XXVIII. PROBLEMS OF NITROGEN CATABOLISM IN INVERTEBRATES.

II. CORRELATION BETWEEN URICOTELIC METABOLISM AND HABITAT IN THE PHYLUM *MOLLUSCA*.

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IN the preceding paper of this series [Baldwin and Needham, 1934] some figures were reported for the uric acid content of the nephridium or kidney of the large land-snail, *Helix pomatia*. It was found that even for a considerable time after the end of the hibernation period, no less than three-quarters of the dry weight of the organ could consist of uric acid. This remarkable retention stimulated a desire to investigate whether other gastropod nephridia showed a like accumulation, and the possibility arose of studying in this way the distribution of uricotelic metabolism. We are accustomed to the view that the use of uric acid as the main vehicle of waste nitrogen excretion may in some way or other be regarded as an adaptation to terrestrial life, and the suggestion has been made [Needham, 1929; 1931; see also Hopkins, 1928] that terrestrial oviparity in particular, where development within the cleidoic egg occurs, is associated with uricotelic metabolism. Hitherto, however, such generalisations have had perforce to rest upon our knowledge of the nitrogen metabolism of vertebrates only. The urea production of fishes, amphibia and mammals has been compared with the uric acid production of reptiles and birds. But the invertebrates may perhaps provide a still more striking opportunity for the correlation of metabolism with habitat. In the insects, where the excretion of uric acid as the main end-product is widespread if not universal, such opportunities, it is true, are not promising, for the Subphylum Insecta is as a whole so typically terrestrial, and the aquatic forms are so obviously secondary, that the boundaries of habitat and metabolism will be the boundaries of the group. In the molluscs, however, we have a morphologically homogeneous population distributed through every conceivable environment; thus the gastropods are to be found in the sea and on its littoral, in brackish-water and fresh water, both rivers and lakes, and on land in surroundings showing every degree of humidity. The lamellibranchs, on the contrary, are mainly marine, with a few fresh-water species. Amphineura, Scaphopoda, and Cephalopoda are wholly marine. With the probability that terrestrial snails are uricotelic organisms now raised almost a certainty [see Baldwin and Needham, 1934] we are evidently at liberty to enquire how far this form of metabolism extends and to correlate within one group of genera the environment and the metabolism.

Strohl [1914, p. 527], in his valuable review of the excretory mechanisms of molluscs, concludes that in general the gastropods are characterised by positive findings of uric acid in their nephridia, but that in the lamellibranchs this substance is always absent, and its place seems to be taken by urea. This statement, it will be seen, is in rough agreement with the expectations which we derive from

facts mentioned in the preceding paragraph. But it rests entirely on the qualitative tests scattered through the literature (which he fully cites). From these it is difficult to draw very far-reaching conclusions, if only for the reason that a positive uric acid test might imply simply the presence of a nuclein metabolism similar to that of mammals and nothing whatever regarding the excretion of the main portion of the waste nitrogen as uric acid. Moreover, it must be admitted that many of the workers reviewed by Strohl employed techniques which would not receive unqualified approval to-day. This vitiates any wide conclusions drawn from the general body of their work.

There are two main ways in which uricotelic metabolism could be proved to exist in molluscs, firstly, by the direct estimation of the various nitrogenous substances excreted by them and the drawing up of a table of nitrogen partition, secondly by the demonstration of the synthesis of uric acid from ammonia by tissue-slices *in vitro*. Unfortunately, both these methods suffer from difficulties, some of which are special to the molluscs. The direct estimation of the end-products, though straightforward enough in the case of terrestrial or fresh-water species, presents technical difficulties if the animals have to be kept in sea-water, especially if a high degree of accuracy is desired. Worse than this, however, is the very erratic nature of the excretion of many species and the possibility that others, although breaking down protein with the ultimate formation of uric acid, never excrete the latter at all. Thus experience in the cultivation of *Helix pomatia* shows us that an emptying of the nephridial contents, with the formation of clay-like granular masses, does not take place more often than once a fortnight or three weeks. Other snails probably excrete more rarely [Simroth and Hoffmann, 1928]. *Cyclostoma elegans*, the terrestrial operculate, possesses a special dermal gland (referred to later) in which uric acid is stored in extremely large amounts, and nothing is known about its excretion. In such a case, the measurement of the types of substance excreted might be very misleading indeed in the absence of other information even if carried on over very long periods. Then with regard to the demonstration of the synthesis of uric acid from ammonia by tissue-slices, difficulties arise owing to the relative slowness of the nitrogen metabolism in gastropods, leading to unduly long experiments. Here other limitations such as the tendency of the hepatopancreas, for instance, to fragmentation, and the danger of bacterial attack, are soon met with. Much further research, too, will be needed before we know the optimum requirements for invertebrate tissues *in vitro*, and there may be a poisoning of the synthesising cells by ammonia. Finally the smallness of many of the most interesting molluscs makes the study of their tissues *in vitro* for the present as good as impossible.

In view of all these considerations, it was decided to attempt a preliminary survey of the molluscs upon the assumption that the existence of uricotelic metabolism in any particular case would be reflected in the degree of retention of uric acid by the nephridium, or what might be called the average load, and to examine accordingly the nephridia of a large number of molluscs, quantitatively estimating the uric acid present. It must be emphasised at the outset that if the possibility that a gastropod could excrete uric acid just as fast as it was formed is realised anywhere in practice, the conclusions of this paper will not hold good, although the comparative data for the uric acid retention of molluscan nephridia will still possess a value. The possibility, however, is here taken to be remote. Judgment would be easier if we knew anything detailed about the rapidity of water-flow through the body of molluscs, but as reviews such as that of Adolph [1930] show, very little is known about the water turnover of the body in species other than the frog and some fishes. There is probably not much difference

between a marine and a terrestrial environment with respect to the water-flow through the body, for since the osmotic pressure of the molluscan body-fluids is well-known to be approximately equal to that of the sea-water in which they live, no favouring osmotic gradient can be envisaged. Studies of the tonicity of the excretions in molluscs, similar to those of Schwabe [1933] for instance, on the nephridial fluid in crustacea, are urgently needed. On the other hand, fresh-water molluscs, such as *Anodonta*, would be expected to have a much higher water turnover and a copious excretion of hypotonic "urine" from the nephridial orifice. These considerations must be borne in mind in relation to the figures tabulated below.

Arguments in favour of the hypothesis that uricotelic metabolism will reveal itself in high uric acid retention may be drawn from the work of Cuénot [1892; 1894; 1899] on excretion in molluscs studied by means of dyes such as indigo-carmin or safranin. If these dyes, he found, were injected into the body of a gastropod or a lamellibranch mollusc, the concretions in the nephridial cells took them up, and an idea of the rate of excretion could be gained from the time taken to lose dye from the nephridium. "For aquatic molluscs" he writes [1899, p. 77] "one can obtain sufficiently precise figures by injecting a series of animals on one day and then opening them week after week until one observes the decoloration of the kidneys, previously tinted with the injected substance. It seems that in general the nephridial cells accumulate for a very long time within themselves the products which they make, and only excrete them at long intervals; thus in *Anodonta* and *Unio* it was not until 140 days after the injection that the coloured concretions with carminate began to disappear." Similar experiments have been carried out by Emeljanenko [1909] on terrestrial gastropods.

The method adopted in the present research was to dissect out the nephridia¹ from a number of animals (live and in good condition), and after grinding in a mortar with clean sand, to bring the uric acid into solution by digesting the mixed tissue for a shorter or longer time over a small flame with the phosphate mixture of Benedict and Hitchcock [1915] in which uric acid has forty times its normal solubility in water. The final volume represented approximately for each 100 ml. 1 g. wet weight of tissue, but this proportion was often departed from, depending upon the amount of uric acid shown to be present in preliminary trials. Precipitation of the proteins was accomplished by 1.5 % uranium acetate, added in the proportion of about 1 to 10 to the boiling digest, but here also the amount varied according to the quantity of protein present. The uric acid was then estimated on an aliquot part of the final solution, which was always crystal clear and usually colourless, by the colorimetric method of Folin [1933]. This method is known to be unsatisfactory in some cases, but Fujiwara and Kataoka [1933] have carefully surveyed the limits of its specificity. Reduction of phosphotungstic acid under Folin's conditions is brought about by many aromatic amines, phenols, indole derivatives, lactic acid, fructose and inulin. It may be legitimately assumed that none of these compounds would be present in the nephridial tissue to such an extent as to interfere seriously with the conclusion that the main bulk of the colour produced is due to uric acid. The analyses made on the whole body are probably more open to doubt.

In some cases the bodies of the gastropods were not removed fresh from their shells, but the attachment of the columella muscle was previously weakened by

¹ For the morphology and cytology of gastropod nephridia see Meisenheimer [1912] and Freitag [1916].

placing them for a few seconds (up to half a minute) in boiling water. It was feared that the partial coagulation of the proteins which occurred might have had the effect of rendering the uric acid more difficult to extract with the hot phosphate mixture after grinding, but experiments made in parallel to test this point on several species showed no difference of significance. In many species the small size necessitated the use of the whole body, and it was found that the shells of the organisms formed a satisfactory substitute for the sand used in grinding. In such cases separate determinations of shell weight and live weight on samples of the group permitted the calculation of the wet weight taken.

The results are summarised in Table I and placed in order of classification and habitat in Table II. From Table I an idea can be gained of the great variation in size between the different species. The whole body of *Hydrobia* weighs but 3 mg. while the nephridium alone of *Buccinum* weighs well over 1 g., and its whole body would amount to a hundred times as much. Some of the nephridia,

Table I. *Uric acid in molluscs.*

Notation; N, nephridium; WB, whole body; RB, rest of body (excluding nephridium); V, visceral mass; M, musculature of body-wall.

			Uric acid		
Animal	Part used	No. taken	Wet weight each g.	mg./g. wet weight	mg./g. dry weight
GASTROPODA					
PULMONATA					
<i>Helix aspersa</i>	WB	3	2.88	1.00	5.43
	WB	3	2.88	0.55	3.00
	WB	33 (young)	0.615	0.48	2.76
	N	20	0.099	29.0	153.0
	N	4	0.09	27.4	167.0
	N	3	0.09	7.8	63.8
	V	3	1.06	0.91	4.43
	V	3	1.65	0.86	4.19
<i>Helicella virgata</i>	WB	40	0.059	1.28	8.05
	WB	25	0.118	3.45	24.8
<i>Helicella itala</i>	WB	9	0.31	0.735	4.63
	WB	6	0.48	0.634	3.98
	WB	6	0.43	0.485	3.05
	N	32	0.0094	31.3	156.0
<i>Cochlicella acuta</i>	WB	56	0.047	3.2	23.0
<i>Bulimulus sporadicus</i>	N	3	0.045	38.3	183.0
<i>Limax maximus</i>	N	1	0.078	30.8	205.0
<i>Limax flavus</i>	N	2	0.019	4.64	31.0
<i>Milax sowerbyi</i>	N	10	0.016	9.9	66.0
<i>Arion ater</i>	N	6	0.064	3.6	36.0
<i>Agriolimax agrestis</i>	N	7	0.036	2.93	20.0
<i>Limnaea stagnalis</i>	N	38	0.030	16.8	137.0
	N	25	0.036	13.4	109.0
	N	29	0.035	11.8	96.5
<i>Planorbis corneus</i>	N	10	0.0145	3.57	19.8
	N	11	0.0106	4.7	26.1
	N	45	0.022	13.3	74.0
	RB	4	0.33	trace	trace
	RB	4	0.32	trace	trace
<i>Limnaea peregra</i>	WB	17	0.048	0.0405	0.245
<i>Ancylastrum fluviatile</i>	WB	93	0.008	0.115	0.58
<i>Onchidella celtica</i>	WB	40	0.206	0.06	0.27
	V	64	0.06	0.098	0.36
	M	64	0.104	0.078	0.49

Table I (cont.).

Table 1 (cont.).				Uric acid	
Animal	Part used	No. taken	Wet weight each g.	mg./g. wet weight	mg./g. dry weight
OPERCULATA					
<i>Cyclostoma elegans</i>	WB	13	0.154	10.15	64.0
	WB	13	0.213	18.2	114.0
<i>Viviparus fasciatus</i>	N	14	0.018	2.24	17.6
	N	6	0.04	6.6	51.6
	RB	4	0.39	1.02	5.15
	RB	4	0.39	1.09	5.5
<i>Bithynia tentaculata</i>	WB	10	0.079	0.765	5.1
	WB	15	0.083	0.73	4.85
<i>Hydrobia jenkinsi</i>	WB	147	0.0034	nil	nil
	WB	895	0.0021	nil	nil
	WB	450	0.0021	nil	nil
<i>Buccinum undatum</i>	N	2	1.2	0.42	4.17
	N	2	0.67	0.64	6.4
	N	2	1.11	0.42	4.2
	N	1	1.3	0.17	1.7
<i>Gibbula umbilicalis</i>	N	18	0.026	0.23	1.92
	N	9	0.022	0.32	2.65
<i>Monodonta crassa</i>	N	6	0.04	0.56	2.8
	N	2	0.057	0.314	1.57
	N	80	0.025	0.108	0.56
<i>Nassa reticulatus</i>	N	22	0.028	0.47	2.94
<i>Purpura lapillus</i>	N	71	0.02	1.42	5.21
	N	50	0.017	1.92	7.05
	N	51	0.011	0.11	0.88
<i>Turritella communis</i>	N	16	0.0056	1.52	6.0
TECTIBRANCHIATA					
<i>Scaphander lignarius</i>	N	2	0.11	0.212	4.64
NUDIBRANCHIATA					
<i>Doto coronata</i>	WB	8	—	trace	trace
<i>Tritonia hombergi</i>	V	1	4.0	0.96	4.2
	V	3	5.3	0.67	2.9
<i>Coryphella rufibranchiata</i>	WB	1	0.09	trace	trace
<i>Eolis papillosa</i>	WB	2	1.19	0.127	1.16
	WB	9	0.44	0.19	2.1
<i>Onchidorus fusca</i>	WB	1	0.16	trace	trace
<i>Doris tuberculata</i>	V	2	0.43	0.096	0.44
ASPIDOBRANCHIATA (non-operculate)					
<i>Patella vulgata</i>	N	6	0.22	0.124	0.58
	N	13	0.24	0.088	0.41
	V	2	2.33	0.086	0.22
LAMELLIBRANCHIATA					
<i>Anodonta cygnaea</i>	N	16	0.198	nil	nil
<i>Pisidium fontinale</i>	WB	16	0.035	nil	nil
<i>Pecten maximus</i>	N	18	0.169	0.09	0.49
	N	24	0.122	0.099	0.54
<i>Pecten opercularis</i>	N	50	0.028	0.29	2.9
	N	60	0.031	0.49	4.9
AMPHINEURA					
APLACOPHORA					
<i>Proneomenia aglaopheniae</i>	WB	3	—	trace	trace
POLYPLACOPHORA					
<i>Lepidochiton cinereus</i>	WB	21	0.06	0.153	0.77
<i>Acanthochitona crinitus</i>	WB	3	0.19	trace	trace

Table II. *General summary of the observations.*

Animal	Locality	Habitat	mg. uric acid/g. dry weight			
			Nephridium	Whole body	Rest of body	Visc. musc.
PULMONATA						
PULMONATA						
<i>Helix pomatia</i> (Linn.)	France	T	810, 660, 785, 720	—	—	—
<i>Helix aspersa</i> (Müll.)	Cambridge, Plymouth	T	153, 167, 64	5.43, 3.0, 2.76	—	4.43, 4.19
<i>Helicella virgata</i> (da Costa)	Speke's Mill, N. Devon and Marloes, S. Pem.	T	—	8.05, 24.8	—	—
<i>Helicella itala</i> (Linn.)	Marloes, S. Pem. and Wicklow, Ireland	T	156	4.63, 3.98, 3.05	—	—
<i>Cochlicella acuta</i> (Müll.)	Marloes, S. Pem.	T	—	23.0	—	—
<i>Bulimulus sporadicus</i>	Ascension, Paraguay	T	183	—	—	—
<i>Limax maximus</i> (Linn.)	Cambridge	(T)	205	—	—	—
<i>Limax flavus</i> (Linn.)	Cambridge	(T)	31	—	—	—
<i>Limax soverbyi</i> (Fér.)	Cambridge	(T)	66	—	—	—
<i>Arion ater</i> (Linn.)	Cambridge	(T)	36	—	—	—
<i>Agriolimax agrestis</i> (Linn.)	Cambridge	(T)	20	—	—	—
<i>Limnaea stagnalis</i> (Linn.)	Cambridge	FW	137, 109, 96	—	—	—
<i>Planorbis corneus</i> (Linn.)	Cambridge	FW	20, 26, 74	—	trace	—
<i>Limnaea peregra</i> (Müll.)	Speke's Mill, N. Devon	FW	—	0.24	—	—
<i>Ancylastrum</i> (<i>Pseudancy-lus</i>) <i>fluviatile</i> (Linn.)	Speke's Mill, N. Devon	FW	—	0.58	—	—
<i>Onchidella celtica</i> (Cuvier)	M.B.L.	SW	—	0.27	—	0.4, 0.5
OPERCULATA						
<i>Cyclostoma</i> (<i>Pomatias</i>) <i>elegans</i> (Müll.)	Gt Gaddesden, Herts	T	—	64, 114	—	—
<i>Viviparus</i> (<i>Paludina</i>) <i>fasciatus</i> (Müll.)	Milton, Cambs.	FW	18, 52	—	5.1, 5.5	—
<i>Bithynia tentaculata</i> (Linn.)	Milton, Cambs.	FW	—	5.1, 4.8	—	—
<i>Hydrobia jenkinsi</i> (<i>Potamopyrgus crystallinus</i>) (Smith)	Watford and Anglesea	FW	—	0, 0, 0	—	—
<i>Buccinum undatum</i> (Linn.)	M.B.L.	SW	4.1, 6.4, 4.2, 1.7	—	—	—
<i>Gibbula umbilicalis</i> (<i>Trochus umbilicatus</i>) (da Costa)	M.B.L.	SW	1.9, 2.65	—	—	—
<i>Osilinus lineatus</i> (<i>Monodonta crassa</i>) (da Costa)	M.B.L. and Hartland	SW	2.8, 1.6, 0.56	—	—	—
<i>Nassarius</i> (<i>Nassa</i>) <i>reticulatus</i> (Linn.)	M.B.L.	SW	2.9	—	—	—
<i>Nucella</i> (<i>Purpura</i>) <i>lapillus</i> (Linn.)	M.B.L.	SW	5.2, 7.05, 0.88	—	—	—
<i>Turritella communis</i> (Risso)	M.B.L.	SW	6.0	—	—	—
<i>Littorina littorea</i> (Linn.)	M.B.L. and Hartland	SW	1.3, 1.6, 1.3	trace, 0.2	—	—
<i>Littorina littoralis</i> (<i>obtusata</i>) (Linn.)	M.B.L. and Blegberry, N. Devon	SW	—	0.3, 0.4	—	—
<i>Littorina rudis</i> (<i>Saxatilis</i>) (Oliv)	M.B.L. and Blegberry	SW	5.1	0.4, 0.5, 1.2, 1.5, 1.5	—	—
<i>Littorina neritoides</i> (Linn.)	M.B.L. and Blegberry	T	—	1.7, 4.3, 0.7, 0.5	—	—
TECTIBRANCHIATA						
<i>Scaphander lignarius</i>	M.B.L.	SW	4.64	—	—	—
NUDIBRANCHIATA						
<i>Idulia</i> (<i>Doto</i>) <i>coronata</i> (Gmelin)	M.B.L.	SW	—	trace	—	—
<i>Sphaerostoma</i> (<i>Tritonia</i>) <i>hombergi</i> (Cuvier)	M.B.L.	SW	—	—	—	4.2, 2.9
<i>Aeolida</i> (<i>Eolis</i>) <i>papillosa</i> (Linn.)	M.B.L.	SW	—	1.2, 2.1	—	—
<i>Archidoris brittanica</i> (<i>Doris tuberculata</i>) (Johnston)	M.B.L.	SW	—	—	—	0.4
<i>Onchidoris fusca</i>	M.B.L.	SW	—	trace	—	—
<i>Coryphella rufibranchiata</i>	M.B.L.	SW	—	trace	—	—
ASPIDOBANCHIATA						
<i>Patella vulgata</i> (Linn.)	M.B.L.	SW	0.58, 0.41	—	—	0.22

Table II (cont.).

Animal	Locality	Habitat	mg. uric acid/g. dry weight			
			Nephridium	Whole body	Rest of body	Visc. musc.
LAMELLIBRANCHIATA						
<i>Anodonta cygnara</i> (Linn.)	Ely	FW	0	—	—	—
<i>Pisidium fontinalis</i> (<i>cinereum</i>) (Alder)	Milton, Cambs.	FW	—	0	—	—
<i>Pecten maximus</i> (Linn.)	M.B.L.	SW	0.49, 0.54	—	—	—
<i>Aequipecten</i> (<i>Pecten</i>) <i>opercularis</i> (Linn.)	M.B.L.	SW	2.9, 4.9	—	—	—
AMPHINEURA						
APLACOPHORA						
<i>Proneomenia aglaopheniae</i> (K.&M.)	M.B.L.	SW	—	trace	—	—
POLYPLACOPHORA						
<i>Lepidochiton</i> (<i>Chiton</i>) <i>cinereus</i> (Linn.)	M.B.L.	SW	—	0.77	—	—
<i>Acanthochitona crinitus</i> (Pennant)	M.B.L.	SW	—	trace	—	—

N.B. The nomenclature adopted in this table follows Ellis [1926] for the terrestrial and fresh-water species, and the Plymouth Fauna List [Marine Biol. Assoc., 1931], as modified by Winckworth [1932; 1934], for the marine ones. Older names are bracketed. Environments are represented by T for terrestrial, (T) for moist or semi-terrestrial, FW for fresh-water, and SW for marine. Under localities M.B.L. stands for English Channel species, collected by the Marine Biological Association at Plymouth. The figures for *Helix pomatia* mostly derive from the previous paper of this series. Correlation of the above results with data on feeding habits may be attempted by consulting the lists of Hirsch [1915], Hunt [1925] and Yonge [1928], but there is no correspondence.

again, are very small; thus those of *Helicella itala* weigh only 9 mg. each, and 20 mg. is an average figure for the nephridia of Trochid marine snails. This involved the taking of large samples, such as nearly a thousand specimens of *Hydrobia* in one case, and from 50 to 100 dissected-out nephridia were a common requirement.

Not until the relation between the results and the environment is brought out, however, do we obtain a coherent picture. In Table II each systematic group is divided into sub-groups according to habitat, terrestrial preceding fresh-water and fresh-water preceding marine.

First of all, the terrestrial pulmonates may be considered. The extremely high retentions of *Helix pomatia* were not encountered in other species, but the uric acid content both of nephridium and whole body is always very high. In *Helix aspersa*¹ the average value for the nephridium would be about 100 and for the whole body 3.5 to 4.0. *H. itala* agrees closely in both ways with *H. aspersa*, but *H. virgata* may contain three times as much in the whole body, as also may *Cochlicella*. All these, as Ellis [1926] says, are typical arid-living forms. At the extreme opposite would be the marine pulmonates, but these are extremely rare, the only convenient English species being *Onchidella celtica*. This very interesting shell-less mollusc (for which see the classical paper of Joyeux-Laffuie [1882]) proved to contain almost no uric acid, its whole body giving a figure of only 0.27 mg./g. dry weight in contradistinction to the amounts from ten to one hundred times as large found in the terrestrial species. It is certain that the high degree of vascularisation of the mantle cavity and the reduction of the gills to vestiges, which characterise the pulmonates, are adaptations to terrestrial life, and *Onchidella* is therefore generally regarded as a return to the sea, *i.e.*

¹ During the dissection of these snails, it was impossible not to be impressed with the large amount of bright yellow pigment excreted by glands in the foot. This was found to be soluble in water but not in chloroform and only irreversibly reducible. Its properties were apparently not those of a flavin or a pterin. The literature is very silent on the subject of gastropod slime-pigments, but there is a reference to them in Krukenberg [1886]. They are now under investigation by my friend Dr Kurt G. Stern.

secondarily aquatic. If, therefore, we may interpret the high retentions seen in the terrestrial pulmonates as evidence of uricotelic metabolism, the condition of affairs in *Onchidella* indicates a far-reaching, if not complete, abandonment of this form of metabolism and a return to ammonia or urea production.

An interesting parallel case to this is to be found in the fresh-water elasmobranchs studied in Siam by Smith and Smith [1931]. The high urea content of elasmobranch blood is now generally believed to be adaptive in character, in that it brings the total osmotic pressure of the internal medium above the external, and so, by forming a favourable osmotic gradient, exempts the fish from the continuous drinking and complicated excretory arrangements found in the teleosts [Smith, *et al.*, 1930, 1; Smith, 1932]. The majority of elasmobranchs are marine, but secondary (or tertiary) penetration into fresh-water environment occurs in certain rather rare cases, such as the saw-fish, *Pristis*. It was accordingly of interest to study the urea content of fresh-water elasmobranch blood, for if no longer required, it might have sunk to a level similar to that of other animals, or on the other hand, if such an evolutionary retrogression were not possible, it might have remained at its usual level¹. In actuality Smith and Smith observed a diminution of about 60 % in all the species studied. If, however, we were to apply this analogy in full to the case of the marine pulmonate, we should have to suppose that a mixed metabolism existed; true uricotelism supplying only a portion of the total excreted nitrogen, but this we may be reluctant to do in view of the all-or-none character of the cases of uricotelic metabolism hitherto known. It is clear that *Onchidella* invites much closer and more detailed attention.

The remainder of the pulmonates divide into two distinct groups (*a*) the aquatic snails of fresh-water and (*b*) the slugs. The fresh-water pulmonates are all, it is generally agreed, secondary, and correspondingly it is found that the uric acid content of some of them closely approaches that of the terrestrial snails; thus a nephridium may have in *Limnaea* 137 mg./g. dry weight and in *H. aspersa* 153. Nevertheless, there seems to be a gradation here reminiscent of what was seen in the case of *Onchidella*, for *Planorbis* nephridia are not as rich in uric acid as the land-snail nephridia, and the water-snail *L. peregra* with the river limpet *Ancylastrum* have an average body content of only 0.41 mg./g. dry weight, as against 8.75 for the land-snails. This low figure, indeed, closely approaches the 0.27 of *Onchidella*. The apparent conclusion is that some of the fresh-water species are still uricotelic while others are not, and that the difference may be associated with the length of time the species have been in fresh-water. The position of *Ancylastrum* would agree with this view, for as it is a pulmonate it must have entered the fresh waters by an overland route, and yet its completely aquatic limpet-like morphology must indicate a very long stay in fresh-water, long enough for it to diverge so widely from its nearest ally *Planorbis*. However, it is to be remembered that a fresh-water environment probably involves a faster current of water through the body than other environments, and the low values in these species may, therefore, be due to great rapidity of excretion. What has already been said, however, about the excretion of molluscs, makes this possibility only indifferently plausible.

Just as the fresh-water snails vary in uric acid content from "terrestrial" values down to very low ones, so also do the slugs. *L. maximus* gives a nephridial value equal to that of any land-snail, but the others more nearly resemble an

¹ The subject of evolutionary retrogression merits more attention from biochemists than it has yet received (*cf.* the papers of Nopcsa [1923] and Blagoveschenski [1929]).

intermediate aquatic form such as *Planorbis*. Here again there are two explanations, either that there is a real retrogression from uricotelic metabolism parallel with the loss of the shell, or that the excretory system is more vigorously flushed with water. In favour of the latter view are the recent experiments of Howes and Wells [1934], who also summarise the previous literature on water turnover of terrestrial organisms. Relatively dry and impermeable skins allow of very little loss (*e.g.* mammals 3 %/day, insects 2 %/day, lizard 5 %/day), moist skins allow more (anurans 10 %/day, urodeles 28 %/day). The earthworm may lose 240 %/day in air, but in subterranean conditions, this capacity for loss is probably not a serious deficiency. The slug, however, loses 58 %/day as a minimum in the motionless state, and if continuously creeping, loses 384 %/day. Correspondingly, although snails such as *Helix* can be kept without water for many months, since they only retire into their shells and lose very little, slugs must be supplied with ample water and must constantly drink. As there is clearly so great a current of water through the skin and the slime-glands it is at least very possible that there is also a great current of water through the nephridium and this would have the effect referred to above. On the other hand, the generalisation, which most oecologists would accept, that on the whole slugs inhabit a moister environment than snails, would be in favour of the former alternative. The work on arginase, reported in the succeeding paper of this series by Baldwin [1935], tends also in the same direction.

We have now to consider the operculate gastropods. Two groups of them, the terrestrial and the marine, give very clear-cut results. In the first place, *Cyclostoma*, the only English representative of its class, shows an enormous retention of uric acid, exceeding even the figures for *H. pomatia*. Its whole body contains uric acid to the same extent as the nephridium alone of the other terrestrial snails. This finding is no doubt due to the presence in *Cyclostoma* of a "concrementary gland" first described by Claparède [1858] and containing dense deposits of uric acid. A lengthy examination of it was made by Meyer [1925], especially with regard to the symbiotic bacteria which it is said to contain. It is not strictly speaking a gland, but a perivascular agglomeration of large cells containing the concretions. Uric acid was first shown to be present in the gland by Barfurth [1884] and according to Meyer's figures, at least 42 % of its dry weight is uric acid (*cf.* the *H. pomatia* nephridium). Other purine bases are believed to be present also in considerable amounts. Very little is known about the actual excretions of *Cyclostoma*, but the elimination of concretions probably takes place at infrequent intervals, and urea has been recognised by the xanthidrol reaction in the nephridial fluid.

Marine operculate gastropods show, without exception, extremely small amounts of uric acid in nephridium or in whole body. The average figure for all species, 3.2 mg./g. dry weight nephridium, contrasts with the immensely high figures for *Cyclostoma* and the land pulmonates (up to 1000). In other words, it is clear that in these species, of which the whelk *Buccinum* is typical, and which possess no trace of terrestrial ancestry, uricotelic metabolism does not exist. Here the question of water turnover is not likely to affect the issue, since the concentration of the external medium allows of no rapid current through the body.

At this stage the general argument may be crystallised as shown in Table III.

The general conclusion that if uricotelic metabolism can be gauged by uric acid retention, uricotelic metabolism is especially associated with terrestrial life, seems unavoidable. And in this case the comparison is between animals all within a comparatively restricted group, the gastropods.

Table III.

Environment	Typical species	Uric acid, mg./g. dry weight	
		Nephridium	Whole body
Terrestrial	Pulm. <i>Helix pomatia</i>	700	(30)
	<i>Helicella itala</i>	150	4
	Operc. <i>Cyclostoma elegans</i>	(1000)	80
Fresh-water	Pulm. <i>Limnaea stagnalis</i>	100	3
	Operc. <i>Viviparus fasciatus</i>	35	1
Marine	Pulm. <i>Onchidella cellica</i>	(0)	0.27
	Operc. <i>Buccinum undatum</i>	4	(0.2)
	<i>Gibbula umbilicalis</i>	2	(0.2)
	<i>Littorina littorea</i>	1	0.2

The figures in brackets are deduced from comparison with other species.

But there remains for consideration the interesting group of fresh-water operculates. It is usually held that these animals, by reason of their gills and lack of mantle vascularisation, must have reached their present position by colonisation up the rivers. We should therefore expect that uricotelic metabolism would not occur in them and that their bodies would be nearly free of uric acid. *Hydrobia jenkinsi* gives a remarkable opportunity of testing this point, for according to Ellis [1926, pp. 31, 78] the species was entirely confined to brackish- and sea-water until near the end of the nineteenth century. Not until 1893 were the first specimens obtained from an inland situation. Subsequently it became abundant in rivers, streams and canals over all the British Isles [see also Boettger, 1931]. In accordance with expectation uric acid was found to be absent from this mollusc, not even detectable amounts being noted in samples from various parts of the country. The phenomenon was sufficiently striking, for where comparable amounts of another gastropod, *Littorina neritoides*, of about the same size (4 mg. individual body weight) would have given deep blue colours, the extract from *H. jenkinsi* was quite colourless. One catch from an Anglesea llyn included some other gastropods; 27 of these (mixed *Limnaea peregra* and *Planorbis albus*) were worked up and gave 1.6 mg./g. dry weight, i.e. similar to other fresh-water pulmonates. The difference cannot, therefore, be purely environmental.

On the other hand, *Paludina* and *Bithynia* did not behave as expected, for both of them contained amounts of uric acid corresponding to the fresh-water pulmonates. Recourse to an explanation based on high water turnover is here impossible, for while that would explain a case in which uric acid was expected but not found, it cannot explain a case in which uric acid is found where it was not expected. There seem to be two explanations which are available (1) that uricotelic metabolism is an adaptation not only to terrestrial but also to fresh-water life, (2) that the fresh-water operculates originated not from the sea direct but by way of a period of terrestrial or semi-terrestrial life, analogous to that which *Cyclostoma* and *Littorina neritoides* are now living.

With regard to the first of these possibilities one of the most general characteristics of the fresh-water fauna, as Carter has shown in numerous studies [e.g. Carter and Beadle, 1930; 1931] is the possession of adaptations for passing periods of unfavourable conditions inactively. These adaptations are particularly necessary in the fauna of the shallower and often stagnant fresh waters, for the danger of desiccation is always present. Many shallow water animals can pass through periods of drought contracted within their shells. Unfortunately for this point of view, the evidence regarding the capabilities of modern fresh-water operculate gastropods is that they possess very poor powers of withstanding

periods of complete dryness. As for the parallel case of the aestivating lung-fishes, it is now definitely known that uricotelic metabolism does not occur in them, but that like the elasmobranchs they can store in their tissues great quantities of urea [Smith *et al.*, 1930, 2]. This mechanism appears to be strictly confined to the fishes.

That the fresh-water operculates originated not directly from the sea but overland in a manner analogous to the fresh-water pulmonates is a suggestion at first sight somewhat more unwelcome to the zoologist, but not by any means impossible on closer inspection. Even in *Cyclostoma* there is a vestigial gill which under suitable conditions might grow again, accompanied by a diminution in the number and size of the blood vessels in the wall of the mantle cavity. And in the periwinkles we see a series of animals the most terrestrial of which is not far removed from the condition of the fresh-water operculates.

The celebrated series of periwinkles affords a particularly good opportunity of testing the ideas here discussed, for within one single genus there is a marked zonation of habitat on the sea-shore, *Littorina littorea* being entirely marine, *L. neritoides* almost wholly terrestrial and two other species, *L. obtusata* and *L. rudis* intermediate. Corresponding with this there is a gradation of adaptations for air-breathing [Carter, 1931]. This is represented in Table II by an arrow, and from the figures accompanying it a gradation in the amount of uric acid contained both in the nephridium and in the whole body, is apparent (Table IV). This is graphically illustrated in Fig. 1. At the bottom of the figure the

Table IV. *The periwinkle series.*

Animal	Part used	No. taken	Wet weight each g.	Uric acid	
				mg./g. wet weight	mg./g. dry weight
<i>Littorina littorea</i>	WB	5	0.48	trace	trace
	WB	20	0.71	0.041	0.205
	N	63	0.027	0.203	1.31
	N	98	0.021	0.255	1.65
	N	26	0.025	0.142	1.31
<i>Littorina obtusata</i>	WB	81	0.082	0.056	0.31
	WB	21	0.08	0.076	0.42
	N	10	0.0085	0.79	5.1
<i>Littorina rudis</i>	WB	66	0.028	0.087	0.41
	WB	154	0.029	0.106	0.50
	WB (large)	42	0.057	—	1.19
	WB (medium)	112	0.026	—	1.54
	WB (small)	232	0.011	—	1.49
<i>Littorina neritoides</i>	WB	140	0.0057	0.338	1.73
	WB	115	0.006	0.851	4.35
	WB	400	0.0063	—	0.71
	WB	755	0.0062	—	0.51

Note. N, nephridium; WB, whole body. Of *L. neritoides* Colman [1932, 1] says it "extends up the rocks several feet above high water mark, where for weeks during a calm summer its sole source of moisture is dew, rain if any, and perhaps the dampness caused by the salt on the rocks." *L. rudis* may also live in positions much above the tide mark. Ellis [1926] lists *L. neritoides* among the terrestrial snails.

percentage intertidal exposure (taken from Colman [1932, 1, 2]) is given, showing that whereas *L. littorea* is never exposed for more than 65 % of the time, *L. neritoides* is never exposed for less than 85 % of the time. Moreover, these figures allow for the splash zone. Above, the uric acid in mg./g. dry weight nephridium and whole body is given. Even if the highest value for *L. neritoides* is set aside, there still remains a definite increase with increasing dryness of habitat. At the

point marked *A* is shown the average figure for all the terrestrial pulmonates (excluding their two highest values), and at *B* the value for the single marine pulmonate. It would be tempting to make the preliminary assumption that *L. rudis* and *L. neritoides* are both uricotelic, while the others are not, and this would accord with the fact that the two first-named species are also ovoviviparous. This raises exceedingly interesting general problems which strongly

Fig. 1.

invite much further work on the nitrogen metabolism of the *Littorinidae*. The literature [*cf.* Turchini, 1923] does not seem to contain a comparative cytological study of their nephridia.

The remainder of Table II contains data about various other forms, all marine, belonging to diverse groups such as the Nudibranchs, the Solenogastres *etc.* They are at one in containing little or no uric acid, agreeing therein with their absence of terrestrial ancestry and with the marine gastropods already mentioned. The lamellibranchs were not studied in any detail, but it was curious that while the two fresh-water forms contained no detectable uric acid, the two Pectinidae had measurable amounts. A repeatable difference seems to exist

between *P. maximus* and *P. opercularis*, the former having somewhat less uric acid in its nephridium than the latter. The *Amphineura* gave uniformly negative results.

A few observations were made upon the developing embryos of *Helix aspersa* which may be of interest in view of the absence of any similar information in the literature. The undeveloped eggs contained no uric acid whatever, but the young snails examined immediately after hatching had 2.76 mg./g. dry weight in the whole body. This is analogous to the uric acid formation which Baldwin [1934] found to go on during the development of the pond-snail, *L. stagnalis*. During the development of these *H. aspersa*, which took 23 days, the wet weight of one individual decreased from 37 to 17.5 mg.; a loss of 19.5 mg. or 53 %; while the dry weight decreased from 6.8 to 5.5 mg., i.e. by 1.3 mg. or 19 %. The loss of water was therefore 48 %¹. The uric acid formed per individual was 0.007 mg. or 0.28 % of the final dry weight (excluding the weight of the shell). The comparison of these figures with those for another terrestrial cleidoic uricotelic form, the hen, is interesting. The hen's egg during its development does not lose so much water (about 15 %) probably owing to its more impermeable shell. On the other hand it loses about 18 % of its original dry weight by combustion—a figure quite close to that for the snail. During development, about 35 mg. of uric acid are formed, or 0.7 % of the final dry weight. This is again a figure of the same order as that for the development of the snail. Further work on this material would well repay the effort involved.

It is hoped that the present work may initiate a series of comparative investigations on nitrogen metabolism in molluscs. The lines of advance which most immediately present themselves are (i) the closer examination of nudibranchs, and tectibranchs such as *Aplysia*, *Philine* and *Pleurobranchus* (*Oscanius*), about which conflicting reports exist in the literature, (ii) direct determinations of the nitrogen partition of the excreta in the *Littorina* series, and in selected cases of special interest such as *Hydrobia* and *Onchidella*, (iii) nephridial determinations on the only English fresh-water Aspidobranchiate *Theodoxus* (*Neritina*) so far unobtainable, and on foreign gastropods such as *Ampullaria* which has gill and mantle-cavity of about the same size, (iv) a study of the rapidity of uric acid elimination after injection of the substance or other purines into terrestrial and aquatic species respectively, (v) a thorough examination of the nitrogen metabolism of cephalopods.

SUMMARY.

1. The uric acid retention of the nephridia and other tissues of a large number of molluscs (especially gastropods) has been studied on the assumption that uricotelic metabolism will indicate itself in this way.

2. The reliability of this assumption for molluscs is discussed in the text. If it is correct, the preliminary survey here reported demonstrates that within the homogeneous group of gastropods uricotelic metabolism is an adaptation to terrestrial life.

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¹ In this connection the results of Carmichael and Rivers on the dehydration of slug eggs are of interest. These eggs will still develop even if their wet weight is lowered 85 % by an environment of low humidity.

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