pericardial fluid; ○, right kidney urine; □, left kidney urine. Expt. 11: ▲, pericardial fluid; ◓, right kidney urine; ◨, left kidney urine.

The results of an experiment where glucose was infused into the circulatory system are presented in the upper portion of Fig. 4. The level of reducing sugar is shown to increase initially in all the body fluids and then to level off. However, only the concentration in the left kidney urine remained consistently lower than in the other body fluids. The results of two additional experiments are summarized in the lower part of Fig. 4. Here again a possible reabsorption of glucose is shown from the urine of the left kidney. It can be seen that the results obtained with the right kidney were less convincing.

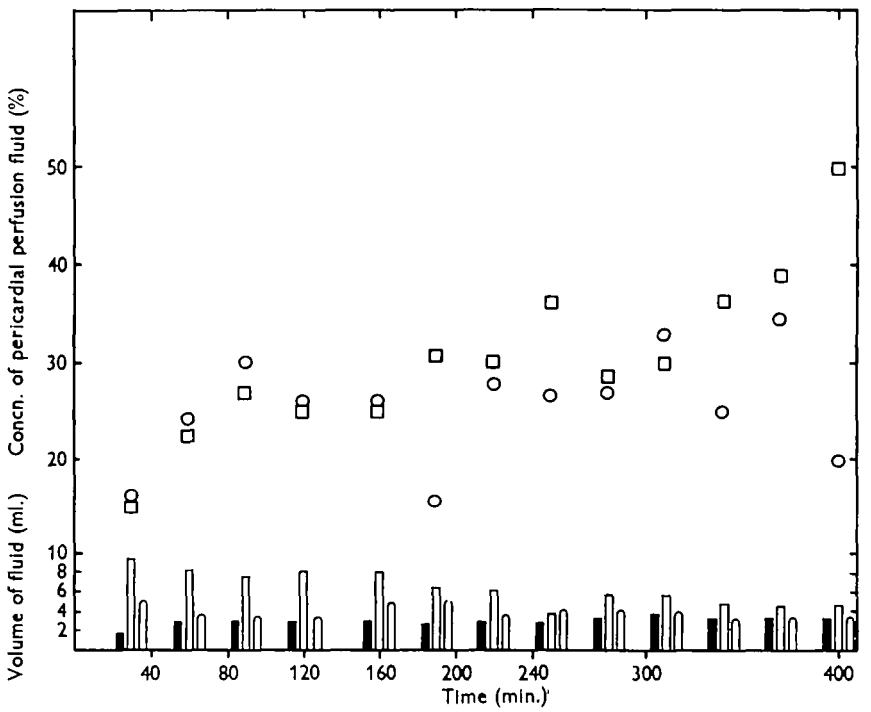

Fig. 5. Upper graph. The T-1824 concentration in the right and left kidney fluids during an experiment in which a T-1824 solution was perfused into the pericardial cavity. Expt. 12: □, right kidney fluid; ○, left kidney fluid. Lower graph. Volume of fluid perfused into the pericardium and volumes of right and left kidney fluid collected during the sampling period. Expt. 12: ▮, volume into pericardium; ▯, volume out right kidney pore; ▯, volume out left kidney pore.

Site of filtration

The results presented so far indicate that the processes of filtration, secretion and reabsorption take place in urine formation. However, the site of filtration has not been identified. For filtration to take place a region of relatively high blood pressure is necessary as well as a suitable surface to restrict the passage of protein. From the morphological accounts of the blood vascular system of the abalone it appears unlikely that the site of filtration is within the renal organs proper. Wegmann (1884), Fleure (1904), Palmer (1907) and Crofts (1929) concur that the principal supply of blood to the right kidney is venous. Crofts (1929) says, 'With the exception of the blood from the rectum, all the deoxygenated blood passes through the portal system of the right renal organ before going to the ctenidia for oxygenation'. She further describes the

arterial supply to the right kidney as being derived from the reno-intestinal artery which originates from the common aortic trunk immediately after it leaves the ventricle. This short artery branches to supply the intestine and provides a small artery to the right kidney.

Although the circulatory pattern of the left kidney is distinctly different from the right, its blood supply is venous also. According to Perrier (1889), Fleure (1904), Palmer (1907) and Crofts (1929), it receives blood which has passed already through the right kidney and been oxygenated in the ctenidia. Crofts (1929) describes the left kidney vessels as originating at the junction of the efferent ctenidial vessel and the

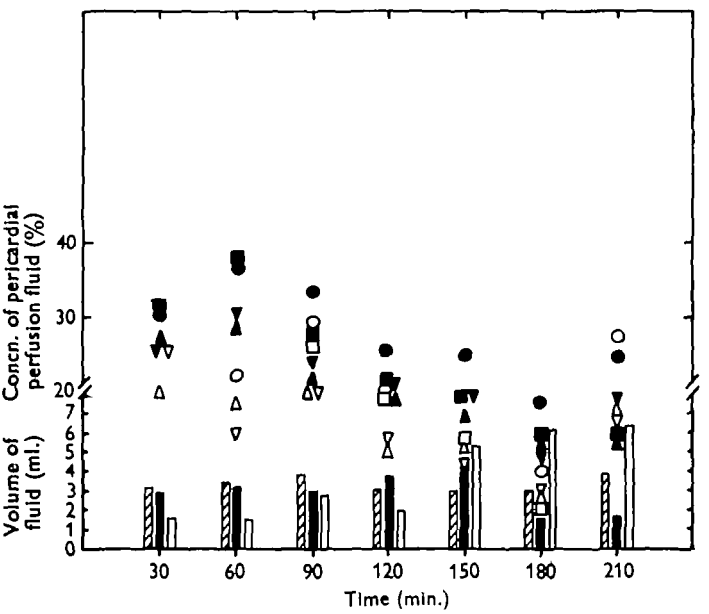

Fig. 6. Upper graph. Concentrations of T-1824, inulin, *p*-amino hippuric acid and glucose in the right and left kidney fluids during an experiment in which these substances were perfused into the pericardium. Expt. 13: ■, T-1824 right kidney fluid; □, T-1824 left kidney fluid; ●, inulin right kidney fluid; ○, inulin left kidney fluid; ▲, *p*-amino hippuric acid right kidney fluid; △, *p*-amino hippuric acid left kidney fluid; ▼, glucose, right kidney fluid; ▽, glucose left kidney fluid. Lower graph. Volume of fluid perfused into the pericardium and volumes of right and left kidney fluid collected during each sampling period. Expt. 13: ▯, volume into pericardium; ▮, volume out right kidney pore; ▯, volume out left kidney pore.

auricle. Perrier (1889) and Palmer (1907) report that they originate from the auricles. Nevertheless, the three agree that the vessels fill with blood during systole and empty during diastole. Although no measurements of the blood pressure in these vessels have been made, it seems improbable, considering the size of the vessels, that there would be sufficient force available here to produce the volume of urine which can be collected from the left renal pore.

Since it seems that the principal supply of blood to the kidneys is under low pressure, another site for filtration must be sought. It seems plausible that the increase in blood pressure during the contraction of the heart could cause fluid to be filtered through the thin walls of the auricles into the pericardial cavity and thence into the

renal cavities via the reno-pericardial canals. An examination of the pericardial fluid data presented in the figures shows that the concentration levels of inulin, PSP, and PAH were essentially the same as in the blood and left kidney fluid. This relationship appears to be independent of time, the rate of urine flow and the blood concentration. It can be concluded from this that the pericardial fluid is a filtrate of the blood. However, unless it can be demonstrated that both reno-pericardial canals are present and functional, the thesis that the urine originates from the pericardial fluid would be difficult to establish.

A careful examination of the pericardial cavity was made with a dissecting microscope. The opening into the left reno-pericardial canal was located readily on the floor of the pericardial cavity adjacent to the left kidney. The right one was located near the antero-dorsal tip of the pericardial cavity. It was possible in the dissected animal to insert thin plastic tubes into the openings and in this way to inject coloured solutions into the renal organs. Once the positions of the canals were located in *Haliotis rufescens* the corresponding structures were found easily in the small black abalone, *H. cracherodii*.

In order to ascertain whether the canals were functional in a living animal, a solution of the dye T-1824 was slowly infused into the pericardial cavity and the fluid was collected from the right and left renal pores. In Fig. 5 the results of such an experiment are presented. The concentrations of T-1824 in the fluids from the renal organs were usually similar even though the volumes of fluid collected from the pores during a sampling period varied considerably.

In Fig. 6 are presented the results of an experiment in which four substances, T-1824, inulin, PAH and glucose were infused simultaneously into the pericardial cavity. In the fluid collected from each renal pore there is a spread in the percentage values of about 7% for the different materials. Even though the inulin and T-1824 values are almost always higher than the PAH and glucose, the significance cannot be evaluated since the results were not verified due to lack of time. In any case both experiments demonstrate that materials introduced into the pericardium are distributed between the two kidneys and reach the outside by way of the renal pores.

DISCUSSION

An investigation of the excretory system of *Haliotis* involves more than a study of the renal organs, for the role of the circulatory system and the relationship to the pericardium become important in the consideration of the dynamics of the system. This becomes apparent in the examination of the process of filtration. Although the results obtained with inulin indicate that filtration takes place, they do not identify the source of the filtrate. Since the pressure necessary for filtration could be supplied by the heart through any of the organs of excretion, the pattern of circulation to the kidneys and related organs was evaluated. From the morphological accounts of the blood-vascular system of the renal organs it appears unlikely that the site of filtration is within the kidneys. The proposal that fluid originates in the pericardium is not new. In 1891 Grobben in his report on the pericardial glands of gastropods says of the structure of the pericardial cells covering the auricles: 'Die flache, platte Gestalt derselben ist einer Abscheidung von Flüssigkeit jedenfalls günstig und ich verweise in dieser Hinsicht bloss auf das Plattenepithel, das in den Malpighi'schen Körperchen der

Vertebratenniere den Glomerulus überkleidet.' Strohl (1913–14) in his review of excretion in molluscs proposes that the stream of liquid that runs from the pericardium through the nephridia might have the function of serving as a vehicle for the solid excretory material. Hoffman (1927) mentions that the pericardium of the pulmonates should not be overlooked as a likely region for water and soluble salt excretion. Crofts (1929) notes that the auricles excrete a watery fluid which becomes part of the pericardial fluid. Picken (1937) has demonstrated in the fresh-water clam, *Anodonta*, a flow of liquid, presumably through the heart, which was nearly free of protein and corresponded in composition to the blood. The experimental results reported here indicate that the pericardial fluid has the proper characteristics to serve as the fluid part of the urine and demonstrate that the fluid can pass into both renal organs. It was not possible to tie off the reno-pericardial canals and in this way to provide final proof that the canals were involved in the passage of fluid. However, from the evidence presented it seems a logical conclusion.

A controversy about the existence of reno-pericardial canals has persisted in the literature for many years. The right canal was described first by Haller (1886), confirmed by Fleure (1902, 1904), Totzauer (1905), Spillman (1905) and Palmer (1907). Its presence was denied by von Jhering (1877), Wegmann (1884), Perrier (1889), von Erlanger (1892) and Crofts (1929). Although the opening of the left canal is quite noticeable, it was not identified by Haller (1886), and Fleure (1902). In a later report Fleure (1904) describes the left canal. Crofts (1929) catheterized the renal pores and injected a small quantity of sea water coloured with carmine into the pericardium. According to her report, the experiment was repeated six times and the colour appeared in the catheter of the left kidney only. Her lack of success in obtaining coloured fluid from both catheters could have been due possibly to the small quantity of fluid injected or could perhaps have resulted because insufficient time was allowed for the coloured solution to appear in the right catheter. It is always possible that *Haliotis tuberculata* differs from *H. rufescens* and *H. cracherodii*. However, Haller (1886), Fleure (1902, 1904) and Spillman (1905) were able to locate the right reno-pericardial canal in *H. tuberculata*. Unfortunately they did not carry out any injection experiments to determine if it was functional.

The results obtained with PSP and PAH demonstrate that as far as the process of secretion is concerned the right and left kidneys differ in their physiological reactions. This was anticipated from the histological findings. Only von Jhering (1877) and Wegmann (1884) considered the cells in the tissues from both kidneys to be similar and filled with the same type of granular secretions. Other investigators (Perrier, 1889; Pelseneer, 1896; Cuénot, 1899; Fleure, 1904; Crofts, 1929) described the tissues from the two kidneys as having cells with different form, size and content and concluded from this that the function of the kidneys differed. Kowalevsky (1889) and Cuénot (1899) using the method of physiological injection found that both the kidneys eliminated coloured materials, but they did not eliminate the same ones. They reported that the right kidney alone eliminated indigo and fuchsin while the left alone eliminated carmine and Chinese ink.

In addition to the right kidney a number of other areas have been considered as being capable of secretion (cf. Crofts, 1929). Those which may contribute products to the pericardial fluid and in this way participate in urine formation are the special cells on

the pericardial wall and the pericardial glands of the atria. Crofts (1929) has described the large oval cells on the ventral and lateral pericardial wall as discharging their contents into the pericardial fluid. She suggests that these cells may secrete the uric acid found in the pericardial fluid. As far as the pericardial glands are concerned Grobben (1891), Spillman (1905) and Crofts (1929) concur that the flattened epithelial cells may function in water elimination. Some investigators (Perrier, 1889; Fleure, 1904; Spillman, 1905) have described small granules in the cells of the pericardial glands. Spillman (1905) considers these granules to be decomposition products of blood cells. Grobben (1891) considers these granules to be of no significance and Crofts (1929) describes the cells as being without granules. Consequently, until further research is conducted, the question as to whether these cells are secretory in nature remains unanswered.

The rate of flow of urine from the animals ranged from about 6 to 21 ml./kg./hr. (0·15 to 0·50 ml./g./24 hr.). This is within the range of values quoted for other marine animals (cf. Martin, 1957). Crofts (1929) describes the rate of excretion from the left organ as much slower than from the right. This observation was not verified by the data presented here on rates of urine flow.

Since a considerable volume of fluid is lost from the animals by way of the urine there would be an additional loss of salts and nutritive material unless reabsorption took place. From the results obtained with glucose infusion it appears that reabsorption of glucose takes place in the left kidney. Although the results with the right kidney were inconsistent the possibility of glucose reabsorption from the right kidney is not excluded. From the data presented in Fig. 4 it may be seen that the concentration of glucose in the right kidney urine was of the same order of magnitude as that of the blood in Expt. 1 and as that of the pericardial fluid in Expt. 11. But in Expt. 10 the ratios of right kidney urine to blood were consistently lower than the ratios of pericardial fluid to blood. It should be noted that the rates of production of urine in Expts. 1 and 11 were high, 18·9 and 10·1 ml./kg./hr. as compared to the lower value of 5·3 for Expt. 10. Possibly at the higher rate of urine flow there was insufficient opportunity for contact with the cells capable of reabsorption.

An interesting question to consider is the role in excretion of the left kidney or papillary sac. The results of physiological injection indicate that it does not take up dye as does a typical depuratory kidney (Kowalevsky, 1889, Cuénot, 1899). Pelseneer (1896) thinks the organ to be phagocytic in nature as only it absorbs the insoluble powders injected. The fact that the interior surface is increased tremendously by formation of papillae has lead Spillman (1905) to the conclusion that it functions in reabsorption. He proposes that the left kidney picks up the decomposition granules which he considers to be produced by the pericardial glands. Crofts (1929) reports that it retains some depurative function because she was able to show 'The reaction of its excretion to uric acid tests is as definite as with the excretion of the right renal organ...'. From the results presented here with PSP and PAH the left kidney does not appear capable of active secretion. However, the results obtained with glucose infusion indicate that it may be capable of reabsorption. As far as its phagocytic activity and participation in the elimination of uric acid or other waste products are concerned, no conclusions can be drawn from the data presented.

SUMMARY

1. Experiments were carried out on the abalone, *Haliotis rufescens*, to discover some of the mechanisms involved in urine formation. Test substances were infused into the blood stream and their concentrations followed in serially taken samples of blood, pericardial fluid and urine from the right and left kidneys.

2. Inulin appears to be filtered since its concentration is essentially the same in samples of blood, pericardial fluid and urine from each kidney. The concentrations of phenolsulphonphthalein and *p*-amino hippuric acid were considerably higher in right kidney urine than in the other fluids, indicating that this kidney is capable of active secretion.

3. Glucose occurred in lower concentrations in the left kidney urine than in blood and pericardial fluid, suggesting a reabsorption of glucose by this kidney.

4. Dye solutions of T-1824 and other materials infused slowly into the pericardial cavity appeared in the urine of both kidneys, suggesting the presence of two functional reno-pericardial canals; presence of both right and left canals was verified by dissection and observation.

5. From the results obtained it appears that the primary step in urine formation is filtration of blood through the walls of the atria into the pericardial cavity. This fluid then passes via the reno-pericardial canals into the kidneys where on the right side substances may be actively secreted into it and in the left kidney substances may be actively reabsorbed.

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