J. Exp. Biol. (1972), 56, 249–261
With 2 text-figures
Printed in Great Britain

THE EVOLUTION OF KIDNEY FUNCTION IN THE NERITACEA (GASTROPODA, PROSOBRANCHIA)

BY COLIN LITTLE
Department of Zoology, University of Bristol

(*Received* 30 *June* 1971)

Gastropod molluscs of the superfamily Neritacea (or order Neritacea: see Morton & Yonge, 1964) are found in marine, freshwater and terrestrial environments; but despite these wide differences in habitat the general anatomical organization is similar in all the members of the group. In particular the organization of the renal system appears to be very similar in marine and freshwater species, and to be not greatly altered in the terrestrial forms. This is surprising in a group subjected to the apparently different problems posed by life in these various environments.

The family Neritidae contains many tropical marine forms, e.g. *Nerita* spp., and freshwater species which are also found in temperate zones, e.g. the European species *Theodoxus fluviatilis*. The terrestrial forms are all tropical and form the family Helicinidae. Very little is known about this family at present, except for an anatomical account (Bourne, 1911). They inhabit damp tropical forests and are often associated with a limestone substrate, although the family as a whole is not confined to limestone areas.

The present paper investigates some of the functions of the kidneys of marine, freshwater and terrestrial species with respect to salt and water balance. Some discussion is given concerning the route by which the Helicinidae have colonized land.

MATERIAL

Marine species (family Neritidae)

Nerita fulgurans Gmelin was obtained from a rocky shore at Virginia Key, Miami, Florida, U.S.A., and was kept in running seawater in the Institute of Marine Sciences, University of Miami. The species lives typically between tidemarks, and is found in purely marine and in brackish habitats (Warmke & Abbott, 1961).

Freshwater species (family Neritidae)

Neritina latissima (Broderip) was obtained from a freshwater stream in Panama, and was kept in aerated water from a freshwater canal in Miami, Florida. This water had the following composition: Na, 1·5 mM/l; K, 0·03 mM/l; Ca, 1·0 mM/l; Cl, 2·0 mM/l.

Theodoxus fluviatilis (L.) was obtained from the River Kennet at Reading, and was kept in well-aerated Kennet water, in Bristol. The snails fed on algal-covered stones from the Kennet and later from the Bristol Avon. At the time that the snails were sampled for haemolymph, the aquarium water had the following composition: Na, 0·6 mM/l; K, 0·24 mM/l; Ca, 2·3 mM/l; Mg, 0·2 mM/l; Cl, 0·6 mM/l. The bulk of the anion fraction was presumably made up by bicarbonate.

Terrestrial species (family Helicinidae)

Eutrochatella pulchella (Gray) was collected from low jagged limestone cliffs beneath scrub forest just north-west of Rio Bueno, on the north coast of Jamaica. The rainfall in this area is given as 'under 50 inches a year' (Scientific Research Council of Jamaica, 1963). This species was also common at the site of collection of *E. tankervillei* (see below).

Eutrochatella tankervillei (Gray) was collected from jagged limestone rocks beneath scrub forest 1 mile east of Balaclava in western Jamaica. The rainfall in this area is 75–100 in. a year.

Alcadia sp. was collected from leaf litter in forest five miles east of Balaclava, western Jamaica.

All the terrestrial species were transported to the Department of Zoology, University of Bristol, England, and were kept in leaf litter with damp limestone rocks. The temperature was maintained at 25 °C.

METHODS

After a hole had been made in the shell to reveal the appropriate areas (see, for example, Little, 1965*a*, 1967), body fluids were sampled using micropipettes, and were centrifuged to remove corpuscles, etc., before analysis. For some analyses, samples from several animals were pooled. Osmotic pressure was measured by the method of Ramsay & Brown (1955). Sodium and potassium were measured, after dilution, by flame photometry; for *Nerita fulgurans*, a Beckman DU spectrophotometer with flame attachment was used, and for the other species a Unicam SP 90 atomic absorption spectrophotometer, using the emission mode.

Calcium and magnesium were determined for *Nerita* by EDTA titrations similar to those described by van Asperen & van Esch (1956) (see Little, 1967). Calcium and magnesium for the other species were measured after dilution, using a Unicam SP 90 in the absorption mode.

Chloride was determined by the potentiometric method of Ramsay, Brown & Croghan (1955). Sulphate was measured by the conductimetric method of Roach (1963), modified by the addition of known amounts of sulphate (see Little, 1967). Bicarbonate was measured by the microdiffusion method of Little & Ruston (1970).

The errors in the methods employed with *Nerita fulgurans* are similar to those calculated by Little (1967).

RESULTS

Marine species

The anatomy of various species of the Neritidae is described by Bourne (1908), and some details are given for *Nerita fulgurans* by Fretter (1965). The kidney consists of two parts: a part filled with lamellae, known as the glandular portion, into whose proximal end opens the canal from the pericardium (the reno-pericardial canal); and a non-glandular part or bladder which connects with the posterior or distal end of the glandular part by a rather wide and diffuse opening, and itself opens into the posterior part of the mantle cavity through the uropore. The rectum, which passes through the glandular and the non-glandular parts of the kidney, is covered by

renal tissue. Posterior to the kidney there is a large extension of the pericardial coelom, termed by Bourne the gonadial coelom. This extension is easily punctured, and in specimens extracted from the shell may easily be overlooked. The whole system is shown diagrammatically in Fig. 1.

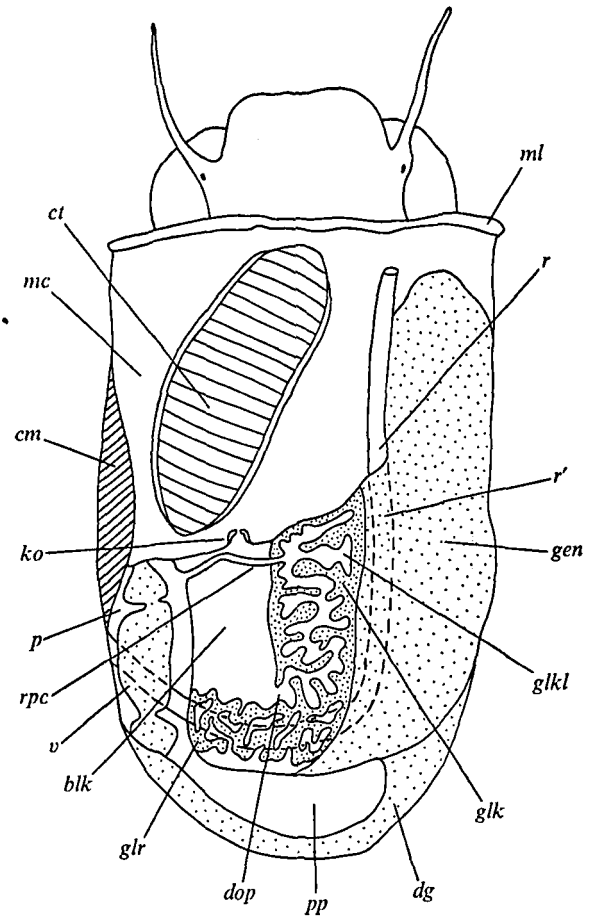

Fig. 1. Highly diagrammatic representation of the renal system in *Nerita fulgurans*, as seen in dorsal view. In life the glandular part of the kidney lies dorsal and not lateral to the bladder. *blk*, Bladder of kidney; *cm*, columellar muscle; *ct*, ctenidium; *dg*, digestive gland; *dop*, distal opening of glandular part of kidney into bladder; *gen*, genital ducts and organs; *glk*, glandular part of kidney; *glkl*, lumen of glandular part of kidney; *glr*, glandular kidney tissue covering the rectum; *ko*, kidney opening or uropore; *mc*, mantle cavity; *ml*, anterior lip of mantle; *p*, pericardium; *pp*, posterior extension of pericardium; *r*, rectum; *r'*, course of rectum through genital tissue; *rpc*, reno-pericardial canal; *v*, ventricle.

For *Nerita fulgurans* living in sea water, analyses were made of haemolymph and of fluid from the bladder of the kidney. The results of these analyses are given in Table 1, and from these it is clear that the fluid produced by the kidney does not differ in ionic composition from the haemolymph. The ionic regulation shown by the snail in sea water is in fact very slight, and presumably is brought about by other means than the production of kidney fluid.

In order to demonstrate any possible osmoregulatory capacity, specimens of *Nerita* were placed for 1 week in a series of diluted sea waters. In the lower concentrations, i.e. 15% and 25% sea water, the snails were first acclimatized by being placed for 2 days in each of the higher concentrations used. For example, the snails acclimatized to 25% sea water spent 2 days in 70%, 2 days in 50%, and then a week in 25% sea water. Measurements of the osmotic pressure of the sea water, the haemolymph and fluid from the bladder of the kidney were then made, and these are shown in Table 2. The snails do not appear to osmoregulate, and the kidney plays no part in controlling the osmotic pressure of the body fluids.

The observations on *Nerita* can be summarized by saying that it is entirely unable

Table 1. *The composition of haemolymph and urine from* Nerita fulgurans *in sea water*

	Osmotic pressure as NaCl (mM/kg water)	Na (mM/kg water)	K (mM/kg water)	Ca (mM/kg water)	Mg (mM/kg water)	Cl (mM/kg water)	SO (mM/kg water)
Sea water	579·3	484·3	11·33	11·20	55·40	554·0	28·43
(No. of observations)	(3)	(3)	(3)	(2)	(2)		(3)
Haemolymph	584·5	487·0	13·12	11·67	55·07	558·2	29·73
±S.E.	±4·1	±3·7	±0·39	±1·18	±0·92	±3·8	±1·05
(No. of observations)	(6)	(6)	(6)	(6)	(6)	(6)	(6)
Urine	581·7	490·0	12·57	11·80	55·52	553·3	28·84
±S.E.	±2·7	±5·7	±0·52	±0·45	±1·25	±3·0	±1·12
(No. of observations)	(6)	(4)	(6)	(6)	(6)	(6)	(5)
Significance of difference between values for haemolymph and urine							
t	0·874	0·465	0·845	0·229	0·314	1·01	0·721
D.F.	10	8	10	10	10	10	9
P	> 0·10	> 0·10	> 0·10	> 0·10	> 0·10	> 0·10	> 0·10

Note: the chlorinity of the sea water varied slightly between some of the sampling times. Concentrations are therefore adjusted by simple proportion to those that would be found at a concentration of chloride in sea water of 19‰ or 554 mM/kg water.

Table 2. *The osmotic pressure of haemolymph and urine from* Nerita fulgurans *in various dilutions of sea water*

	Osmotic pressure as NaCl, mM/kg water			
Medium	Medium	Haemolymph	Urine	Condition of the snails
100% SW	557	561 (3)	559 (3)	Mobile
70% SW	364	370 (3)	365 (3)	Mobile
50% SW	264	266 (3)	266 (3)	Mobile, but not very active
25% SW (direct)	136	139 (2)	139 (2)	Immobile, but attached to substrate
25% SW (acclimatized)	136	145 (3)	144 (3)	Mobile, but not very active
15% SW (acclimatized)	78	80 (2)	81 (2)	Immobile, but attached to substrate

Numbers in parentheses show the number of samples.

to osmoregulate, and that it regulates ions only to a very minor degree. The kidney plays absolutely no part in controlling the ionic or osmotic composition of the haemolymph.

Freshwater species

The renal system of freshwater neritids is remarkably similar to that of the marine forms (see Bourne, 1908), both in organization and in the gross appearance of the tissues. The excretory system of *Theodoxus* (as *Neritina*) is described by Lenssen (1902).

Table 3. *The concentration of body fluids from* Neritina latissima

	Haemolymph	Pericardial fluid	Fluid from glandular part of kidney: proximal	Fluid from glandular part of kidney: distal	Fluid from bladder	Aquarium water
O.P. as NaCl (mM/l)	49·8	49·9	49·4	35·1	31·6	4·0
±S.E.	±1·4	±1·7	±2·0	±3·2	±2·8	
(No. of observations)	(8)	(7)	(7)	(7)	(8)	
Cl mM/l	40·3	39·0	42·0	—	23·0	2·0
(No. of observations)	(2)	(3)	(1)		(3)	

Table 4. *The composition of haemolymph from* Theodoxus fluviatilis

	O.P. as NaCl (mM/l)	Na (mM/l)	K (mM/l)	Ca (mM/l)	Mg (mM/l)	Cl (mM/l)	HCO_3 (mM/l)
Mean	52·5	45·0	2·2	2·3	2·9	32·8	11·3
±S.E.	±1·6	±1·4	±0·1	±0·1	±0·2	±2·7	±0·3
(No. of observations)	(13)	(6)	(6)	(6)	(6)	(6)	(6)

In order to evaluate the osmoregulatory ability of the kidney of *Neritina latissima*, samples of fluid were taken from the visceral blood sinus, the pericardium, the anterior and posterior parts of the glandular portion of the kidney, and from the bladder. Because of the small volumes collected, measurements were in the main restricted to osmotic pressure, but a few measurements of chloride concentration were made. All these values are shown in Table 3, in which it is evident that salts are removed from the renal fluid during its passage through the glandular part of the kidney, the change in anion content being completely accounted for by the removal of chloride. This is in great contrast to the situation in the marine species, in spite of the similarity in structure at the gross level.

It was not possible to make analyses of the ionic composition of the haemolymph of *Neritina*, but later measurements were made on the English freshwater form, *Theodoxus fluviatilis*. In this case haemolymph was removed by passing a paraffin-filled pipette into the pericardium, through the ventricle and into the auricle. The beating of this latter helped to force haemolymph into the capillary. The haemolymph composition is shown in Table 4. The osmotic pressure is similar to that of *Neritina*, and the present figures compare well with those found by Neumann (1960). Neumann found a variation from 44 mM/l in starved snails to 59–62 mM/l in feeding snails. The details of the composition of the haemolymph are discussed later.

Terrestrial species

As in the Neritidae, the kidney of the Helicinidae consists of a glandular and a non-glandular part; but although the relationships of the various parts of the renal system remain unchanged, the anatomical arrangement is different because of a dorsal elongation of the body and a consequent rotation of most of the organs of the body through 90° (see Bourne, 1911). A diagram shows the approximate arrangement in *Eutrochatella pulchella* (see Fig. 2).

The only species available in quantity was *E. pulchella*. Initial study of this species showed that it was possible to sample haemolymph, pericardial fluid, fluid from the bladder, and fluid from the glandular portion of the kidney. To sample the latter it

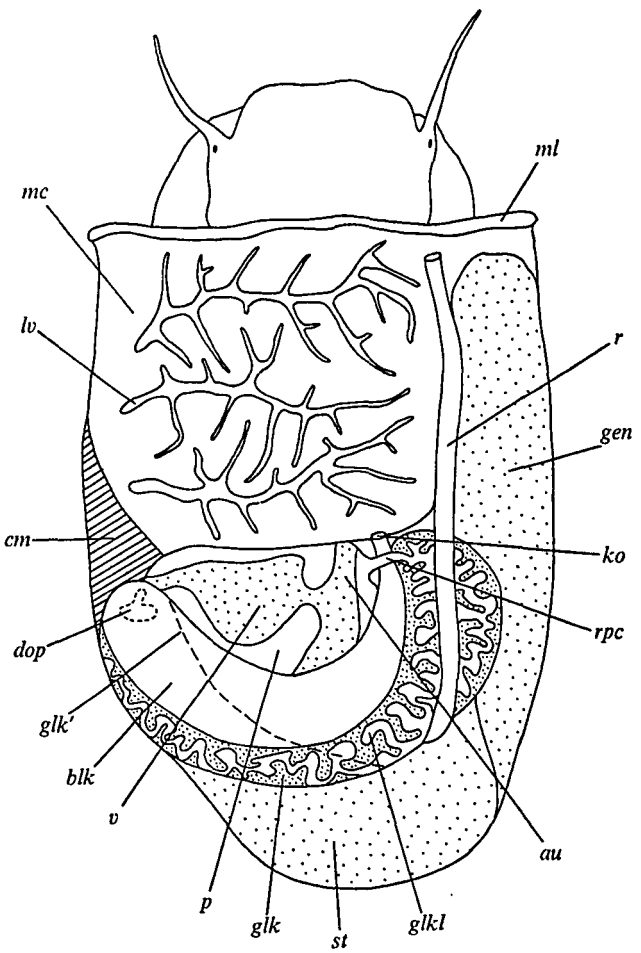

Fig. 2. Highly diagrammatic representation of the renal system in *Eutrochatella pulchella*, as seen in dorsal view. *au*, Auricle; *blk*, bladder of kidney; *cm*, columellar muscle; *dop*, distal opening of glandular part of kidney into bladder; *gen*, genital ducts and organs; *glk*, glandular part of kidney; *glk'*, outline of that region of the glandular part of the kidney which is folded underneath the bladder; *glkl*, lumen of glandular part of kidney; *ko*, kidney opening or uropore; *lv*, blood vessels in the 'lung'; *mc*, mantle cavity or 'lung'; *ml*, anterior lip of mantle; *p*, pericardium; *r*, rectum; *rpc*, reno-pericardial canal; *st*, stomach; *v*, ventricle of heart.

was necessary to pass a micropipette through the bladder, and then through the opening from the distal part of the glandular portion of the kidney. In addition to these samples, when the snails were handled, fluid was produced at the aperture of the shell, and it was assumed that this was fluid which originated in the kidney and had been passed through the mantle cavity. It would therefore represent final urine. The species in question is a small one, having a shell diameter of only approximately 7 mm, and a total weight of about 0·15 g; and it proved impossible to obtain sufficient fluid to measure more than the osmotic pressure. Such measurements as were obtained are given in Table 5.

Table 5. *The osmotic pressure of body fluids from* Eutrochatella pulchella

	Haemolymph	Pericardial fluid	Fluid from glandular part of kidney (distal)	Fluid from bladder	Fluid from shell mouth (final urine)
O.P. as NaCl (mM/l)	48·0	43·5	44·0	30·0	23·9
±S.E.	±1·6	±2·5	±2·4	±3·9	±3·3
(No. of observations)	(10)	(6)	(6)	(7)	(12)

The observations of osmotic pressure show that fluid in the bladder is much more dilute than haemolymph, and that what may be termed final urine is more dilute still, having approximately half the salt concentration of the haemolymph. The question of where this salt reabsorption occurs is difficult to answer from the results shown. It might be concluded that this reabsorption occurs in the bladder, but the one result obtained from *E. tankervillei* given below (see Table 6) suggests that it occurs in the glandular portion of the kidney. One explanation of this apparent difference is that the glandular part of the kidney is highly contractile, and that since it took longer to sample fluid from *E. pulchella* than from *E. tankervillei*, most of the dilute urine was expressed from the glandular part of the kidney of the former before fluid from that part was sampled. This would lead to the sampling of fluid which had only just entered the kidney, and which would be expected to be equal to the pericardial fluid in concentration. However, the matter cannot be regarded as settled.

The general trend of the results for *E. pulchella* was confirmed by a few observations on *Alcadia* sp. For three specimens, mean osmotic pressures were as follows: haemolymph, 43·0 mM/l; fluid from the bladder, 36·0 mM/l; and fluid from the shell mouth, 17·5 mM/l.

Study of the larger form, *E. tankervillei* (shell diameter approximately 1·8 cm), was limited by the small number of specimens. However, the larger volumes obtained allowed some measurements to be made of the concentrations of ions, as well as of osmotic pressures, and these are given in Table 6. Both the haemolymph and the final urine of this species are much more dilute than in *E. pulchella*. The dilution of the urine appears to be due mainly to the reabsorption of sodium, chloride, and bicarbonate, there being little if any reabsorption of calcium and magnesium. The relatively high concentrations of potassium and magnesium in final urine are not obviously explicable.

The figures given in Table 6 must also be discussed in relation to the sites at which salts are reabsorbed. They certainly suggest a gradual reabsorption of salt first in the

glandular part of the kidney, then in the bladder, and finally again in the mantle cavity, as the fluid proceeds to the outside. This may indeed be the case, but it is also conceivable that during handling of the snails fluid is moved along the renal system much faster than would be normal. This would mean that every compartment would contain, during sampling, fluid of a higher concentration than would normally be found. If this were the case, salt reabsorption would be occurring in the two parts of the kidney but not in the mantle cavity. The alternative is that the results obtained represent the normal state, in which case we must postulate a considerable degree of reabsorption by the mantle epithelium. On the present evidence no decision can be reached as to which of these two possibilities is correct.

Table 6. *The composition of body fluids from* Eutrochatella tankervillei

	O.P. as NaCl (mM/l)	Na (mM/l)	K (mM/l)	Ca (mM/l)	Mg (mM/l)	Cl (mM/l)	HCO_3 (mM/l)
Haemolymph	36·8	26·5	1·2	3·2	1·5	23·7	12·5
±S.E.	±0·5	±0·6	±0·1	±0·2	±0·1	±0·6	
(No. of observations)	(6)	(5)	(5)	(6)	(5)	(5)	(3)
Pericardial fluid	36·0	—	—	3·0	1·6	23·0	—
(No. of observations)	(3)			(2)	(1)	(1)	
Fluid from glandular part of kidney (distal)	25·0	17·0	—	3·3	—	—	—
(No. of observations)	(1)	(1)		(1)			
Fluid from bladder	15·3	—	—	—	—	—	—
(No. of observations)	(3)						
Fluid from shell mouth (final urine)	7·2	3·3	4·1	2·7	2·2	0·7	5·0
±S.E.	±1·3						
(No. of observations)	(6)	(3)	(2)	(3)	(2)	(3)	(1)

DISCUSSION

It has been shown in the present study that the kidney of the marine *Nerita fulgurans* is not involved in either ionic or osmotic regulation. In this it resembles the archaeogastropod *Acmaea scutum*, in which, when the snails are properly equilibrated with the external medium, there is no significant difference between the composition of haemolymph and the urine even in dilute or concentrated sea waters (Webber & Dehnel, 1968). Differences between haemolymph and urine which are found in the first 6 h after being placed in a new salinity can presumably be explained by a time-lag effect, since urine is produced only very slowly by marine gastropods. The situation differs somewhat in the mesogastropod *Strombus gigas* (Little, 1967), where the kidney fluid has higher concentrations of calcium and potassium than pericardial fluid or haemolymph; but even in this case it seems unlikely that the kidney contributes significantly to the snail's overall ionic regulation. In summary, it appears that in marine gastropods the renal system is not employed in ionic regulation. It must be presumed, then, that it is primarily concerned with the elimination of particulate matter (e.g. Brown & Brown, 1965), organic and especially nitrogenous compounds, although very little reliable information is available on this subject (see review by Potts, 1967).

The situation in the freshwater *Neritina latissima* is in striking contrast. Here the

kidney appears similar in gross morphology to that of the marine *Nerita*, but the glandular portion is now important in salt reabsorption. Certainly chloride is reabsorbed, and presumably sodium, although which ion is actively transported is not known. The only other examination that has been made of the kidney fluids of freshwater neritids is that of Neumann (1960). Neumann was unable to demonstrate a difference between the osmotic pressures of haemolymph and kidney fluid in *Theodoxus fluviatilis*, but he attributed this to technical difficulties. The salt-reabsorbing capacity found in *Neritina latissima* is certainly normal for freshwater molluscs (see, for example, van Aardt, 1968; Florkin & Duchâteau, 1949; Little, 1965*b*; Picken, 1937). It is particularly interesting in the case of the neritids because it suggests that a comparative examination of the fine structure of the marine and freshwater forms could provide an excellent opportunity for the study of the fine-structural basis of salt transport: presumably the structures involved in organic and nitrogenous excretion would be similar in both forms, and the main differences would be due solely to a difference in the capacity to transport salts.

The terrestrial helicinids such as *Eutrochatella* have a renal system which is organized in basically the same way as those of the marine and freshwater neritids. The glandular portion of the kidney differs, however, in its appearance, in that the tissue contains large numbers of white granules; it is also somewhat more fragile than that of the aquatic forms. Nevertheless, it appears to be very similar in function to its homologue in the freshwater species, if the conclusions here made concerning its capacity to reabsorb salts are accepted. The functions of the bladder and of the mantle epithelium in osmotic regulation are much more in doubt. However, it seems likely that some salt reabsorption may well go on in the mantle cavity. This view is supported by two different observations. The first is that the ureter of the freshwater mesogastropod *Viviparus* is important in salt reabsorption (Little, 1965*b*) and this ureter is in fact derived from the mantle surface and not from the kidney (Johansson, 1950). The second observation is that of Krogh (1939) that many freshwater molluscs can take up chloride from very dilute solution; and it seems probable that the absorbing surface is part of the mantle. If the view of the mantle cavity as a possible modifier of excretory fluid in terrestrial helicinids is valid, it may be well to bear in mind the possibility that it may be important similarly in freshwater and even in marine gastropods. This would, of course, make an accurate assessment of reabsorptive capacities in aquatic forms even more difficult than it is at present.

The ecology of the helicinids is at present entirely undocumented, and it may therefore be appropriate to summarize some brief personal observations on their habitats in Costa Rica and Jamaica, and to suggest how the present comparison with aquatic relatives may throw some light on how their physiological properties are linked to their distribution and habits. Helicinids are found in predominantly damp environments in the tropics, mainly on trees or on limestone rocks; but a few species live in damp leaf litter as do several other groups of terrestrial prosobranchs. Those that live on trees are not limited to limestone areas, and have not as yet been investigated; for the most part they have non-calcareous shells. Ground-living species are restricted to limestone. All species are active only when conditions are very humid, and it is not known for how long they can aestivate. All helicinids have a vascularized mantle cavity (Bourne, 1911) which acts as a 'lung', although the opening is wide and there is no pneumostome

as in the snails of the subclass Pulmonata such as *Helix*. Such vascularization is foreshadowed in some marine species of *Nerita*, which utilize air when out of water (Fretter, 1965).

The restriction of helicinids to damp habitats fits well with their renal physiology, since the kidney functions very much like that of freshwater neritids. There is no evidence that the kidney can aid in restricting water loss by producing a concentrated urine, and indeed the possibility of reabsorption of salts in the mantle cavity makes this unlikely. There is often some fluid in the mantle cavity when the animals are active, so that the rate of water loss must be high. The lower concentration of both haemolymph and urine in *E. tankervillei* than in *E. pulchella* is probably related to the greater

Table 7. *Comparison of the ionic composition of the haemolymph of* Nerita fulgurans, Theodoxus fluviatilis *and* Eutrochatella tankervillei

	Nerita		*Theodoxus*		*Eutrochatella*	
	mM/kg	% cations or anions	mM/l	% cations or anions	mM/l	% cations or anions
Osmotic pressure...	584·5	—	52·5	—	36·8	—
Cations						
Na	487·0	83·2	45·0	85·7	26·5	72·0
K	13·1	2·2	2·2	4·2	1·2	3·3
Ca	11·7	2·0	2·3	4·4	3·2	8·7
Mg	55·1	9·4	2·9	5·5	1·5	4·1
Anions						
Cl	558·2	95·0	33·0	62·8	23·7	64·4
SO_4	29·7	5·1	N.D.	?	N.D.	?
HCO_3	N.D.	?	11·3	21·5	12·5	34·0

N.D., not determined.

The figures for '% cations or anions' are calculated as if the osmotic pressure were made up, for *Nerita*, of 584·5 mM/l of cations and 584·5 mM/l of anions; and on a similar basis, but with figures of 52·5 mM/l and 36·8 mM/l for *Theodoxus* and *Eutrochatella* respectively.

and more constant rainfall in areas occupied by the former (see Materials). The limitation of the species that have been studied to limestone areas may be reflected in the inability of the snails to reabsorb calcium and magnesium from the urine (Table 6).

One way in which the origin of these terrestrial forms may be approached is by a comparison of the composition of the haemolymph with that of the related aquatic forms (Table 7). The haemolymph of *Theodoxus* and that of *Eutrochatella* show many obvious similarities, both in the absolute values for many ions and in the proportions of the ions. Two of the largest differences are found in the figures for calcium and bicarbonate. The concentration of calcium in *Theodoxus* haemolymph is surprisingly low for a freshwater prosobranch (cf. 5·7 mM/l for *Viviparus*, Little, 1965; 6·6 mM/l for *Pomacea depressa* and 7·2 mM/l for *P. lineata*, Little, 1968). That of *Eutrochatella* is also low, but here it provides a greater percentage of the cations. The situation for bicarbonate is very similar, and this suggests that the two ions are linked, as indeed is likely because of the equilibrium with the shell and the limited solubility of calcium carbonate (see Potts, 1954). The situation is different in the marine *Nerita* where the calcium level is much higher, being approximately in equilibrium with sea water. The

figures for bicarbonate in *Nerita* haemolymph are not available, but would probably be similar to those of other marine prosobranchs such as *Strombus* (10·2 mM/l, Little, 1967), i.e. about the same concentration as in *Theodoxus* and *Eutrochatella*. This constancy of bicarbonate levels in a variety of habitats is presumably tied up with the respiratory physiology of the snails.

In general there are not many similarities between the composition of the haemolymph in *Theodoxus* and *Eutrochatella* on the one hand and that of *Nerita* on the other, and this suggests a close relationship of the two former types to each other, and perhaps a more distant relationship of these to the marine forms. This in turn suggests that the terrestrial forms have evolved from the freshwater ones, and not directly from the marine neritids. The evidence discussed above concerning the renal physiology of the three types also supports this view. The marine *Nerita* is incapable of producing dilute urine, and it may be suggested that a terrestrial line evolved directly from such forms would also be unable to reabsorb salts from the kidney fluid; certainly this is the case in the terrestrial relatives of *Littorina* (personal communcation from Miss T. J. Rumsey). In contrast, the freshwater forms produce a dilute urine, and the selection pressure acting to bring about the acquisition of this new function by the kidney is in this case an obvious one, whereas it is by no means obvious in snails colonizing land via the littoral zone. This capacity for production of dilute urine acquired in fresh water has been retained by the terrestrial helicinids; it is certainly an appropriate property for animals living in rain-soaked tropical forests.

The products of nitrogenous excretion have not been considered in the present investigation, and little is known on this subject with regard to neritaceans. A brief report by Fischer & Brunel (1953) has shown that while neritids living in the lower part of the marine intertidal zone are ammonotelic, at least one species which lives at the top of the shore is uricotelic. It would therefore be expected that the terrestrial forms would have become uricotelic; but the work of Speeg & Campbell (1968) showing that aestivating pulmonate snails evolve considerable quantities of gaseous ammonia shows the need for a reinvestigation of the generalization that the colonization of land is linked with uricotelism.

SUMMARY

1. The marine form *Nerita fulgurans* is unable to osmoregulate, and regulates ions only to a very minor degree. The kidney plays no part in controlling the ionic or osmotic composition of the haemolymph.

2. The freshwater neritid *Neritina latissima* produces a dilute urine. Salts are removed from the urine by the glandular part of the kidney. The composition of haemolymph in the freshwater *Theodoxus fluviatilis* has been analysed. It contains surprisingly little calcium.

3. Observations on terrestrial species of *Eutrochatella* and *Alcadia* showed that these snails all produce a dilute urine. The site of salt reabsorption is not clear, but the results indicate that it may be reabsorbed by the glandular part of the kidney, the bladder, and also by the mantle epithelium. The haemolymph of the terrestrial species is similar in composition to that of *Theodoxus*.

4. The renal systems of marine, freshwater and terrestrial neritaceans are compared.

Without much gross structural reorganization from the marine species, the kidneys of the freshwater forms have been developed into organs for reabsorbing salts from the urine. The terrestrial species have probably evolved from these freshwater ones, and have retained the production of a dilute urine; this is certainly appropriate because they live in very damp environments.

A large number of people and organizations have made this study possible, and I wish to record my thanks to all of these. Dr V. Fretter, of the Department of Zoology, University of Reading, very kindly advised me on some points concerning the anatomy of the marine neritids. The work on these and on the freshwater *Neritina* was done at the Institute of Marine Sciences, University of Miami, Florida. I am indebted to Dr F. M. Bayer of this Institute for supplying these freshwater *Neritina*, and to Dr W. J. Byas of the Smithsonian Institution for identifying them. Dr Elizabeth Andrews of Bedford College, London, very kindly helped me to obtain the *Theodoxus* from Reading.

The collecting expeditions to Jamaica and Costa Rica which provided the helicinids and allowed some observations on their habitats were partly financed by grants from the Organization for Tropical Studies, U.S.A., and from the Royal Society. I am particularly grateful to Dr and Mrs J. L. Munro of the Department of Zoology, University of the West Indies, Kingston, Jamaica, for their help and hospitality during my visits to Jamaica. Dr T. L. Farr very kindly allowed me to make use of the facilities of the Institute of Jamaica. Help in the collecting expeditions was given by Mr J. D. Richard and Mr J. Eggert, to whom I am most grateful.

Although I have not incorporated all their suggestions, I am grateful to Dr T. E. Thompson, Dr A. E. Dorey and Mr A. J. Underwood for their comments on various parts of the manuscript.

REFERENCES

VAN AARDT, W. J. (1968). Quantitative aspects of the water balance in *Lymnaea stagnalis* (L.). *Netherlands J. Zool.* **18**, 253–312.

VAN ASPEREN, K. & VAN ESCH, I. (1956). The chemical composition of the haemolymph in *Periplaneta americana*. *Arch. néerl. Zool.* **11**, 342–60.

BROWN, A. C. & BROWN, R. J. (1965). The fate of thorium dioxide injected into the pedal sinus of *Bullia* (Gastropoda: Prosobranchiata). *J. exp. Biol.* **42**, 509–19.

BOURNE, G. C. (1908). Contributions to the morphology of the group Neritacea of aspidobranch gastropods. I. The Neritidae. *Proc. zool. Soc. Lond.* **1908**, 810–87.

BOURNE, G. C. (1911). Contributions to the morphology of the group Neritacea of the aspidobranch gastropods. II. The Helicinidae. *Proc. zool. Soc. Lond.* **1911**, 759–809.

FISCHER, P. H. & BRUNEL, A. (1953). Relation entre l'habitat des gastéropodes et leur processus d'excrétion. *C. r. somm. Séanc. Soc. Biogéogr.* **30**, 36–37.

FLORKIN, M. & DUCHÂTEAU, G. (1949). Sur l'osmoregulation de l'anodonte (*Anodonta cygnea* L.). *Physiologia comp. Oecol.* **1**, 29–45.

FRETTER, V. (1965). Functional studies of the anatomy of some neritid prosobranchs. *J. Zool., Lond.* **147**, 46–74.

JOHANSSON, J. (1950). On the embryology of *Viviparus* and its significance for the phylogeny of the Gastropoda. *Ark. Zool.* A.S. **1**, 173–7.

KROGH, A. (1939). *Osmotic Regulation in Aquatic Animals*. Cambridge University Press.

LENSSEN, J. (1902). Système nerveux, système circulatoire, système respiratoire et système excréteur de la *Neritina fluviatilis*. *Cellule* **20**, 289–331.

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.

LITTLE, C. (1967). Ionic regulation in the queen conch, *Strombus gigas* (Gastropoda, Prosobranchia). *J. exp. Biol.* **46**, 459–74.

LITTLE, C. (1968). Aestivation and ionic regulation in two species of *Pomacea* (Gastropoda, Prosobranchia). *J. exp. Biol.* **48**, 569–85.

LITTLE, C. & RUSTON, G. (1970). A method for determining total carbon dioxide in small volumes of liquid. *J. exp. Biol.* **52**, 395–400.

MORTON, J. E. & YONGE, C. M. (1964). Classification and structure of the Mollusca. In *Physiology of Mollusca*, vol. 1 (ed. K. M. Wilbur and C. M. Yonge). London: Academic Press.

NEUMANN, D. (1960). Osmotische Resistenz und Osmoregulation der Flussdeckelschnecke *Theodoxus fluviatilis* L. *Biol. Zbl.* **79**, 585–605.

PICKEN, L. E. R. (1937). The mechanism of urine formation in invertebrates. 2. The excretory mechanism in certain Mollusca. *J. exp. Biol.* **14**, 20–34.

POTTS, W. T. W. (1954). The inorganic composition of the blood of *Mytilus edulis* and *Anodonta cygnea*. *J. exp. Biol.* **31**, 376–85.

POTTS, W. T. W. (1967). Excretion in the molluscs. *Biol. Rev.* **42**, 1–41.

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.

ROACH, D. K. (1963). Analysis of the haemolymph of *Arion ater* (Gastropoda, Pulmonata). *J. exp. Biol.* **40**, 613–23.

SCIENTIFIC RESEARCH COUNCIL OF JAMAICA. (1963). *The Rainfall of Jamaica*. Jamaica: S.R.C.

SPEEG, K. V. & CAMPBELL, J. W. (1968). Formation and volatilization of ammonia gas by terrestial snails. *Am. J. Physiol.* **214**, 1392–402.

WARMKE, G. L. & ABBOTT, R. T. (1961). *Caribbean Seashells*. Narberth, Pennsylvania: Livingston Publishing Company.

WEBBER, H. H. & DEHNEL, P. A. (1968). Ion balance in the prosobranch gastropod, *Acmaea scutum*. *Comp. Biochem. Physiol.* **25**, 49–64.