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AESTIVATION AND IONIC REGULATION IN TWO SPECIES OF *POMACEA* (GASTROPODA, PROSOBRANCHIA)*

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Aestivation and hibernation by terrestrial pulmonate gastropods appear to be commonly occurring processes, and have been investigated from various aspects (e.g. Duval, 1930; Fischer, 1931; Arvanitaki & Cardot, 1932; Wells, 1944; Vorwohl, 1961). The resistance to desiccation of freshwater pulmonates has also been examined (von Brand, McMahon & Nolan, 1957; Klekowski, 1963). Similar phenomena in the prosobranch gastropods have received very little attention, at least partly because the terrestrial prosobranchs are common only in tropical areas. One family of prosobranchs, the Ampullariidae, is amphibious, and this family has representatives such as *Pila* in India, and *Pomacea* in South America; some short papers have been published on *Pila globosa* and *P. virens*. Prashad (1928) documents some of the ecology, and Meenakshi (1964) gives some information about the factors involved in the start and cessation of aestivation. During the aestivation period, which normally lasts about 6 months, metabolism is apparently anaerobic, and lactic acid accumulates in the tissues (Meenakshi, 1956). Saxena (1955) examined the function of the kidney during aestivation, and concluded that the anterior chamber of the kidney was responsible for storing uric acid, while the posterior chamber stored relatively little; uric acid from both chambers was slowly excreted when the animals were returned to water.

The present paper considers two American members of the Ampullariidae, *Pomacea lineata* (Spix) from Brazil, and *P. depressa* (Say) from Florida, U.S.A. Aestivation has been investigated from the point of view of the changing composition of the blood, and the function of the kidney in regulating this composition. These factors have been examined before aestivation, during its onset, and through the aestivation period up to the time of re-hydration. Some comparisons are made with *Viviparus viviparus*, an entirely freshwater form in the same superfamily, the Architaenioglossa.

MATERIAL AND METHODS

Material

Specimens of *Pomacea lineata* (Spix) were collected from the reservoir at São José do Rio Prêto, in the State of São Paulo, Brazil. The water temperature was about 25° C., the probable annual range being from 20 to 25° C. The water was either still or very slowly flowing, and contained much *Eichornia*, on which the *Pomacea* were found.

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P. depressa (Say) were collected in pools and canals near the Everglades, in southern Florida, U.S.A. *P. lineata* were maintained in the Departamento de Fisiologia, Universidade de São Paulo, in aerated tap water, and were fed on lettuce leaves. They survived well when the temperature was kept at 20° C or higher. Some specimens were transported to the Institute of Marine Sciences, Miami, Florida, where they were kept in aerated water from the Tamiami Canal. *P. depressa* were kept under the same conditions at Miami.

Sampling methods

Samples of haemolymph (from the efferent branchial vein), pericardial fluid, and fluid from the posterior chamber of the kidney were taken by making a hole in the shell over the posterior part of the mantle cavity, and inserting fine glass pipettes into the appropriate chambers. Samples of the order of 200–300 μl. were transferred to Polythene centrifuge tubes, and cells and debris were spun down. Samples from the anterior chamber of the kidney were more difficult to obtain, since this part is covered with a thick layer of black pigment, is largely filled with lamellae, and is strongly contractile when touched. Small samples were obtained by the use of glass micropipettes partially filled with mineral oil, which were inserted between the lamellae after the pigment layer had been removed with filter paper. Larger samples were obtained by scraping off the pigment layer, patching the hole in the shell with paraffin wax, and sampling later, when the kidney had relaxed.

For estimation of uric acid in the tissues, the appropriate parts were dissected out under a binocular microscope, washed for 10 sec. in iso-osmotic Ringer solution, carefully blotted dry, and then dried to constant weight at 104° C. Uric acid was extracted as described below.

Analytical methods

Osmotic pressure was measured with a Fiske osmometer having an attachment needing about 200 μl. of fluid, in São Paulo. In Miami, the method of Ramsay & Brown (1955) was used with samples of the order of 10^{-3} μl.

For sodium and potassium, a Beckman DU spectrophotometer with flame attachment and photomultiplier was used. 10 μl. of fluid was used for sodium (dilution 1:500), and 50 μl. for potassium (dilution 1:100).

To measure calcium concentrations, an EDTA titration with calcein as indicator and cyanide to block interference from other divalent cations was employed. This is a Beckman/Spinco micro-adaptation of the method of Diehl & Ellingboe (1956).

Chloride was estimated by the method of Ramsay, Brown & Croghan (1955), using a volume of approximately 1 μl.

Total protein was determined by the Beckman/Spinco micro-adaptation of the methods of Kingsley (1939) and Gornall, Bardawill & David (1949). The colour developed by a modified biuret reagent was measured at 450 mμ, using a Beckman model 151 spectrocolorimeter in São Paulo, and 50 μl. cells in a Beckman DU spectrophotometer in Miami.

Uric acid in body fluids was determined by adding sodium carbonate and dilute phosphotungstic acid to a tungstic acid filtrate, and measuring the absorbance of the resulting blue colour at 650 mμ. This is a Beckman/Spinco micro-adaptation of the method of Caraway (1955). Determinations were carried out on centrifuged samples;

but samples thoroughly stirred to suspend particulate matter gave only very slightly higher readings.

For the determination of uric acid in tissues, the tissues were first homogenized in 0·5 % lithium carbonate, and centrifuged. The supernatant was analysed as for body fluids, and the precipitate was extracted repeatedly until no further uric acid was detected in the supernatant. The lung and the anterior chamber of the kidney showed no interfering pigments, but the posterior chamber of the kidney contained a yellow pigment which in some cases may have produced a reading which was slightly too high.

Urea was measured by the Beckman/Spinco micro-adaptation of the method of Fearon (1939) and Friedman (1953). In this method a protein-free filtrate is heated with diacetyl monoxime and the colour is developed with arsenic-sulphuric acid, which also oxidizes the interfering hydroxylamine that forms. Absorbance is read at 475 mμ.

To obtain the wet weight of snails individuals were removed from water, dried with tissue paper, and the mantle cavity was allowed to drain for 5 min. They were then weighed, on a Mettler balance in São Paulo, and on a Voland balance in Miami. The shell and operculum were then removed, dried to constant weight, and their combined weight was subtracted from the total weight.

Portions of tissue were weighed to 0·1 mg. on a Voland Analytical Balance.

RESULTS

(1) *Snails living in fresh water*

Composition of haemolymph and of fresh waters

The composition of the natural fresh waters involved, and that of water in the aquaria, is given in Table 1. The aquarium water was usually changed every few days, but nevertheless varied a good deal in composition.

The composition of haemolymph from *Pomacea depressa* and *P. lineata* is given in Table 2. All animals had been in fresh water for at least 30 days, and were feeding.

Table 1. *Composition of fresh waters and aquarium waters*

	Na (mM/l.)	K (mM/l.)	Ca (mM/l.)	Cl (mM/l.)
Reservoir, Rio Prêto, São José, Brazil	0·17	0·11	0·22	1·0
Aquarium water, São Paulo, Brazil	0·08–0·45	0·03–1·25	0·06–0·17	1·0–3·0
Aquarium water, Miami, Florida (from the Tamiami Canal)	1·50–1·53	0 03–0·48	1·01–1·80	< 1·0–3·0

Although the osmotic pressure (O.P.) of haemolymph from *P. depressa* is higher than the O.P. of that from *P. lineata*, the proportions of ions are similar in both cases; except that the concentration of chloride is relatively higher in *P. depressa* (74 % of O.P.) than in *P. lineata* (63 % of O.P.). The analyses of urea and uric acid show great variations as can be seen from the large S.E. Many samples gave zero readings for both substances.

The composition of both haemolymphs is very similar to that of the Indian *Pila globosa* (Saxena, 1957), although the concentration of potassium is only half that found in *Pila*. This is unlikely to reflect dietary effects, since both forms are vegetation

feeders. Although the O.P. of the haemolymph is higher than that of the related *Viviparus*, the general features of its composition are similar (Little, 1965*a*).

The reno-pericardial system

The anatomy of the reno-pericardial system of *Pila* has been described by Prashad (1928), and that of *Pomacea*, which is basically very similar, has recently been very carefully examined by Andrews (1965). It is more complicated than that of the Viviparidae, because the kidney consists of two parts. A view of the surface of the body in the region of the kidney is shown in Fig. 1. The pericardium connects with the cavity of the posterior chamber of the kidney by the reno-pericardial canal, the opening of

Table 2. *Composition of body fluids from* Pomacea depressa *and* P. lineata *in fresh water*

	O.P. as NaCl (mM/l. ±S.E.)	Na (mM/l. ±S.E.)	K (mM/l. ±S.E.)	Ca (mM/l. ±S.E.)	HCO_3 (mM/l. ±S.E.)	Cl (mM/l. ±S.E.)	Protein N (mgN/ 100 ml. ±S.E.)	Uric acid N (mgN/ 100 ml. ±S.E.)	Urea N (mgN/ 100 ml. ±S.E.)
P. depressa									
Haemolymph	69·7	55·7	3·0	6·6	19·0	52·0	—	0·04	—
	±1·5	±1·8	±0·2	±0·4	—	±2·0	—	±0·04	—
	(6)	(6)	(6)	(6)	(2)	(6)	—	(6)	—
P. lineata									
Haemolymph	64·7	49·8	2·4	7·2	23·4	41·3	247	0·09	0·07
	±1·7	±1·3	±0·1	±0·5	±1·7	±1·8	±26	±0·03	±0·03
	(7)	(7)	(7)	(7)	(7)	(7)	(7)	(7)	(6)
Pericardial fluid	62·5	50·1	2·5	5·5	—	45·0	53	0·03	—
	±1·9	±1·2	—	±1·2	—	—	—	—	—
	(4)	(4)	(2)	(4)	—	(3)	(3)	(1)	—
Fluid from posterior chamber of kidney	61·0	49·4	2·1	5·0	24·3	40·4	37	0·18	0·05
	±2·0	±1·4	±0·1	±0·4	±2·6	±2·0	±9	±0·05	±0·03
	(7)	(7)	(7)	(7)	(4)	(7)	(7)	(6)	(5)
Fluid from anterior chamber of kidney (left side)	50·5	46·0	1·7	2·9	—	26·8	30	0·20	—
	±3·6	—	—	—	—	—	—	—	—
	(4)	(3)	(3)	(3)	—	(3)	(1)	(2)	—
Fluid from anterior chamber of kidney (right side)	26·0	25·1	1·2	2·0	—	17·5	12	0·05	0·30
	±5·6	±3·5	—	±0·4	—	±4·2	—	—	—
	(5)	(4)	(2)	(4)	—	(6)	(2)	(2)	(2)
'Final urine'	15·1	—	—	2·4	—	10·1	—	—	—
	±1·8	—	—	±0·1	—	±1·0	—	—	—
	(4)	—	—	(5)	—	(15)	—	—	—

Figures in parentheses denote the number of observations.

which into the posterior chamber is very close to the canal connecting posterior and anterior chambers; this latter canal opens into the anterior chamber near its left side. The posterior chamber is a large sac with the tissue forming a close-knit roof. The anterior chamber is smaller, and is almost filled by lamellae. It opens into the back of the mantle cavity through a pore near its right side, at the posterior end of a deep slit.

Table 2 gives the composition of fluids taken from the pericardium, and from the posterior and anterior chambers of the kidney of *P. lineata*. The last column, 'final

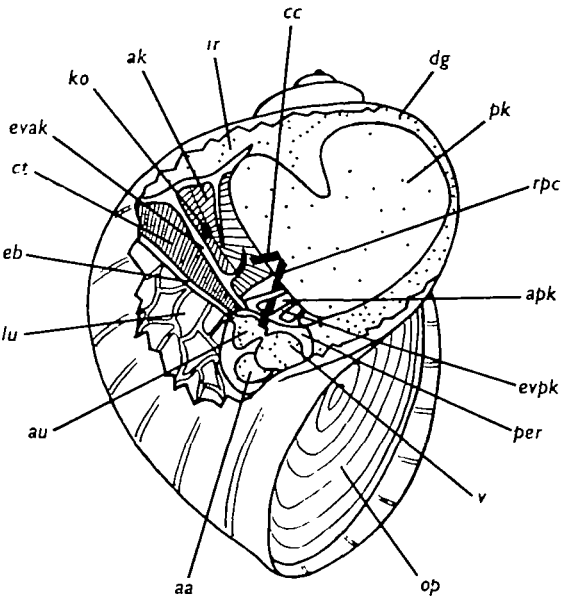

Fig. 1. Diagrammatic surface view of some of the organs of *Pomacea* which are exposed when the shell is partially removed. The sites of canals connecting the pericardium and the posterior chamber of the kidney, and the posterior and anterior chambers, as well as the kidney opening, are shown in black as if they were visible through the tissues. *aa*, Aortic ampulla; *ak*, anterior chamber of the kidney; *apk*, transparent surface of the anterior part of the posterior chamber of the kidney; *au*, auricle; *cc*, site of canal connecting posterior and anterior chambers of the kidney; *ct*, ctenidium; *dg*, digestive gland; *eb*, efferent branchial vein; *evak*, efferent vein of anterior chamber of kidney; *evpk*, efferent vein of posterior chamber of kidney; *ir*, site of intestine and rectum; *ko*, site of kidney opening into mantle cavity; *lu*, lung; *op*, operculum; *per*, pericardium; *pk*, dorsal surface of posterior chamber of kidney; *rpc*, site of reno-pericardial canal; *v*, ventricle.

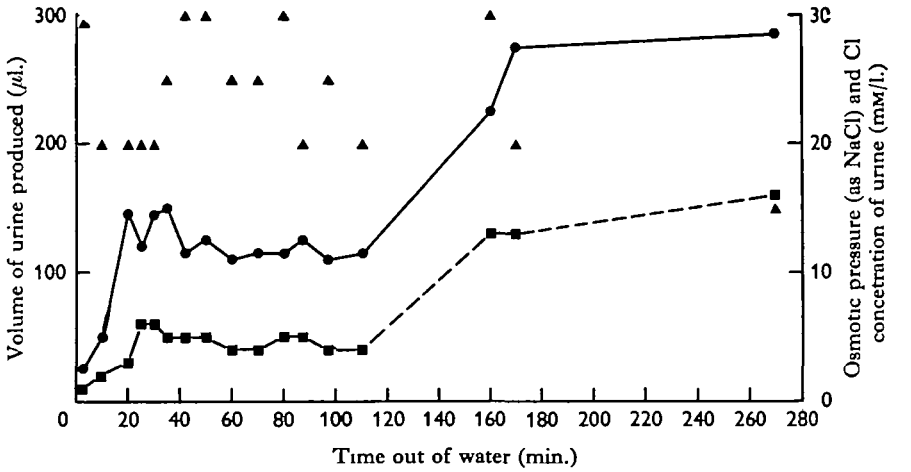

Fig. 2. Flow of fluid from the mantle cavity of a snail taken out of water (*P. lineata*). ●, Osmotic pressure of fluid; ■, chloride concentration of fluid; ▲, volume of individual pulses of fluid.

urine', requires some explanation. It proved impossible to sample fluid from the kidney pore, because of its inaccessible position. However, when snails were taken out of water, and allowed to crawl on a dry surface, a flow of liquid came, in pulses, from the mantle cavity. An example of the characteristics of this fluid is given in Fig. 2. Since the O.P. and the concentrations of chloride and calcium formed a plateau for some time, before rising as the flow began to diminish, it was assumed that this 'plateau' fluid was normal urine; although it is of course possible that this urine is in fact modified from that produced while the animal is in water.

It is apparent that if excretory fluid passes through the reno-pericardial system in the order suggested in Table 2, some of the processes that modify its composition can be localized in various organs. Protein is removed in the passage from haemolymph to pericardium, and with the similarity in ionic composition of the two fluids, this suggests that pericardial fluid is formed by ultrafiltration. The posterior chamber of the kidney appears to add uric acid to the fluid coming from the pericardium; it may absorb some potassium, but otherwise the composition of its fluid is similar to pericardial fluid. In contrast, the anterior chamber of the kidney reabsorbs ions as fluid moves into it and across from the left to the right side, producing a hypo-osmotic urine; but it does not appear to add uric acid to the urine. Indeed, the few figures available suggest that the concentration of uric acid decreases from left to right, while that of urea increases; but lack of enough samples must preclude conclusions from these results.

Table 3. *The rate of urine production of* Pomacea lineata (*i.e. the rate of production during the 'plateau' period, out of water*)

Snail	Wet weight of tissues (g.)	Rate of urine flow (μl./g./min.)
g	27	1·11
h	28	0·50
q	29	1·14
o	28	1·07
Mean ± S.E.		0·96 ± 0 15.

Final urine is somewhat more dilute than fluid taken from the anterior chamber of the kidney, which is not surprising since samples were obtained dorsally, and this fluid must pass still more kidney lamellae before reaching the kidney opening.

The site of reabsorption of calcium is difficult to locate. Some calcium does not pass into the pericardium, presumably because it is bound to protein. The fluid in the posterior chamber of the kidney appears to have the same concentration of calcium as pericardial fluid, but fluid in the left side of the anterior chamber has a considerably lower concentration. No further reabsorption of calcium occurs in the anterior chamber, although sodium, potassium and chloride are further reabsorbed.

An approximation of the rate of production of urine by *P. lineata* has been made by measuring the volume of the fluid produced by animals out of water during the 'plateau' period. Figures are given in Table 3. The rate of production of this fluid is similar to the rate of urine production in *Viviparus* (Little, 1965*b*).

(2) *The onset of aestivation*

When specimens of *Pomacea* are removed from water and left on a dry surface, there is a period of up to about 15 hr. during which they may crawl about, some almost constantly, some sporadically. Urine is produced copiously up to about 4 hr., and more, infrequently up to about 2 days. Up to about 4 days, and occasionally up to 8 days, the

Table 4. *Weight loss during the onset of aestivation* (Pomacea lineata)

Initial wet weight of tissues (g.)	Rate of weight loss as % initial wet weight/hr.				
	0–4 hr.	4–15 hr.	15–50 hr.	50–100 hr.	250–1250 hr.
23·55	2·25 (0·92)	0·32	0·088	0·006	0·006
30·20	2 20 (1·08)	0 47	0 034	0 008	0·007
19·02	2·40 (1 51)	0 27	0·154	0·044	0·016
22 20	2·32 (0·90)	0 75	0·106	0·058	0·008
26 37	1·13 (0 43)	1 05	0·163	0·018	0·006
	Mean 2 06 (0·97)	0·57	0·109	0·027	0·009
	±S.E. ±0 23 (0.17)	±0·15	±0·020	±0 010	±0 002
		Actual weight loss as % initial wet weight.			
	Mean 8·2	6·3	4·2	1·3	8·4
	±S.E. ±0·9	±1 6	±1·1	±0·5	±1·9

Figures in parentheses denote water lost as urine.

Table 5. *Weight loss up to the time when a constant rate of weight loss begins* (Pomacea lineata)

Initial wet weight of tissues (IW) (g.)	Wet weight of tissues at start of constant rate of weight loss (CW) (g.)	Time until CW (days)	Weight loss at CW as % of IW (%)
32·68	25 23	8	22·78
21·84	17·54	4	19·69
24·11	21·55	1	10·64
36·29	31 86	2	12·19
29·88	23·67	2	20·79
32·00	27·92	2	12·76
25·54	22·25	6	13·28
24 70	20·00	5	19·02
25·06	20·60	5	17·80
24 34	19·92	5	18·18
29·43	22·98	4	21·94
27·19	21 80	5	19·85
26·20	20 04	6	23·52
19·02	15·03	6	26·21
22·20	16·30	6	26·59
27·37	19·84	6	22·26
			Mean ± S.E. 19.22 ± 1·24

operculum closes loosely, allowing loss of water by evaporation; but by the eighth day the operculum is firmly closed, and the rate of water loss assumes a low and constant rate. This is the state known as aestivation. The rate of water loss during these periods was measured in five specimens of *P. lineata*, and the results are shown in Table 4.

In the initial 4 hr., loss in the urine represents about 50% of the water lost; the other 50% being lost in mucus and by evaporation.

From Table 4 it can be seen that at the end of 4 days about 20% of the initial wet weight has been lost. Since it seemed possible that the loss of a certain percentage of body water might act as a trigger for the onset of aestivation, the weight loss up to the time when the rate of weight loss becomes constant (i.e. the time of onset of true aestivation) was measured for a series of animals (Table 5). The percentage weight loss has a mean of 19·22%, ranging from 10·64 to 26·59%. Low results may possibly be linked with short times before aestivation, but do not appear to be correlated with the size of the animal. Certainly the percentage weight loss before aestivation is a more constant factor than the time involved.

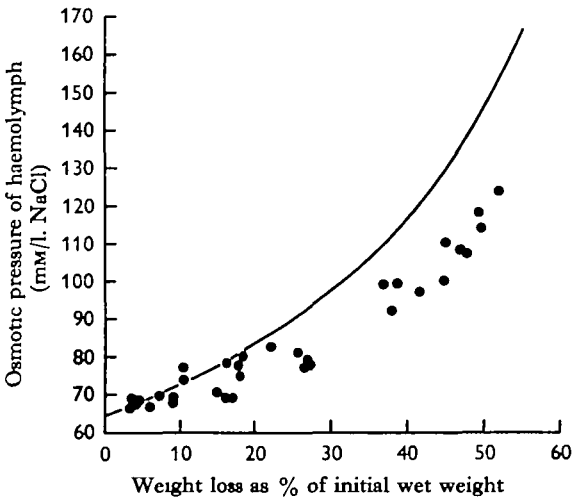

Fig. 3. The relationship of water loss to the calculated and observed osmotic pressures of the haemolymph. The curve represents the theoretical rise in osmotic pressure, based on an initial osmotic pressure of 64·7 mM/l. NaCl, and an initial water content of 89·82%. The points represent individual observations on *P. lineata*.

(3) *Aestivation*

Survival, weight loss and changes in osmotic pressure

The slow decrease in weight which occurs after the operculum has been finally closed continues at a relatively constant rate until death, or until the animal comes into contact with water. A few individuals of *P. lineata* died after 3 days, but this appeared to be due to rupture of blood vessels following very violent withdrawal into the shell. Occasional *P. lineata* died at 30 and 100 days, but the majority lived for over 100 days, and some lived for over 400 days. Typical rates of loss of weight during this time are shown in the last column of Table 4. The mortality rate of *P. depressa* was considerably higher than that of *P. lineata*, probably because the operculum very seldom exactly fitted the mouth of the shell.

If the weight loss reflected solely evaporation of water, and if the intracellular fluids were iso-osmotic with the haemolymph, the osmotic pressure of the latter would rise in proportion to the percentage loss of body water. The theoretical rise in O.P. is

shown in Fig. 3; the curve starts from a basal O.P. equivalent to the mean found in *Pomacea* in fresh water (from Table 2), and is calculated on the basis of a water content for *P. lineata* of 89·82 %. Measurements showed 89·82 % ± S.E. 1·09 (7 animals). The measured changes in O.P. coincide well with this curve up to a 10 % weight loss, suggesting that the loss of urine does not remove a significant fraction of the ionic constituents; while above a 20 % weight loss (i.e. during aestivation proper) the measured points fall well below the theoretical curve. At 50 % weight loss measured O.P.S are 30 mM/l. NaCl below the curve. Possible explanations could be that some small osmotically active particles are removed from solution and combined into bigger molecules, or that a large amount of metabolic water is produced.

Table 6. *Constituents of the haemolymph of* Pomacea lineata *in fresh water and aestivating* Pomacea lineata

(*a*) Protein and uric acid

	Protein	Uric acid
mg. N/100 ml., in fresh water, ±S.D. (no. of observations)	247 ± 69 (7)	0·09 ± 0·11 (7)
mg. N/100 ml., aestivating, ±S.D. (no. of observations)	267 ± 84 (9)	0·35 ± 0·22 (10)
mg. N/100 ml., calculated for aestivating state from 49·11 % loss of body water, ±S.D. (no. of observations)	486 ± 135 (7)	0·18 ± 0·42 (7)
Significance of difference between observed and calculated values for aestivating state		
t	3 726	1·025
n	14	15
P	0·01–0·001	0·4–0·3

(*b*) Ions

	Na (mM/l.)	K (mM/l.)	Ca (mM/l.)	Cl (mM/l.)	HCO_3 (mM/l.)
In fresh water (if O.P. were 100 mM/l. NaCl) ±S.D. (no. of observations)	76·99 ± 3·57 (7)	3·74 ± 0·52 (7)	11·05 ± 1·64 (7)	63·97 ± 7·47 (7)	35·77 ± 4 88 (7)
Aestivating (if O.P. were 100 mM/l. NaCl) ±S.D. (no. of observations)	80·42 ± 2·72 (10)	4·05 ± 0·76 (10)	10·78 ± 2·34 (10)	71·44 ± 3·72 (10)	25·64 ± 4 69 (5)
Significance of difference between snails in freshwater, and aestivating snails					
t	2·140	1·78	0·248	2·553	5 181
n	15	15	15	15	10
P	0 05–0·02	0·10–0·05	0·9–0 8	0·05–0·02	< 0 001

Changes in composition of the haemolymph

The concentrations of protein and of uric acid in the haemolymph of snails living in fresh water and of those that have aestivated long enough to have lost a mean 49·11 % of their original body water are compared in Table 6*a*. Theoretical values for aestivating snails were calculated assuming a directly proportional relationship between rise in concentration and percentage water loss. When theoretical and observed results are compared, the observed concentration of protein is found to be significantly lower than the predicted concentration, so that the level of protein is in some degree 'regulated'. It is, in fact, almost identical in snails in fresh water and in aestivating snails. The observed concentration of uric acid is apparently higher than the theoretical value, but is not shown to be significantly so; one reason for this may be that the variation in concentration in both series of observations is very great.

Since, during the onset of aestivation, ions are lost in the urine (and probably in the

mucus), the changes in concentration of ions during aestivation have been calculated as changes in relation to the O.P., and not as changes in absolute values. For both snails in fresh water and aestivating snails with O.P.s of 90–120 mM/l. NaCl the concentrations of ions in the haemolymph have been re-calculated as they would be if the O.P. were 100 mM/l. NaCl. These values are compared in Table 6*b*. In aestivating snails, the proportions of sodium and chloride are higher, whereas those of potassium and calcium are not significantly higher, than in snails in fresh water. The increase in concentration of chloride coincides with the fall of bicarbonate, the other major anion; but the increase in concentration of sodium during aestivation must be related to the decrease of some unmeasured cation which presumably moves into the cells.

Table 7. *Composition of body fluids from aestivating* Pomacea lineata

	O.P. as NaCl (mM/l.)	Na (mM/l.)	K (mM/l.)	Ca (mM/l.)	Cl (mM/l.)	HCO_3 (mM/l.)	Protein N (mg. N/100 ml.)	Uric acid N (mg. N/100 ml.)
Haemolymph ± S.E.	100·0	80·4	4·0	10·8	71·4	35·8	258	0·35
(no. of observations)	—	±0·9	±0·2	±0·7	±1·2	±2·1	±28	±·07
	(10)	(10)	(10)	(10)	(10)	(5)	(9)	(10)
Pericardial fluid ± S.E.	97·9	84·1	3·9	6·2	75·7	—	10	0 05
(no. of observations)	±0·2	±2·9	—	±0·2	±3·9	—	—	—
	(6)	(5)	(2)	(5)	(5)	—	(2)	(2)
Fluid from posterior	98·5	80·4	4·1	10·1	70·1	—	35	1 82
chamber of kidney ± S.E.	±0 5	±1·0	±0·2	±1·0	±1·9	—	±12	±0·80
(no. of observations)	(10)	(10)	(9)	(10)	(10)	—	(6)	(10)
Fluid from anterior	84·7	—	—	—	53·6	—	—	—
chamber of kidney ± S.E.	±2·9	—	—	—	—	—	—	—
(no. of observations)	(4)	—	—	—	(2)	—	—	—

The O.P. of the haemolymph of all snails was between 90 and 120 mM/l. NaCl. Concentrations of ions are converted by simple proportion to those that would be found if the O.P. were 100 mM/l. NaCl. Concentrations of protein and uric acid are not so altered.

Changes in the fluids of the reno-pericardial system

The composition of body fluids from aestivating snails with O.P.s of the haemolymph between 90 and 120 mM/l. NaCl (mean 105 mM/l. NaCl) is given in Table 7. Concentrations of ions have been re-calculated as they would be if the O.P. of the haemolymph were 100 mM/l. NaCl; concentrations of protein and uric acid have not been altered.

The pericardial fluid appears much the same in relation to the haemolymph as it does in snails living in fresh water, except that the concentration of calcium is low. In the posterior chamber of the kidney the concentrations of most ions are similar to those in the pericardial fluid, but here the concentration of calcium is higher, and equal to the concentration in the haemolymph. The concentration of uric acid is also high, being five times as concentrated as in haemolymph, in contrast to the situation in animals in fresh water. The volume of fluid in the posterior chamber of the kidney is much reduced, being 100–200 μl., instead of 1–2 ml. as is usual in snails in fresh water.

The composition of this fluid in the posterior chamber of the kidney may be examined a little more closely. Although the total water loss from the snails is approxi-

mately 50%, loss from the posterior chamber may be up to 90%; yet the fluid is still iso-osmotic with the haemolymph, so that salts must have moved out until equilibrium was attained. The high concentration of calcium is therefore unexpected, since much of the calcium in haemolymph is bound (Schoffeniels, 1951) and only ionic calcium would be expected to reach the lumen of the kidney. The situation of uric acid is such that, although the concentration is five times that in haemolymph, this cannot be considered an 'accumulation' when the volume change of up to × 10 is taken into account.

The anterior chamber of the kidney contains very little fluid, but this is slightly hypo-osmotic to other fluids, so that some reabsorption appears to be continued.

The accumulation of uric acid

The measurements made on *Pila* by Saxena (1955) suggested that uric acid was accumulating in aestivation as a major end-product of nitrogen metabolism. Saxena found that the anterior chamber of the kidney produced relatively more uric acid than the posterior chamber. In the present study preliminary investigations showed that the anterior chamber of the kidney of *Pomacea* contained white granules around the afferent blood vessels, and that these had a high uric acid content. Since these granules were found round blood vessels in the lung and round those over the digestive gland (where one measurement showed 240 mg./g. dry weight of blood vessel), it was assumed that they were not directly associated with renal tissue; and an attempt was made to remove the major blood vessels from the anterior chamber before analysis. This attempt was never more than partly successful, although a similar proportion was probably removed each time. It seems likely that the uric acid recorded for the anterior chamber consisted mostly of these granules. In contrast, no such granules were seen in the posterior chamber of the kidney, so that all the uric acid present there may be considered to be in renal tissue.

The specimens of *P. lineata* available consisted of a number that had aestivated for 100–250 days; and some that had aestivated for about 80 days, later recovering in fresh water for 40–50 days. Snails in both these series were of similar size. For comparison, specimens of *P. depressa* living in permanent freshwater ponds and canals were used; these had almost certainly never aestivated. Estimations were made of the uric acid in both chambers of the kidney, and of that in the lung as an example of accumulation in vascularized tissue. The figures for these three tissues (Table 8) are discussed below.

Anterior chamber of the kidney. The total weight of uric acid in the anterior chamber is similar in aestivating snails and in those recovering from aestivation in fresh water. Although the concentration appears higher in aestivating snails, this could be due to a reduction in dry weight caused by reabsorption of tissue proteins. The concentration of uric acid in the anterior chamber of *P. depressa* is similar to that in recovering *P. lineata*, the lower total weight possibly reflecting a relatively smaller kidney. Uric acid may, therefore, be accumulated during aestivation, but it is excreted very slowly or not at all afterwards.

Posterior chamber of the kidney. The total weight of uric acid is reduced after recovery in fresh water, and the concentration is also decreased. Since values for *P. depressa* in fresh water are similar to those for *P. lineata* recovering in fresh water, it appears that the small quantity of uric acid accumulated in aestivation is all released when the snails return to fresh water.

Lung. A large quantity of uric acid is found in the lung of aestivating *P. lineata* and in those recovering from aestivation. As in the anterior chamber of the kidney, the concentration appears higher after aestivation, but this may merely reflect tissue reabsorption. The values for *P. depressa* that have never aestivated are strikingly lower: less uric acid is present, in lower concentrations. These observations suggest that uric acid in the lung is in a similar state to that in the anterior chamber of the kidney, being accumulated during aestivation, but being only slowly or never ex creted afterwards.

Table 8. *Uric acid in the tissues of the kidney and the lung*

	Pomacea lineata					
	After 100–250 days of aestivation		In fresh water 40–50 days, after previously aestivating 80 days		*P. depressa* In freshwater, collected from permanent ponds	
	Total wt. uric acid (mg.)	Concentration (mg./g. dry wt.)	Total wt. uric acid (mg.)	Concentration (mg./g. dry wt.)	Total wt. uric acid (mg.)	Concentration (mg./g. dry wt.)
Anterior chamber of kidney	0·338 ±0·058 (10)	48·27 ±7·16 (10)	0·397 ±0·097 (7)	21·65 ±5·06 (7)	0·138 ±0·018 (6)	20·64 ±4·14 (6)
Posterior chamber of kidney	0·291 ±0·043 (10)	20·04 ±3·44 (10)	0·131 ±0·007 (7)	7·63 ±2·22 (7)	0·093 ±0·016 (6)	4·23 ±0·71 (6)
Lung	27·81 ±6·58 (6)	325 ±58 (6)	35·21 ±4·27 (7)	209 ±27 (7)	2·08 ±0·39 (6)	17·90 ±6·92 (6)

Figures are given as mg. or mg./g. dry wt. ± S.E. (number of observations).

In summary, it appears that uric acid accumulates to a slight degree in the anterior chamber of the kidney, and to an enormous degree in the lung and probably other well-vascularized tissues; and that it is never, or only very slowly, excreted from these tissues. In contrast, although uric acid accumulates only to a slight degree in the posterior chamber of the kidney, this uric acid is able to be excreted later, suggesting that the posterior chamber is the normal site of excretion of uric acid. This was suggested by the first comparisons of haemolymph and fluid from the posterior chamber (Table 2), and is further discussed in the next section.

(4) *Recovery from aestivation*

When an aestivating individual of *Pomacea* is placed in water, the operculum opens slightly within 30 min. The subsequent time elapsing before the snail becomes active may be less than 1 hr. if aestivation has lasted only a few days, or up to 5 hr. if the aestivation period has been long. An example of the changes in composition of body fluids during the recovery period is given in Fig. 4. The haemolymph becomes diluted to its normal O.P. in 12–24 hr., and often falls below this normal for some time, before rising to normal again after 48–72 hr. The fluid in the posterior chamber of the kidney follows these changes, staying iso-osmotic with the haemolymph, while the fluid in the

anterior chamber rapidly becomes hypo-osmotic, reaching its normal low value within 24 hr.

The excretion of uric acid in large concentrations appears as a temporary phase, not lasting longer than 24 hr. (Fig. 4*b*). There is some evidence of mobilization of uric acid in the haemolymph, but the initial high concentration in the posterior chamber of the kidney is at least partly due to the flushing out of the high concentrations formed there during aestivation (see Table 7). The concentration of uric acid in fluid from the

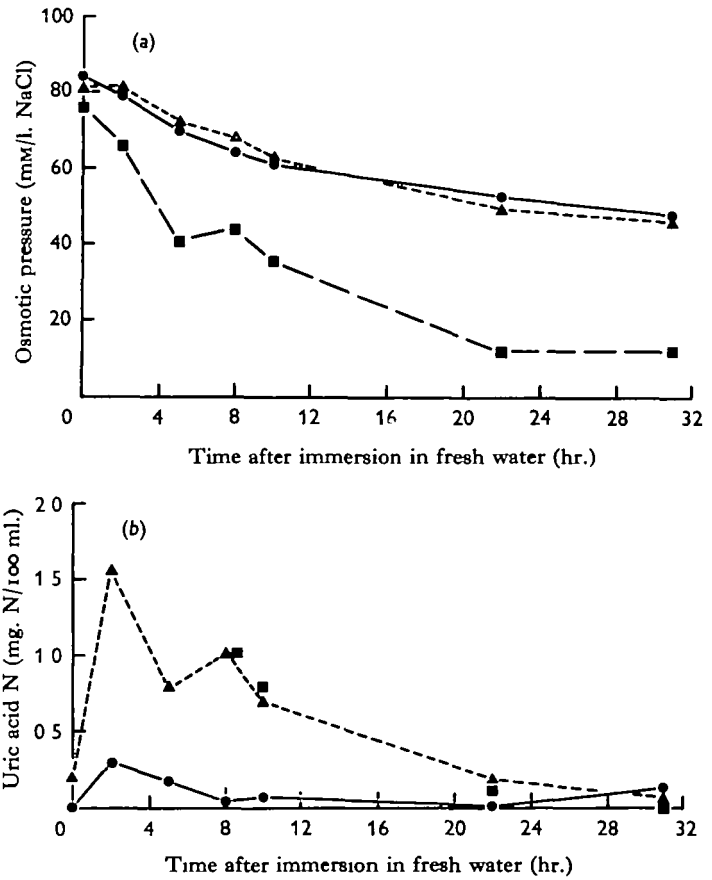

Fig. 4. The changing composition of body fluids during recovery from aestivation. (*P. lineata*) (*a*) Osmotic pressure; (*b*) uric acid. ●, Haemolymph; ■, fluid from anterior chamber of kidney; ▲, fluid from posterior chamber of kidney.

anterior chamber of the kidney is only sometimes slightly higher than that in the posterior chamber. This is probably a reflexion of the fact that fluid in the anterior chamber at any one time is derived from fluid that was in the posterior chamber some time earlier. Certainly there is no evidence that the anterior chamber passes uric acid into the fluid received from the posterior chamber.

As discussed in the previous section, the uric acid in the tissues of the posterior chamber of the kidney appears to be soon excreted, while that in other tissues is more firmly bound.

DISCUSSION

The functions and homologies of the kidney

The anatomy of the kidney of *Pomacea* has been very well described by Andrews (1965). She found that the cells of the posterior chamber contained many concretions, and showed positive tests for uric acid. Very little musculature was observed. The present examination has shown that it is this posterior chamber that excretes uric acid, passing it on to the anterior chamber. It has a large cavity which may act as an organ of water storage during aestivation, but it is not contractile and it does not reabsorb salts or otherwise perform any ionic regulation.

The anterior chamber of the kidney contains no uric acid in the cells, but these cells have a very close association with the blood spaces, and Andrews suggested that it might have an osmoregulatory function. This has now been confirmed, and indeed the reabsorption of salts produces a very dilute urine, having approximately one-quarter of the concentration of the haemolymph. The anterior chamber does not appear to add uric acid to the urine. Saxena (1955) supposed, on the contrary, that uric acid accumulated in the anterior chamber of the kidney of *Pila* during aestivation, and that uric acid only moved into the posterior chamber when the anterior one was full. The accumulation of uric acid in the anterior chamber of the kidney of *Pomacea* is in the form of granules, around the blood vessels, and this produces very great variation between individuals in the amount of uric acid found (Table 8). This great variation must cast suspicion on the conclusions of Saxena, because although he apparently showed an almost perfect decrease in the amount of uric acid in the anterior chamber, with time after the end of aestivation, he only used individual animals. The situation in *Pila* must, then, remain unsettled, but in *Pomacea* the accumulation of uric acid is greatest around the blood vessels, and especially in such vascular tissues as the lung.

The homologies of the two chambers of the kidney of Pilids have been much disputed (see Andrews, 1965, for references). On the basis of the present investigation, it is certainly the anterior chamber that most resembles the kidney of the Viviparidae: it is this part which is osmoregulatory, and it is also this part which is highly contractile and therefore is presumably the active member in moving fluid through the renal system (see Little, 1965*b*). The posterior chamber appears to be correlated with the aestivating habit, providing a water store, and a means of eliminating uric acid.

The onset of aestivation

Duval (1930) suggested that activity, in *Helix pomatia*, was related to the degree of hydration, and that water loss causing an increase of O.P. from 0·3 to 0·4° C. also caused the start of aestivation. Wells (1944) disputed the relationship between hydration and activity, but was more concerned with the termination of aestivation, which he said could only be caused by direct sensory stimulation, and not by hydration of the tissues. Meenakshi (1964) suggested, for *Pila*, that water loss activated a receptor system, which then caused aestivation. The present examination of *Pomacea* showed a relatively constant loss of water (19·22% ± S.E. 1·24) before aestivation, which is very suggestive of some kind of hormonal control. It is interesting that this loss of water in

Pomacea corresponds to an increase in O.P. of about 33 %, which is the same figure given by Duval for *Helix*.

In an earlier paper Meenakshi (1956) compared the concentrations of magnesium and calcium in the blood of *Pila* in fresh water, and of *Pila* during aestivation, showed that both calcium and magnesium rose in concentration by $\times 1{\cdot}5 - 1{\cdot}7$, and suggested that this might be a causal factor in the onset of aestivation. However, it must be noted that in *Pomacea* the calcium rise of $\times 1{\cdot}5$ found during aestivation can be explained entirely by the decrease in water content of the snail. Meenakshi mentions further experiments concerning the injection of a sterol fraction from the cerebral ganglion of aestivating animals, which causes a rise in the concentrations of calcium and magnesium, and a decrease in oxygen consumption; but she gives no details.

Physiological processes during aestivation

Meenakshi (1956) investigated the rate of respiration of *Pila*, and found no detectable uptake of oxygen during aestivation. Lactic acid accumulated in the tissues, and the glycogen reserves became depleted. In contrast, the terrestrial pulmonate *Helix pomatia* appears to respire aerobically during aestivation (Fischer, 1931), and the freshwater pulmonate *Australorbis glabratus* utilizes oxygen during desiccation (von Brand *et al.*, 1957). Interestingly, the heart of *Pomacea* is seen to beat if a hole is cut in the shell during aestivation; but it is not known whether this is due to a stimulating effect of the operation, or whether the heart beats throughout aestivation.

During aestivation the O.P. of the haemolymph of *Pomacea* may rise to twice its normal value, i.e. to about 120 mM/l. NaCl, after about a 50 % weight loss. This is some 30 mM/l. NaCl lower than predicted on the basis of an initial water content of 89·82 %, and is equivalent to the value predicted from a 40 % weight loss (Fig. 3). In this sense, then, there is 'osmoregulation' during aestivation. Corresponding figures are available for the freshwater pulmonate *Coretus corneus* during desiccation (Klekowski, 1963). At a 45 % weight loss the O.P. of the haemolymph is approximately 138 mM/l. NaCl (0·447° C.); on the basis of an initial water content of 89·80 %, and an initial O.P. of 74 mM/l. NaCl (0·256° C.), the calculated value would be 148 mM/l. NaCl. The difference is not nearly as great as in *Pomacea*; but the time involved is much shorter, being only about 46 days compared to 200 for *Pomacea*. Burton (1964) notes that the range given by various authors for the O.P. of the haemolymph of *Helix pomatia* under different conditions is from 0·21 to 0·62° C. (approx. 60–180 mM/l. NaCl.) and Meyer & Thibaudet (1937) measured losses of up to 44 % of the initial weight, but unfortunately these figures cannot be correlated. It is, however, interesting to see that Meyer & Thibaudet showed that *H. pomatia* died after similar periods of aestivation and of hibernation, although water loss was much greater during aestivation.

The accumulation of uric acid during aestivation is such that *Pila* must be considered truly uricotelic during this phase (Saxena, 1955). *Pomacea* likewise accumulates large quantities of uric acid. In this it appears to be similar to *Helix pomatia*; and even the freshwater prosobranchs *Bythinia tentaculata* and *Viviparus viviparus*, and the freshwater pulmonate *Limnaea stagnalis*, have large quantities of uric acid in the nephridium (Needham, 1935). Needham suggested that the capacity to synthesize uric acid could have arisen as an adaptation to living in temporary bodies of water,

but he preferred the suggestion that it arose during a previous phase of semi-terrestrial life. In the case of *Pomacea* it could be said that in fact the animal combines these two modes of existence.

SUMMARY

1. The ionic composition of the haemolymph, and the concentrations of uric acid and protein, have been determined for the amphibious prosobranchs *Pomacea lineata* and *P. depressa*. Ionic composition of the haemolymph is similar to that of freshwater gastropods.

2. The urine is decidedly hypo-osmotic to the haemolymph, reabsorption of ions occurring in the anterior chamber of the kidney. The rate of production of urine is approximately 1 μl./g./min. at 25° C.

3. The onset of aestivation appears to be related to a loss of 20 % of the normal wet weight of the tissues. The loss of weight during aestivation averages 0·009 % of the initial wet weight/hr., and aestivation may continue for over 400 days.

4. During aestivation the osmotic pressure of the haemolymph may rise to twice its normal value; but this is 30 mM/l. NaCl less than that predicted from weight losses. The relative composition of the haemolymph alters little, except that the percentages of sodium and chloride increase.

5. Uric acid accumulates round the blood vessels during aestivation, especially in the lung. Relatively little accumulates in the two chambers of the kidney, and only that in the posterior chamber is excreted later. Since the volume of fluid in the posterior chamber falls to about 10 % of its normal value, while the total loss of weight of the snail is about 50 %, the fluid in the posterior chamber acts as a water reserve.

6. Recovery from aestivation occurs in about 24 hr., when the snails are placed in water. The posterior chamber of the kidney excretes high concentrations of uric acid during this time.

7. The characteristics of aestivation are discussed, and compared with those shown by the Pulmonata.

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REFERENCES

ANDREWS, E. B. (1965). The functional anatomy of the mantle cavity, kidney and blood system of some pilid gastropods (Prosobranchia). *J. Zool.* **146**, 70–94.

ARVANITAKI, A. & CARDOT, H. (1932). Sur les variations de la concentration du milieu intérieur chez les mollusques terrestres. *J. Physiol. Path. gén.* **30**, 577–92.

VON BRAND, T., MCMAHON, P. & NOLAN, M. O. (1957). Physiological observations on starvation and desiccation of the snail *Australorbis glabratus*. *Biol. Bull. mar. biol. Lab., Woods Hole* **113**, 89–102.

BURTON, R. F. (1964). Variations in the volume and concentration of the blood of the snail, *Helix pomatia* L., in relation to the water content of the body. *Can. J. Zool.* **42**, 1085–97.

CARAWAY, W. T. (1955). Determination of uric acid in serum by a carbonate method. *Am. J. clin. Path.* **25**, 840–5.

DIEHL, H. & ELLINGBOE, J. (1956). Indicator for titration of calcium in presence of magnesium, using disodium dihydrogen ethylenediamine tetraacetate. *Analyt. Chem.* **28**, 882–4.

DUVAL, M. (1930). Concentration moléculaire du sang de l'escargot. Ses facteurs, ses variations, influence de l'état d'activité de l'animal. *Annls Physiol Physicochim. biol.* **6**, 346–64.

FEARON, W. R. (1939). The carbamido diacetyl reaction: a test for citrulline. *Biochem. J.* **33**, 902–7.

FISCHER, P. H. (1931). Recherches sur la vie ralentie de l'escargot (*Helix pomatia*). *J. Conch., Paris* **75**, 5–100, 112–200.

FRIEDMAN, H. W. (1953). Modification of the determination of urea by the diacetyl monoxime method. *Analyt. Chem.* **25**, 662–4.

GORNALL, A. G., BARDAWILL, C. J. & DAVID, M. M. (1949). Determination of serum proteins by means of the biuret reaction. *J. biol. Chem.* **177**, 751–66.

KINGSLEY, G. R. (1939). The determination of serum total protein, albumin, and globulin by the biuret reaction. *J. biol. Chem.* **131**, 197–200.

KLEKOWSKI, R. Z. (1963). Water balance and osmoregulation in the snail *Coretus corneus* (L.) under conditions of desiccation and in diluted seawater. *Polskie Archwm Hydrobiol.* **11**, 219–40.

LITTLE, C. (1965*a*). Osmotic and ionic regulation in the prosobranch gastropod mollusc, *Viviparus viviparus* Linn. *J. exp. Biol.* **43**, 23–37.

LITTLE, C. (1965*b*). The formation of urine by the prosobranch gastropod mollusc, *Viviparus viviparus* Linn. *J. exp. Biol.* **43**, 39–54.

MEENAKSHI, V. R. (1956). Physiology of hibernation of the apple-snail *Pila virens* (Lamarck). *Curr. Sci.* **25**, 321–2.

MEENAKSHI, V. R. (1964). Aestivation in the Indian apple snail *Pila*. I—Adaptation in natural and experimental conditions. *Comp. Biochem. Physiol.* **11**, 379–86.

MEYER, P. & THIBAUDET, M.-A. (1937). Les variations du poids pendant l'hibernation et l'estivation des Hélices. *C. r. Séanc. Soc. Biol.* **124**, 182–5.

NEEDHAM, J. (1935). Problems of nitrogen catabolism in invertebrates. II. Correlation between uricotelic metabolism and habitat in the phylum Mollusca. *Biochem. J.* **29**, 238–51.

PRASHAD, B. (1928). Anatomy of the common Indian apple-snail, *Pila globosa*. *Mem. Indian Mus.* **8**, 91–153.

RAMSAY, J. A. & BROWN, R. H. J. (1955). Simplified apparatus and procedure for freezing-point determinations upon small volumes of fluid. *J. sci. Instrum.* **32**, 372–5.

RAMSAY, J. A., BROWN, R. H. J. & CROGHAN, P. C. (1955). Electrometric titration of chloride in small volumes. *J. exp. Biol.* **32**, 822–9.

SAXENA, B. B. (1955). Physiology of excretion in the common Indian apple-snail, *Pila globosa* (Swainson). Part I. *J. Anim. Morph. Physiol.* **2**, 87–95.

SAXENA, B. B. (1957). Inorganic ions in the blood of *Pila globosa* (Swainson). *Physiol. Zoöl.* **30**, 161–4.

SCHOFFENIELS, E. (1951). Distribution du calcium diffusible et non-diffusible dans le plasma sanguin de l'Anodonte. *Archs int. Physiol.* **59**, 49–52.

VORWOHL, G. (1961). Zur Function der Excretionsorgane von *Helix pomatia* L. und *Archachatina ventricosa* Gould. *Z. vergl. Physiol.* **45**, 12–49.

WELLS, G. P. (1944). The water relations of snails and slugs. III. Factors determining activity in *Helix pomatia* L. *J. exp. Biol.* **20**, 79–87.