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IONIC REGULATION IN THE QUEEN CONCH, *STROMBUS GIGAS* (GASTROPODA, PROSOBRANCHIA)*

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The haemolymph of marine molluscs, with the exception of the cephalopods, is similar but not identical to sea water in inorganic composition. The analyses of various workers are well summarized by Robertson (1965). In general, the concentrations of sodium, magnesium and chloride are the same in haemolymph and in sea water; potassium and calcium are more concentrated in haemolymph; while sulphate is less concentrated in haemolymph than in sea water. In the cephalopods ionic regulation is more marked than in other molluscan classes; concentrations of sodium and chloride differ from the concentrations in sea water, and the accumulation of potassium and elimination of sulphate are very pronounced.

Up to the present the mechanisms of ionic regulation in marine molluscs have been examined only in the cephalopods, where the kidneys have been shown to be at least partly responsible. The present study is an examination of the inorganic composition of the haemolymph of the large tropical gastropod *Strombus gigas* Linnaeus (Mesogastropoda, Strombidae). From analyses of various body fluids, and their movements, some suggestions are made concerning the mechanisms by which the composition of the haemolymph is regulated.

MATERIAL AND METHODS

Specimens of *Strombus gigas* Linnaeus were collected from the shallow water surrounding the southern Florida Keys. Adult specimens, with a shell up to 30 cm. in length and having a thick, well-formed lip, and specimens that were as long but without the lip, were used. The animals were maintained alive at the Institute of Marine Science, Miami, in large aquaria supplied with running sea water. This water is taken in directly from Bear Cut, and the chloride content varied from 517 to 562 mM/kg. water. The chloride value was checked for several days before body fluids were sampled, to ensure that the animals were in true equilibrium with the sea water; from day to day it did not vary more than 6 mM/kg. water and sampling days were chosen so that variation over the last 3 days was no more than 2 mM/kg. water.

In order to be able to sample body fluids and to make measurements of potential difference, a large area of the body whorl was exposed by cutting away the shell with a rotary saw. The shell was replaced with a wax layer that could be removed at any time. Since the shell is to a large degree involute, the configuration of the whorls was somewhat altered from the normal to allow manipulation of the body whorl without disturbance of the mantle and head, which areas are highly sensitive to touch. Figure 1

* Contribution No. 794 from the Marine Laboratory, Institute of Marine Science, University of Miami.

shows an animal prepared for sampling and indicates the positions of some of the organs beneath the shell. Wax patches were inserted further round the shell to expose the stomach. The anatomy of *Strombus* is described by Little (1965*b*).

Body fluids were withdrawn in fine glass pipettes, the fluid being always kept under mineral oil. Samples were centrifuged immediately to remove amoebocytes and other particulate matter, and were then transferred under oil to clean containers. Analyses were carried out as soon as possible, but in the event of samples being stored overnight they were placed in a refrigerator at about 4° C.

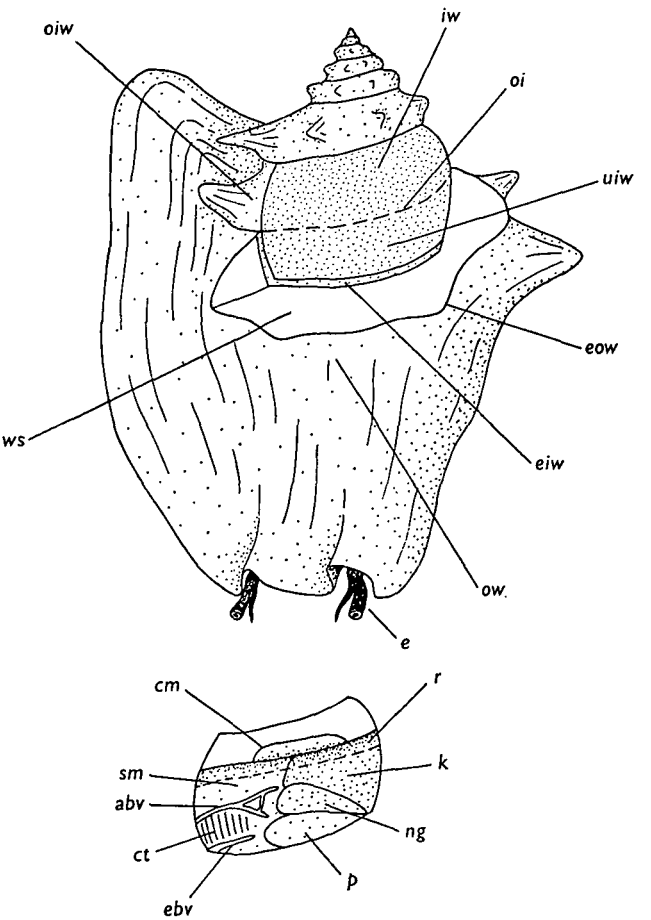


Fig. 1. Top view of *Strombus* in normal position to show placing of wax patches. The inset shows the disposition of organs beneath the stippled area. *abv*, afferent branchial vein. *cm*, columellar muscle. *ct*, ctenidium. *e*, eyestalk, *ebv*, efferent branchial vein. *eiw*, remaining edge of inner whorl. *eow*, cut edge of outer whorl. *iw*, previously exposed surface of inner whorl, now substituted with wax. *k*, kidney-tissue proper. *ng*, nephridial gland. *oi*, original site of insertion of outer on inner whorl. *oiw*, unaltered outer surface of inner whorl. *ow*, outer surface of outer whorl. *p*, pericardium. *r*, rectum. *sm*, dorsal surface of mantle cavity. *uiw*, previously unexposed surface of inner whorl, now substituted with wax. *ws*, wax septum, added to separate inner and outer whorls.

Estimation of ion concentrations

Sodium and potassium were estimated using a Beckman DU Spectrophotometer with a photomultiplier and flame attachment. Dilutions in distilled water were approximately 1:500 for sodium and 1:250 for potassium.

Calcium and magnesium were determined by EDTA titrations similar to those described by van Asperen & van Esch (1956), in which magnesium is found as the difference between a value for total divalent cations and a calcium value. Cyanide was used to prevent interference from other divalent cations such as iron and copper. The titration value for calcium includes strontium, which in sea water of 19‰ chlorinity has a concentration of 0·1 mM/kg. water. On the supposition that the strontium concentration will be similar in the various fluids, this value has been subtracted from the titration values to give the calcium concentration.

Chloride was determined potentiometrically by the first method of Ramsay, Brown & Crogham (1955).

Table 1. *Estimated errors in analytical methods*

	Na	K	Ca	Mg	SO_4	HCO_3
Sea water* as calculated from Cl value (mM/kg. water)	475·4	10·07	10·34	54·17	28·56	2·37
Sea water as measured (mM/kg. water)	472·4	10·05	10·36	55·70	28·01	2·63
± S.E.	±1·6	±0·01	±0·03	±0·36	±0·68	±0·19
No. of observations	8	9	5	5	8	6
Mean error in measured value (%)	−0·63	−0·20	+0·19	+2·82	−1·93	+10·97
Maximum errors (mM/kg. water)	−10·4	−0·61	−0·39	−0·07	−2·96	−0·37
	+ 3·4	+0·41	+0·81	+2·63	+1·96	+1·03

*This sea water has a chloride content of 19‰, or 554·4 mM/kg. water.

Sulphate was determined by the conductimetric method of Roach (1963). Since this method is more accurate at higher concentrations of sulphate relative to the total concentration of salts, a known amount of sodium sulphate was added to the titration vessel. In the measurement of sulphate in some fluids, notably stomach fluid, excessive frothing occurred; some samples were therefore deproteinized with ethyl alcohol, and the apparatus was recalibrated using sulphate standards similarly treated with alcohol. Aside from the frothing, protein appeared to have no effect on estimation of sulphate, since stomach fluid (which normally gives a reading of almost zero sulphate), when dialysed against sea water, gave values similar to those of the dialysing sea water.

Bicarbonate was estimated by the microdiffusion method of Conway (1962).

The water content of samples was measured by drying overnight at 104° C., and ion concentrations are expressed as mM/kg. water. All values given in this paper are converted to those equivalent to sea water of 19‰ chlorinity.

The values for sea water were calculated from the chloride content (Barnes, 1954), but were also measured directly by the above methods in an attempt to estimate the probable accuracy of the methods. The chloride titrations were repeatable to within 2·5 mM/kg. water (max. variation). Table 1 shows errors as estimated by this method,

but these can only be considered as giving a rough estimate of the errors involved in the measurement of biological samples, since these latter probably contain many interfering substances not present in sea water.

Measurement of pH

A Beckman capillary electrode was used in conjunction with a Keithley electrometer. 50–100 μl. samples were aspirated into the electrode, which was placed in a water-bath at 22° C. The electrode was calibrated with Beckman buffers which are supplied as accurate to within $\pm 0{\cdot}03$ pH.

Dialysis

1–2 ml. samples of haemolymph were placed in small bags of cellulose dialysis tubing, which were then lowered into a large volume of sea water until the liquid levels were equal on both sides of the membrane. The sea water was covered to reduce evaporation, and was stirred at intervals. Haemolymph was removed and analysed, together with a sample of sea water, after approximately 14 hr. at room temperature. After this time there was no visible change in the samples, and volumes withdrawn were approximately equal to those at the start of dialysis.

Measurement of potential difference

Animals were placed in insulated bowls containing about 5 cm. of sea water, so that the area of tissue exposed by removal of the wax patch was above the sea water level. Potentials were measured with mercury-calomel-sat. KCl electrodes (Beckman) connected to a Keithley electrometer (input impedance $10^{14}\,\Omega$), which at maximum sensitivity reads 1·0 mV. for full-scale deflexion. The electrodes were fitted with polythene tubes filled with sat. KCl and agar, which could be introduced into the afferent or efferent branchial veins. There was no need to tie in the cannulae, as the tubes were large enough at their widest diameter to fill the blood vessels. The area surrounding the insertion of the cannulae was scrupulously dried with soft tissue. Zero potential was recorded as the reading when both electrodes were in sea water. Measurements were made at 22° C.

Estimation of ^{14}C-inulin

Carboxyl-^{14}C-inulin was used to follow movement of fluids. The volumes of samples of body fluids were measured in small self-adjusting pipettes, and samples were transferred to planchets. Spreading was assisted by dilution with distilled water, and samples were dried down under an infra-red lamp. Samples of sea water (1·22 ml.) were taken in a 2 ml. syringe. ^{14}C was counted using a Nuclear-Chicago gas-flow detector. The background count was about 15 c.p.m., except in some experiments in which a low-background counter (background 1·5 c.p.m.) was used.

The samples of sea water contained enough salt to cause appreciable self-absorption; and a separate calibration curve for these samples was constructed by adding 1·22 ml. of sea sater to samples having a known count rate, and recording the reduced count rate. From this curve the true count rate could be read off.

RESULTS

Composition of the haemolymph

The haemolymph of *Strombus* is a bright blue colour, presumably due to the presence of haemocyanin. The 'protein' content is given in Table 2. The pH is 7·91 (Table 3), which value is not significantly different from that of sea water ($P > 0{\cdot}50$).

The inorganic composition of the haemolymph is given in Table 4. The osmotic pressure has not been measured, but it can be assumed that it is equal to that of sea water, since this is so for all marine molluscs that have been examined (e.g. the data compiled by Robertson, 1964). At the bottom of Table 4 are given the potential

Table 2. *'Protein'* content of body fluids*

	% of wet weight	± S.E.	Observations (no.)
Haemolymph	3·37	±0·35	20
Pericardial fluid	0·76	±0·17	8
Kidney fluid	2·43	±0·42	10
Stomach fluid	8·01	±0·65	10
Rectal fluid	1·35	±0·21	11

* 'Protein' = (total dry wt. − dry wt. of salts) expressed as a % of total wet wt. Since drying was carried out at 104° C, this value may include some water of hydration.

Table 3. *pH of body fluids*

	pH	± S.E.	Observations
Sea water	8·01	±0·06	10
Haemolymph	7·91	±0·06	12
Pericardial fluid	7·74	±0·06	6
Kidney fluid	7·41	±0·07	8
Stomach fluid	5·87	±0·16	9
Rectal fluid	7·95	±0·06	10

Table 4. *Relation of the composition of haemolymph to that of sea water*

	I Na	II K	III Ca	IV Ca (unoperated animals)	V Ca (pericardial fluid)	VI Mg	VII HCO_3	VIII Cl	IX SO_4
Concentration in sea water (mM/kg. water)	475·4	10·07	10·34	10·34	10·34	54·17	2·37	554·4	28·56
Concentration in haemolymph (mM/kg. water)	495·9	10·90	11·03	10·77	9·92	58·28	10·16	557·8	20·48
± S.E.	±5·4	±0·09	±0·28	±0·21	±0·20	±0·68	±0·36	±4·3	±0·94
No. of observations	12	12	8	8	9	8	12	12	12
Mean calculated Nernst potentials (mV)	−1·05	−2·00	−0·63	−0·50	+0·54	−0·92	—	+0·15	−4·22
± S.E.	±0·28	±0·34	±0·27	±0·25	±0·21	±0·15	—	±0·21	±0·61
Significance of difference between measured P.D.s (from Table 5) and calculated P.D.s									
t	0·451	1·752	1·963	2·505	6·563	1·407	—	4·918	4·110
D.F.	19	19	15	15	16	15	—	19	19
P	>0·50	0·1–0·05	0·1–0·05	0·05–0·01	<0·001	0·2–0·1	—	<0·001	<0·001

differences that would exist between haemolymph and sea water if these two fluids were in passive equilibrium, as calculated from the Nernst equation,

$$E = \frac{RT}{zF} \ln \frac{C_1}{C_2}.$$

R is the gas constant (8·314 V. coulombs/degree.mole), T is the absolute temperature, z the valency, and F the Faraday. C_1 and C_2 are the concentrations of the ions in haemolymph and in sea water, the potentials being calculated with respect to the haemolymph. These potentials may be compared to the potential as measured *in vivo*, which is shown in Table 5. The comparison is considered in more detail later, but

Table 5. *Potential difference (mV) between haemolymph and sea water at a temperature of* 22° C.

−1·10	−1·15
−0·75	−2·10
−0·60	−1·15
−1·10	−1·35
−1·90	Mean −1·24 ± 0·17 (S.E.)

Table 6. *Concentration of ions in haemolymph and dialysed haemolymph*

	Na	K	Ca	Mg	Cl	SO_4
Concentration in sea water (mM/kg. water)	475·4	10·07	10·34	54·17	554·4	28·56
Concentration in haemolymph before dialysis (mM/kg. water)	503	11·1	11·2	60·2	577	20·1
Concentration in haemolymph after dialysis (mM/kg. water)	499	10·6	11·1	60·6	574	27·9
Concentration in normal haemolymph as % of conc. in dialysed haemolymph*	100·5	105·0	101·0	99·9	100·4	72·8
± S.E.	±0·9	±1·6	±2·9	±1·2	±0·5	±3·4
No. of observations	7	7	5	5	9	8
Significance of the difference from 100%						
t	0·556	3·125	0·351	0·083	0·800	8·000
P	>0·10	0·05–0·02	>0·10	>0·10	>0·10	<0·001

*Calculated for individual animals, not from mean values.

here it is shown that the potentials for chloride and sulphate are significantly different from the measured potential. This suggests that chloride is actively accumulated and sulphate eliminated, while calcium may be actively eliminated and potassium may be actively accumulated. No Nernst potential can be calculated for bicarbonate, since this ion may be considered a metabolic product, and there will be a net efflux from the body; a Nernst equation will hold only if there is no net flux across the membrane.

A second method that has been used to obtain an estimate of the degree of ionic regulation, in marine invertebrates that do not osmoregulate, is that of dialysis of the haemolymph against sea water, and comparison of the original concentration of ions with the concentration of ions after dialysis (Robertson, 1939, 1949, 1953). Such values are given for *Strombus* in Table 6. These suggest active accumulation of potassium, and active elimination of sulphate, but no regulation of chloride or calcium.

The difference between these two approaches is discussed later, but here it is sufficient to note that both suggest as a major feature the elimination of sulphate, and both agree that potassium may be accumulated; major differences lie in the interpretation of the situation for calcium and chloride.

Sites of exchange of water and ions

Three sites at which loss and uptake of water and ions are to be expected have been investigated: the renal and digestive systems most extensively, and the secretion of mucus but briefly.

(*a*) *The reno-pericardial system*

It is probable that in all aquatic molluscs urine is formed by ultrafiltration of haemolymph through the wall of the heart into the pericardium, and that this is followed by modification of the composition of this filtrate in the kidney (Martin, 1957; Harrison, 1965; Little, 1965.) The pericardial fluid of *Strombus* is colourless and less

Table 7. *Composition of pericardial and kidney fluids compared to haemolymph*

	Na	K	Ca	Mg	HCO_3	Cl	SO_4
Concentration in haemolymph (mM/kg. water)	495·9	10·90	11·03	58·28	10·16	557·8	20·48
± S.E.	±5·4	±0·09	±0·28	±0·68	±0·36	±4·3	±0·94
No. of observations	12	12	8	8	12	12	12
Concentration in pericardial fluid (mK/kg. water)	478·2	13·43	9·92	56·96	10·07	555·6	19·38
± S.E.	±3·6	±0·87	±0·20	±0·64	±0·52	±9·3	±1·77
No. of observations	9	9	11	11	9	10	8
Concentration in kidney fluid (mM/kg. water)	477·7	13·33	14·97	58·14	10·27	561·5	21·19
± S.E.	±6·2	±0·91	±1·37	±0·75	±0·39	±2·2	±1·26
No. of observations	9	9	11	11	9	10	8

viscous than the haemolymph, and had a lower 'protein' content (Table 2). Its inorganic composition (Table 7) is similar to that of the haemolymph, but it contains more potassium and less calcium; and the pH is slightly lower (Table 3). The inorganic composition and lowered protein content suggest that pericardial fluid could be an ultrafiltrate of the haemolymph.

Fluid withdrawn from the kidney is a light brown colour, and is apparently similar in composition to pericardial fluid in that it has a raised concentration of potassium compared to the haemolymph (Table 7). It also has a raised concentration of calcium compared to the haemolymph, and is more acid than either this or pericardial fluid, while the 'protein' content is higher than that of the latter (Table 2).

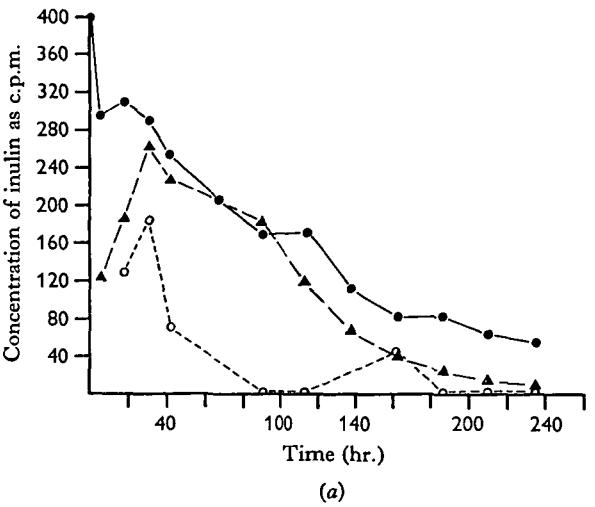
The possible function, in regulation of the inorganic composition of the haemolymph, of the pericardium/kidney system will be discussed later. Superficially, at least, it appears that this system excretes calcium and potassium, which does not fit the concept of active accumulation of potassium suggested by the measurements of electrochemical potential.

The flow of fluid through the renal system has been investigated by injection of ^{14}C-inulin into the haemolymph and subsequent sampling of various fluids. 1 ml. of haemolymph was withdrawn from the afferent branchial vein through a No. 27 hypodermic needle, and was replaced with 1 ml. of a solution of inulin in Ringer

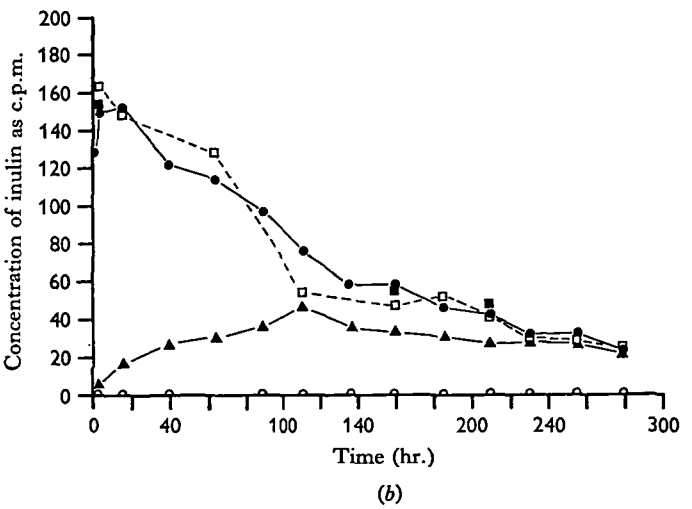
Table 8. *Composition of Ringer solutions*

	NaCl	$MgCl_2$	Na_2SO_4	$CaCl_2$	KCl	$NaHCO_3$
Ringer solution for haemolymph	430	58	20	10	11	10
Ringer solution for stomach fluid	480	53	—	20	11	—

All values are given for anhydrous salts, as mM/kg. water.
These solutions are adequate for use when the sea water has a chlorinity of 19‰.



(*a*)



(*b*)

Fig. 2. Concentrations of inulin as c.p.m. in body fluids, after injection of ^{14}C-inulin into the haemolymph at zero time. ● haemolymph, ■ pericardial fluid, □ kidney fluid, ▲ stomach fluid, ○ rectal fluid.

solution. The composition of this latter is given in Table 8. The results of two typical experiments are given in Fig. 2*a* and *b*. At all points the concentrations of inulin in haemolymph, pericardial fluid and kidney fluid are approximately equal, supporting the idea of filtration into the pericardium.

In these experiments the animals were kept in 10 l. of aerated sea water in polythene bowls, and samples of sea water were taken at intervals. From the accumulation of inulin in the sea water over successive 24 hr. periods, and on the assumption that the concentration of inulin in haemolymph is equal to the concentration in final urine, the rates of urine production have been calculated for four animals and are given in Table 9.

Table 9. *The rate of production of urine*

Animal	Wet wt. tissue (g.)	Mean vol. urine per 24 hr. (ml.)	No. of obs.	Mean rate of production of urine (ml./kg/ hr. ± S.E.)	Blood volume (ml.)	Blood volume (% wet wt.)	Mean rate of production of urine (% blood vol./24 hr.)
A	300	21·0	6	2·5 ± 0·3	197·1	65·7	10·6
B	239	23·5	2	4·1 ± 0·6	165·0	69·0	14·3
C	241	17·8	9	3·2 ± 0·5	169·6	70·4	10·5
E	345	19·6	12	2·4 ± 0·4	185·6	53·8	10·6
Mean				3·0 ± 0·4		64·6 ± 3·8	11·5 ± 0·9

Table 10. *Composition of fluids from stomach and rectum*

	Na	K	Ca	Mg	HCO_3	Cl	SO_4
Stomach	479	10·9	19·2	53·1	0·9	568	0·14
± S.E.	±3·9	±0·46	±2·25	±1·02	±0·29	±4·7	±0·13
No. of observations	9	9	9	9	8	10	7
Rectum	468	12·9	9·4	56·6	13·9	559	23·5
± S.E.	±7·4	±0·48	±0·26	±0·36	±1·17	±4·0	±1·34
No. of observations	8	8	11	11	8	7	12

All values are given as mM/kg. water ± S.E. (no. of observations)

Since a known quantity of inulin was injected into the haemocoel, the inulin volumes can be calculated. If it is assumed that these volumes represent haemolymph volumes, the rate of urine production can also be expressed as a percentage of these. Such results are also collected in Table 9.

(*b*) *The digestive system*

Fluid from the stomach is dark brown and viscous, and can often be obtained in relatively large volumes (e.g. 10 ml. from a fully grown specimen). It has a high 'protein' content (Table 2). The most striking features of its inorganic composition (Table 10) are the very low concentrations of bicarbonate and sulphate, and the high concentration of calcium. The concentrations of other ions are similar to their concentrations in the haemolymph. The very low bicarbonate concentration is compatible with the low pH (Table 3), and the latter may also account for the calcium values, since 'sand' grains were often observed in the stomach, and 'sand' in the Miami area is to a great degree calcareous. The low sulphate concentration (in almost every sample sulphate was undetectable) must be due to active regulation.

Since the stomach fluid has been examined with a view to estimating the function

of the stomach in ionic regulation, the origin and fate of this fluid is of great importance. Traditionally, the molluscan digestive system is regarded as functioning in the following way. Food may be partly digested in the oesophagus by secretions from the salivary glands. In the stomach it is further acted upon by secretions from the digestive gland, and by enzymes liberated from the crystalline style in those forms that have

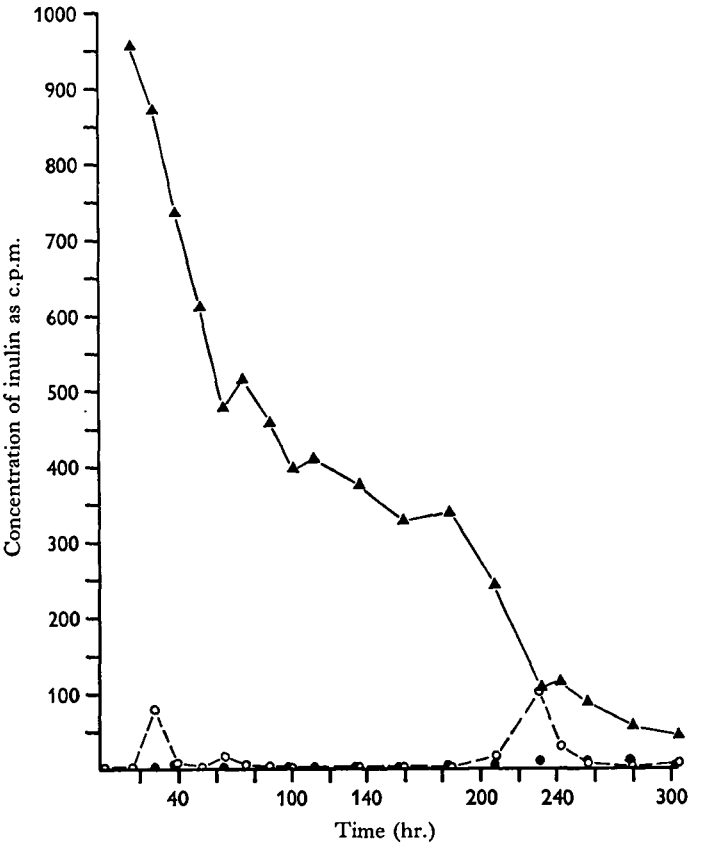


Fig. 3. Concentration of inulin, as c.p.m., in stomach fluid, rectal fluid and haemolymph, after injection into the stomach of ^{14}C-inulin at zero time. ▲ Stomach fluid, ○ rectal fluid, ● haemolymph.

such a structure. Ciliary sorting in the stomach moves small particles into the digestive gland, where they are absorbed and mostly digested intracellularly, and rejected matter is passed into the intestine; from there it moves down the rectum to the outside. The rate of loss of fluid from the rectum, and the modification of the composition of fluid as it passes down the intestine, have been briefly examined.

The composition of fluid collected from the rectum is given in Table 10. This shows that if the fluid has been derived from stomach fluid much calcium has been removed, and potassium, sulphate and bicarbonate added. Sodium, magnesium and chloride appear to be approximately in passive equilibrium between haemolymph, stomach fluid and rectal fluid, although without knowing the electrochemical gradients it is impossible to be certain of this. The 'protein' content of rectal fluid is much lower than that of stomach fluid (Table 2), and the pH is considerably higher (Table 3).

The movement of fluid from the stomach through the intestine and rectum has been followed by the injection of ^{14}C-inulin. When inulin is injected into the haemolymph (Fig. 2*a*, *b*), the concentration if inulin in stomach fluid may or may not rise sharply. In either case the concentration in rectal fluid remains relatively low, but may show occasional peaks up to the concentration in the stomach. In no case does the rectal fluid show a concentration of inulin approaching that in the haemolymph.

Table 11. *Rate of loss of fluid from the stomach*

Animal	Wet wt. tissue (gm.)	Rate of loss of fluid over successive 24 hr. periods (ml./kg.)	Mean rate of loss (ml./kg./hr. ± S.E.)
D	322	7·04 7·68 0·00 4·80 4·52 2·60 0·00	0·16 ± 0·05
F	297	1·92 1·60 1·92 2·20 3·92 0·00 2·48 2·68 5·44	0·10 ± 0·02

When inulin is injected into the stomach there is an apparently rapid drop in concentration after the first reading, presumably due to mixing (Fig. 3); and thereafter the concentration decreases slowly. The concentration in haemolymph stays low, but in rectal fluid it shows occasional peaks. This suggests that some fluid passes down the intestine from the stomach, but, since the concentration of inulin always falls again, this stomach fluid must be flushed out by another fluid containing little inulin. The two possible candidates for such a fluid appear to be external sea water and a secretion from the rectal walls. This latter seems unlikely, since in the experiments in which inulin is injected into the haemolymph no inulin is found in the rectal fluid unless the concentration in the stomach is already high (Fig. 2*a*). However, to test for the possibility of a rectal secretion the following observations were made. From two individuals the rectum and intestinal loop that lies in the kidney cavity were isolated and flushed out with sea water. The anal ends were tied off and 1·0 ml. of sea water (which does not by any means fill the cavity) containing methylene blue was injected into the intestinal ends. These ends were tied off, and the preparations were placed in aerated sea water for 13 hr. After this time there was no evidence of any dye in the sea water, but the volumes of fluid when withdrawn were 0·9 and 0·8 ml., suggesting that there had been no secretion into the rectal cavity.

It therefore appears probable that part of the fluid in the rectum is in fact sea water drawn in from outside. This idea is supported by similarities in inorganic composition between sea water and rectal fluid and by differences between rectal fluid and stomach fluid (Table 10); in particular, the lower calcium content, and higher bicarbonate

and sulphate contents of rectal fluid, combined with higher pH, as compared to stomach fluid, suggest a greater affinity with sea water than with fluid originating in the stomach.

The rate of loss of stomach fluid to the exterior can be calculated, since the concentration of inulin in stomach fluid is known, as is the inulin lost to the sea water. If there is indeed net secretion into the intestine and rectum, then this rate of loss also represents the true rate of net loss of fluid from the rectum, i.e. it does not take into account the sea water that flushes out this fluid and the faeces. Rates are calculated for two animals in Table 11.

(*c*) *Mucus*

No detailed analyses have been made of mucus, but one sample from the mantle cavity was digested in nitric acid and redissolved in the original volume of water. The concentration of calcium was 10·0 mM/kg. water, and that of magnesium 52·5 mg/kg. water, which values are similar to those of haemolymph. Sulphate had a concentration of 127 mM/kg. water, much in excess of that in sea water or haemolymph.

DISCUSSION

Ionic composition of the haemolymph and active regulation

The composition of the haemolymph of *Strombus* is similar to that described for other marine gastropods (see Robertson, 1965), in that the concentration of potassium is raised, and that of sulphate is lowered, when haemolymph is compared with haemolymph dialysed against sea water. For other molluscs these figures have been interpreted as an active regulation of these ions. However, active transport of an ion must be defined as movement of that ion against the electrochemical gradient (see Dainty, 1962, for discussion); and movement against a chemical gradient is therefore not evidence of active transport. This has been recently emphasized by Croghan, Curra & Lockwood (1965). In the present paper it is shown that even in *Strombus*, where differences in composition between body fluids and sea water are relatively slight and the potential difference is small, measurement of both is of the greatest importance.

From Table 4 it appears, as previously discussed, that chloride is actively accumulated, and sulphate eliminated, while sodium and magnesium are in passive equilibrium. Potassium and calcium show differences between calculated and measured potentials at the 10%, but not at the 5%, probability level. The question of the regulation of calcium may be examined a little further. The figures given in column III of Table 4 are for animals in which part of the shell had been replaced by wax some days before sampling. Since it seemed possible that such a procedure might affect the rates of calcium exchange, samples were also taken from animals without prior operation (column IV). In this case the mean calculated potential is significantly different from the measured potential at the 5% level, indicating active excretion of calcium. It is suggested that part at least of this excretion may be represented by the secretion of the shell-forming area of the mantle. One further point is that much calcium in the haemolymph of molluscs may be bound to protein (Robertson, 1965; Schoffeniels, 1951); and the concentration of calcium in true solution should be equal to the concentration in pericardial fluid if this is an ultrafiltrate of the haemo-

lymph. When this latter figure is substituted in the Nernst equation (Table 4, column V), the calculated potential becomes highly significant, suggesting excretion of calcium as a major process of ion regulation.

The reno-pericardial system in ion regulation

The composition of pericardial fluid, with the exception of the concentration of potassium, is compatible with the theory that it is produced by ultrafiltration of the haemolymph, as has been shown for other aquatic molluscs (e.g. Harrison, 1965; Harrison & Martin, 1965; Little, 1965*a*). This theory is supported by the fact that concentrations of inulin are virtually the same in haemolymph, pericardial fluid and kidney fluid. The reno-pericardial canal is open and cilia beat towards the renal end of the canal, so that a flow of fluid into the kidney is probable. The kidney itself consists of two organs projecting into one cavity, and the reno-pericardial canal opens near to the renal pore (for anatomy see Little, 1965*b*); so it is uncertain whether the fluid sampled is 'final' urine, fluid recently entered from the pericardium, or a secretion of the kidney.

In *Pecten*, Robertson (1949) found that the concentrations of potassium and sulphate in kidney fluid were essentially the same as in the haemolymph. If the fluid that has been collected from *Strombus* can be considered 'final' urine, the kidney is excreting relatively more calcium and potassium than other ions. The source of potassium appears to be within the pericardium (Table 7), that of calcium within the kidney. Any reabsorption of ions within the kidney is most likely to occur in the nephridial gland, since this organ consists of many complex lamellae resembling the reabsorptive kidneys of the fresh-water gastropods *Viviparus* (Little, 1965*a*) and *Pomacea* (Little, unpublished observations). Strunk (1935), working with the neogastropod *Buccinum undatum*, came to the conclusion that the kidney-tissue proper excretes dyes and uric acid, while the nephridial gland reabsorbs glucose and albumin.

The apparent differential loss of ions via the kidney is small, due to the relatively slight differences in concentration of ions between haemolymph and kidney fluid, and the low rate of flow of urine. The range for this is 2·4–4·5 ml./kg./hr., as compared to a range found by Harrison (1965) for *Haliotis* of 6–21 ml./kg./hr., and an average value for *Octopus* of 2·6 ml./kg./hr. (Harrison & Martin, 1965). In *Strombus* a volume equivalent to the blood volume is excreted in a period of about 9 days, whereas the potassium in the haemolymph is excreted in 7 days, and the soluble fraction of calcium in 6 days.

The replacement of water and ions lost via the urine in animals that are iso-osmotic with sea water has been discussed by Shaw (1961) for *Carcinus* and by Robertson (1949) for molluscs. No evidence can be added here except that in a large number of feeding and starving animals that were dissected no definite fluid was found in the oesophagus of any individual; suggesting that water is probably not swallowed as it is in marine teleosts (Smith, 1930).

The digestive system in ionic regulation

Some stomach fluid is lost through the rectum, although it is not certain whether it is much altered in composition apart from being mixed with sea water. The rate of loss is very low compared to the rate of loss of urine: 0·10–0·16 ml./kg./hr. In comparison, Potts & Todd (1965) found the rate of loss of rectal fluid from starved *Octopus*

to be 5–10 ml./hr., which becomes 0·5–1·0 ml./kg./hr. if the animals are assumed to be about 10 kg. (as, for example, in Potts, 1965). The ionic composition of rectal fluid from *Octopus* differs from the stomach fluid of *Strombus* in that it contains much sulphate. In *Strombus* the sulphate appears to be added only when sea water flushes out the rectum.

The mechanism of formation of stomach fluid is difficult to assess because of the slow rate at which this fluid is lost and replaced. If, for example, the volume of fluid is 10 ml. (an average volume obtained by opening the stomach), and the rate of loss is as high as 0·16 ml./kg./hr. (see Table 11), it would take 250 hr. for all the fluid to be eliminated from the stomach. During this time the concentration of inulin injected into the haemolymph will have changed enormously (e.g. Fig. 2) and inulin ratios become impossible to estimate. The flow of inulin from stomach fluid into haemolymph appears to be much slower than that in the opposite direction (Figs. 2, 3), even allowing for the great differences in volume between haemolymph and stomach fluid. This suggests that it is not primarily moving by diffusion (for discussion see, for example, Ramsay, 1958). The possibility of filtration is suggested by the similar concentrations of sodium, potassium, magnesium and chloride in haemolymph and stomach fluid; but there appears no obvious source of a high hydrostatic pressure to cause such filtration. The low concentration of sulphate in stomach fluid is presumably due to active reabsorption; while the high concentration of calcium and low concentration of bicarbonate have been tentatively linked with the low pH. The high percentage of organic material must be produced by secretion, but the mechanism of formation of the bulk of stomach fluid has not been explained.

Mucus

The isolated sample of mucus that has been analysed showed a high concentration of sulphate; which is to be expected since the mucus of other marine gastropods contains sulphated muco-polysaccharides (Hunt & Jevons, 1966; Stacey & Barker, 1962). However, there may be differences between molluscan groups, and as noted by Machin (1964) for *Helix aspersa* there is often more than one type of mucus secreted. In *Strombus* there are certainly two types: the white mucus of the hypobranchial gland, and a clear mucus on the body surface.

It is tempting to suggest that mucus secretion may be concerned in producing the low concentration of sulphate in the haemolymph; but this process cannot be directly linked with the idea of an active transport of sulphate, since in the secretion of sulphated polysaccharides the sulphate is covalently bound to the polysaccharide, and is not passed out in ionic form.

SUMMARY

1. The concentrations of sodium, potassium, calcium, magnesium, bicarbonate, chloride and sulphate in the haemolymph of *Strombus* have been measured. Greatest differences between concentrations in haemolymph and sea water were found for bicarbonate and sulphate.

2. Haemolymph has been compared with haemolymph dialysed against sea water, and the comparison suggests that sodium, calcium, magnesium and chloride are in passive equilibrium; potassium is actively absorbed, and sulphate is actively eliminated.

3. Nernst potentials have been calculated for the measured concentration ratios of ions in haemolymph and sea water, and have been compared with the potential difference measured between haemolymph and sea water *in vivo*. These observations show that sodium and magnesium are in passive equilibrium; chloride is taken up actively (significant at the 5% level of probability) and potassium may be taken up actively (significant at the 10% level); calcium and sulphate are actively eliminated (significant at the 5% level). The differences between these conclusions and those derived from dialysis experiments are discussed.

4. Analyses have been made of the composition of pericardial and kidney fluid, and the rate of loss of urine has been measured. It is concluded that pericardial fluid is produced by ultrafiltration from the haemolymph; the mean rate of production of urine is low, at 11·5% of the blood volume per day (3 ml./kg./hr.), but small differences of concentration between haemolymph and sea water indicate that calcium and potassium must be lost at a relatively faster rate than other ions.

5. Analyses have been made of the composition of stomach fluid. This contains almost no sulphate or bicarbonate, but the concentrations of other ions are similar to those of haemolymph, except for calcium, which is probably derived from calcareous sand grains. The basic mechanism of formation of stomach fluid has not been explained. The rate of loss of stomach fluid to the exterior is very slow (0·10–0·16 ml./kg./hr.), but this fluid is rinsed out of the rectum by sea water drawn in from the outside.

6. An analysis of one sample of mucus from the mantle cavity suggests that mucus glands may be responsible for the low concentration of sulphate in the haemolymph.

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