diagnosis as soon as possible. Where free fluid is demonstrated in the peritoneal cavity, diagnostic aspiration and culture may be helpful in planning treatment for a successful outcome.

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REFERENCES

1. Fonkalsrud, E. W., Ellis, D. G. and Clatworthy, H. W. (1966): J. Pediat. Surg., **1**, 227.
2. Birtch, A. G., Coran, A. G. and Gross, R. E. (1967): Surgery, **61**, 305.
3. Scott, J. E. (1963): Arch. Dis. Childh., **38**, 120.
4. Rickham, P. P. (1955): *Ibid.*, **30**, 23.
5. Thelander, H. E. (1939): Amer. J. Dis. Child., **58**, 371.
6. Thore, M. (1846): *De la Péritonite chez les Nouveau-nes*. Paris: Arch.ves générales de Médecine.
7. Churchill, F. (1850): *On the Diseases of Infants and Children*, p. 497. Philadelphia: Lea & Blanchard.
8. Leading Article (1968): Brit. Med. J., **4**, 340.
9. Pickett, L. K. (1969): *Nelson's Textbook of Pediatrics*, 9th ed. p. 820. Philadelphia: W. B. Saunders.
10. Parrish, R. A., Sherman, R. T. and Wilson, H. (1964): Ann. Surg., **159**, 244.
11. Tsingoglou, S. and Wilkinson, A. W. (1971): Arch. Dis. Childh., **46**, 452.
12. Allen, R. G. and Wrenn, E. L. jnr (1969): J. Pediat. Surg., **4**, 3.
13. Cywes, S. (1971): S. Afr. Med. J., **45**, 1134.
14. Corkey, J. J., Dubowitz, V., Lister, J. and Moosa, A. (1968): Brit. Med. J., **4**, 345.
15. Lé Orme, R. and Eades, S. M. (1968): *Ibid.*, **4**, 349.
16. Lloyd, J. R. (1969): J. Pediat. Surg., **1**, 77.
17. Parsons, J. M., Miscall, B. G. and McSherry, C. K. (1970): Surgery, **67**, 841.

Phylogenetic Aspects of Purine Metabolism*

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SUMMARY

Primitive aquatic invertebrates excrete ammonia as the end-product of purine metabolism. They possess all enzymes necessary for the breakdown of purines to ammonia. In the evolutionary line leading to man there occurred successive losses of enzymes of purine catabolism, resulting in different nitrogenous end-products Urease was lost early in vertebrate evolution, and urea is the end-product of purine metabolism in lower vertebrates. In reptiles, uric acid became the predominant end-product of amino acid as well as purine metabolism. The enzymes of uric acid catabolism disappeared, while the levels of enzymes of uric acid biosynthesis were raised in order to cope with a greatly increased turnover. Some properties of these enzymes also changed. In primitive reptiles, the switch to uric acid excretion is incomplete, and mammals, which evolved from them, excrete predominantly urea. They retained uricase, which converts uric acid to allantoin. This enzyme has, however, been lost in the evolution of anthropoids, in which uric acid is the end-product of purine metabolism.

S. Afr. Med. J., **46**, 993 (1972).

We know since the experiments of Schoenheimer[1] that all body constituents are replaced from time to time. Every organism, therefore, must possess a mechanism for breaking down purines, which are constituents of nucleic acids and nucleotides in every cell. In primitive aquatic animals, the final nitrogenous end-product of purine metabolism (and, incidentally, of amino acid metabolism) is ammonia. The reason for the breakdown of purines before excretion is twofold. Firstly, any organism exercises economy of energy; therefore, all organic compounds are broken down, and their carbon portions are oxidized to CO_2 and water, yielding energy. Secondly, and this applies specifically to purines, they must be converted to end-products which are easily disposable. Purines are relatively insoluble substances; ammonia is readily soluble. Therefore, an aquatic animal can dispose of ammonia much more readily than of purine compounds.

Fig. 1 shows the pathways of metabolism of purine nitrogen in primitive aquatic animals. Nitrogen is incorporated into the purine ring from amino acids via inosine monophosphate (IMP). When it leaves the nucleotides, it goes via hypoxanthine and xanthine to uric acid, and from there through allantoin and urea to ammonia. Two of the carbon atoms are 'salvaged' and either converted to glycine or oxidized to yield energy. It is important to remember that only a small percentage of the total nitrogen turnover actually passes by this route (Fig. 2, top); the rest, that derived from amino acids, goes directly to ammonia.

At some stage in the early evolution of vertebrates, these animals lost the enzyme urease. It is not easy to say why they lost it. One possibility is that they needed to accumulate urea as 'osmotic ballast' in a salt water environment. Another is that ammonia became too toxic an end-product for their evolving brains to tolerate. Both explanations have their weaknesses, but the fact remains that urease is not found in the Vertebrate phylum. This means that urea had become the end-poduct of purine metabolism. Often, as in Elasmobranchs and in Amphibia, this is also the main end-product of amino acid metabolism (Fig. 2, bottom). Sometimes, as in Teleosts, ammonia con-

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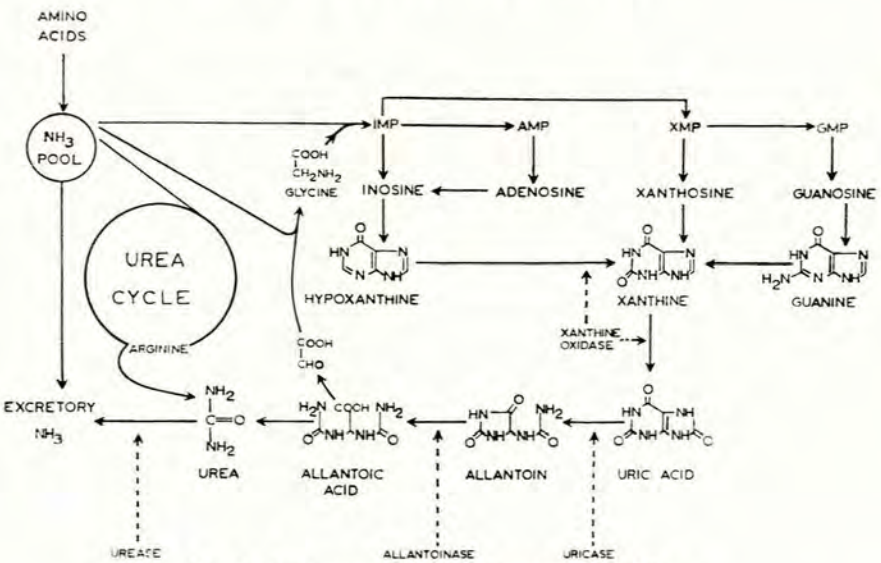

Fig. 1. Pathways of waste nitrogen metabolism.

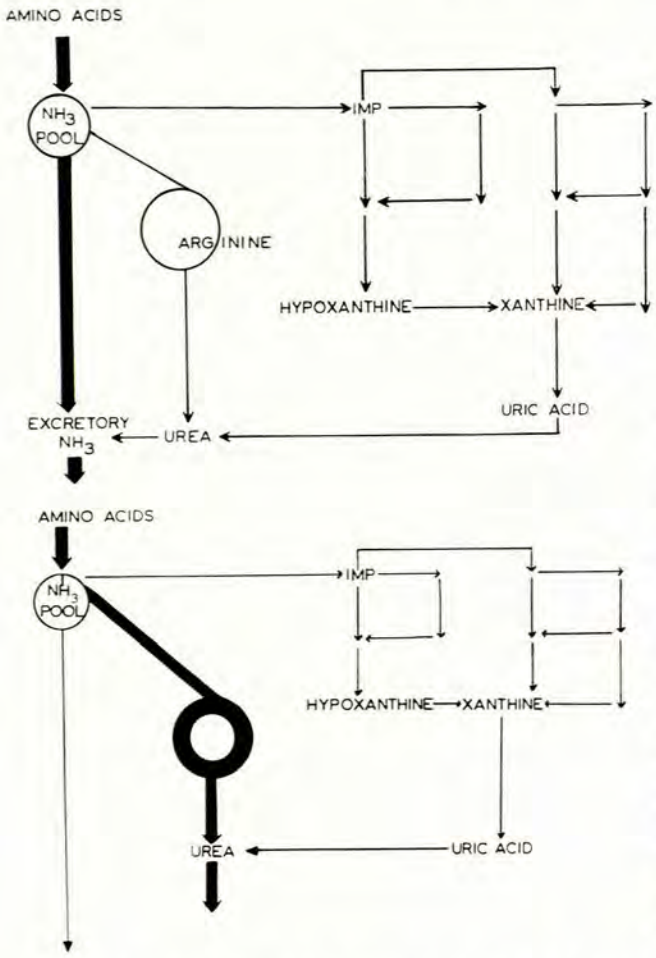

Fig. 2. Pathways of waste nitrogen metabolism in different animals. Above: **Aquatic invertebrates.** Below: **Elasmobranchs and Amphibia.**

tinues to be the main end-product of amino acid metabolism, though purines are degraded no further than urea.

Amphibia were the first land animals, and in them, the excretion of urea represents an adaptation to terrestrial life. As mentioned before, ammonia is too toxic to be the nitrogenous end-product in any terrestrial animal, which cannot get rid of it fast enough as a result of the water shortage to which it is subjected. Urea is thus a detoxification product of ammonia. An alternative detoxification product is uric acid, which we have already met as an intermediate of purine breakdown. In the course of evolution, different groups of land animals adopted different detoxification products of ammonia. Amphibia, and the mammals, like us, chose urea; land worms, land snails, insects, reptiles and birds chose uric acid. Whenever uric acid is chosen as a nitrogenous end-product, the enzymes breaking down uric acid must disappear. Moreover, the bulk of the waste nitrogen, not just a small percentage, is now channelled through the purines (Fig. 3, centre). Spiders, just to be different, chose another purine, guanine.

The big advantage of uric acid to terrestrial animals is its very low solubility. This means that it can be excreted virtually without loss of water. Removal of water from the urine merely leads to crystallization of uric acid and its excretion as a solid deposit. There is a reverse side to the medal: the insolubility of uric acid can lead to precipitation in undesired places, resulting in renal calculi or gout. The latter disease is shared by man with the uric acid-excreting chickens. The other drawback is, of course, a waste of energy involved in synthesizing a waste product. This is emphasized by the results of some measurements of the excretion of crocodiles (Table I).[2] These animals have secondarily become aquatic, and are again able to excrete large amounts of ammonia (Fig. 3, bottom).

Let us look more closely at the transition to uric acid excretion as an example of a molecular evolutionary change. We can see that it consists of two parts. Firstly, there is a loss of the enzymes of uric acid breakdown. This

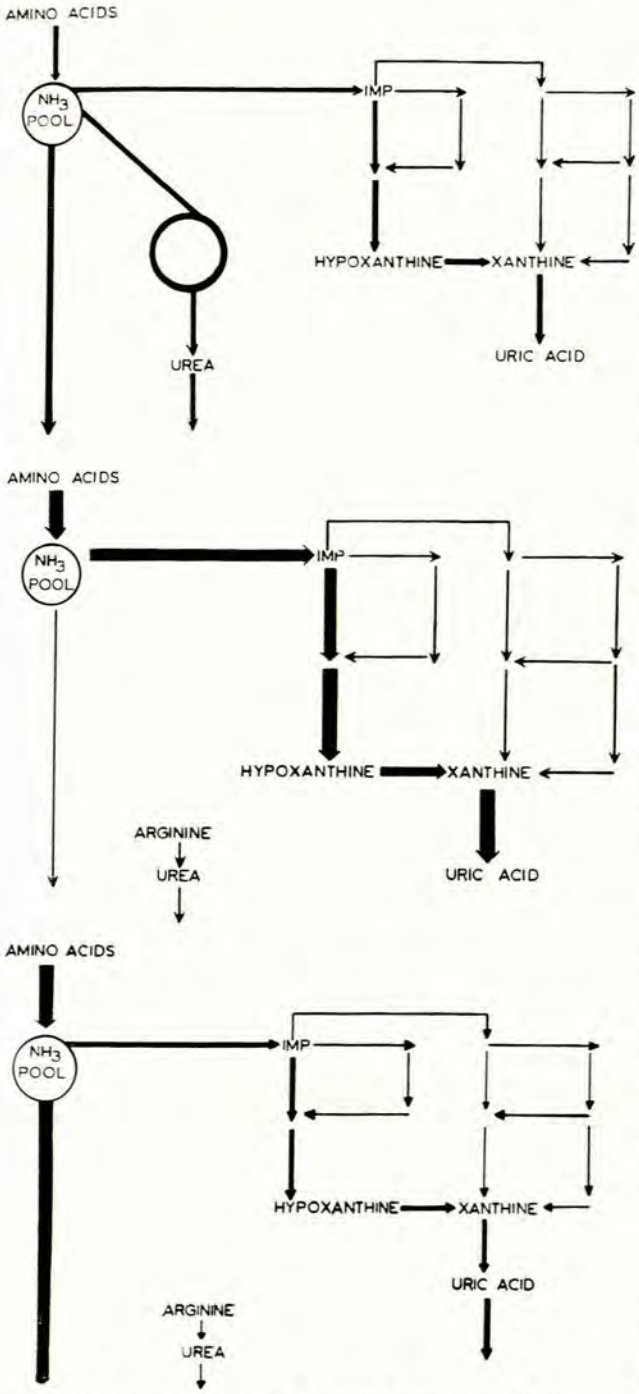

Fig. 3. Pathways of waste nitrogen metabolism in reptiles and terrestrial invertebrates. Above: **Chelonia.** Centre: **lizards, snakes, and terrestrial invertebrates.** Below: **crocodiles.**

loss, if it is really a loss of genetic information, is essentially irreversible, according to the Law of Dollo. As we shall see, this fact assumes a somewhat sinister significance when considered in relation to ourselves. Secondly, there is a change of function of an enzyme system from the purpose of purine biosynthesis to that of detoxifying mechanism. No totally new enzymes are acquired. A change of function is a well-accepted concept in morphological evolution, and it holds just as well for biochemical processes. As in morphology, its value is that it makes much easier the appearance of a new metabolic function.

A change of function does not mean merely the rewriting of a metabolic flow sheet. Very real biochemical changes are associated with it. The mechanism of purine biosynthesis now has to cope with the entire nitrogen turnover of the organism. Understandably, this requires much higher levels of the enzymes involved. Table II shows the results of some measurements made in our laboratory of the levels of xanthine oxidase in the livers and kidneys of various reptiles. The levels in lizard kidney are much higher than in mammalian liver. Another interesting fact is that lizards have xanthine oxidase only in the kidney; snakes have it both in liver and kidney,[3,4] an interesting distinction between the two groups which might have diagnostic value for systematists. It implies that, in snakes, some of the uric acid must be formed in the liver and circulated in the bloodstream before being excreted. In lizards, on the other hand, hypoxanthine, which is formed in the liver, circulates in the bloodstream.[5] The latter compound, being more soluble, appears preferable; as circulating uric acid carries the greater chance of forming insoluble precipitates leading, for example, to gout. In birds, the pigeon appears to have xanthine oxidase mainly in the kidney,[6] while the chicken has it in both organs. As mentioned before, gout among chickens is a well-known disease.

Besides changes in level of enzymes, there can be changes in properties. Thus 'xanthine oxidase' in birds[7] and reptiles,[4] unlike mammals, is actually unable to react with molecular oxygen. This is indicated by the fact that methylene blue has to be added to an extract containing this enzyme before any oxygen uptake due to hypoxanthine oxidation can be observed (Fig. 4). In our experiments it reacted with methylene blue as intermediate hydrogen acceptor. *In vivo* it probably uses NAD.[8] This would have the advantage that some of the energy invested in the biosynthesis of IMP is actually recovered by reducing the nucleotide, and generating energy by the usual process of oxidative phosphorylation. In animals which have a high turnover of purines, this economy is well worth while.

The most primitive of today's reptiles, the Chelonia, have not entirely switched to uric acid excretion. All 3 compounds, ammonia, urea, and uric acid, can be excreted by them in large amounts (Fig. 3, top). The predominant end-product is correlated with the environment (Table III).[9,10] Fully aquatic species excrete mainly ammonia; semi-aquatic ones excrete urea. In the terrestrial species, urea or uric acid may predominate, though those species which prefer a very dry environment tend to excrete a larger proportion of uric acid. We have, therefore, no clear picture of what was excreted by primitive reptiles, and, more especially, by the mammal-like reptiles. This point is of considerable interest, because mammals lack allantoinase and allantoicase. They, therefore, do not degrade uric acid beyond allantoin. This loss does not make sense except it be associated with uric acid excretion

somewhere along the evolutionary line. Fortunately for them, the mammals did not lose uricase. They are, therefore, able to convert the highly insoluble uric acid into the somewhat more soluble allantoin. (Fig. 5, top). This applies to all mammals, except the anthropoids, to which we belong. These animals have lost uricase (Fig. 5, bottom) apparently by accident. This exposes us to the dangers associated with hyperuricaemia. Fig. 6 summarizes the evolutionary trend which has brought us to our present state.

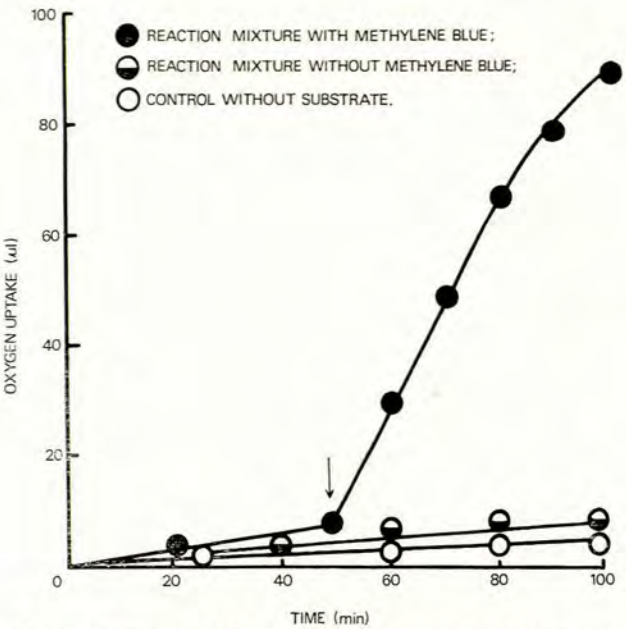

Fig. 4. Oxygen uptake of a liver homogenate extract of *Boaedon fuliginosus*, with hypoxanthine as substrate. The reaction mixture contained tissue extract, and final concentrations of 3 mM hypoxanthine, 40 mM sodium phosphate buffer, pH 7,4, and 0,68 mM methylene blue where indicated. Oxygen uptake was measured manometrically in a Warburg apparatus. An arrow marks the point at which the substrate was tipped in from the side arm of each flask.

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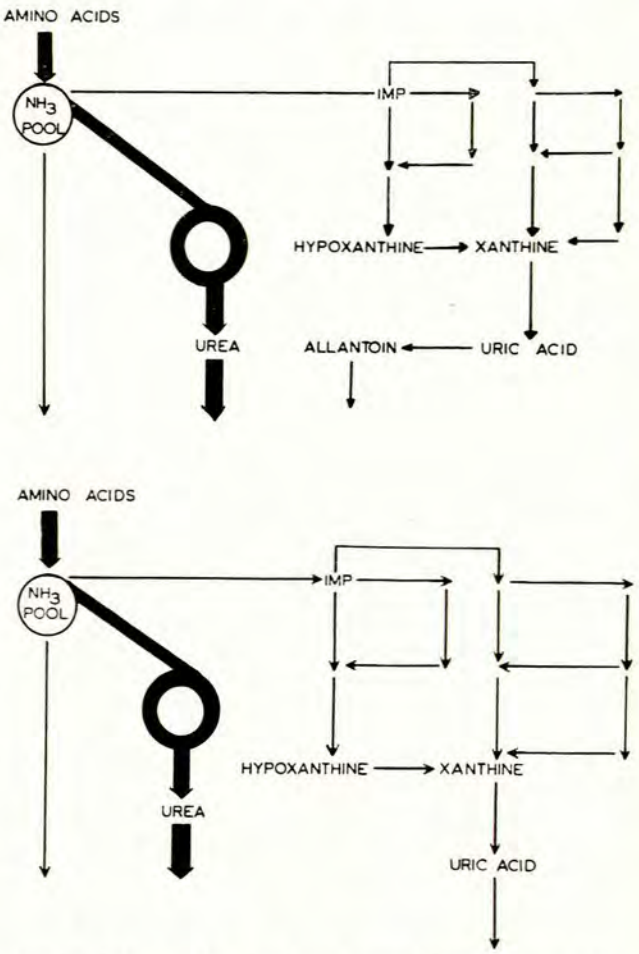

Fig. 5. Pathways of waste nitrogen metabolism in mammals. Above: **Most mammals.** Below: **Anthropoids.**

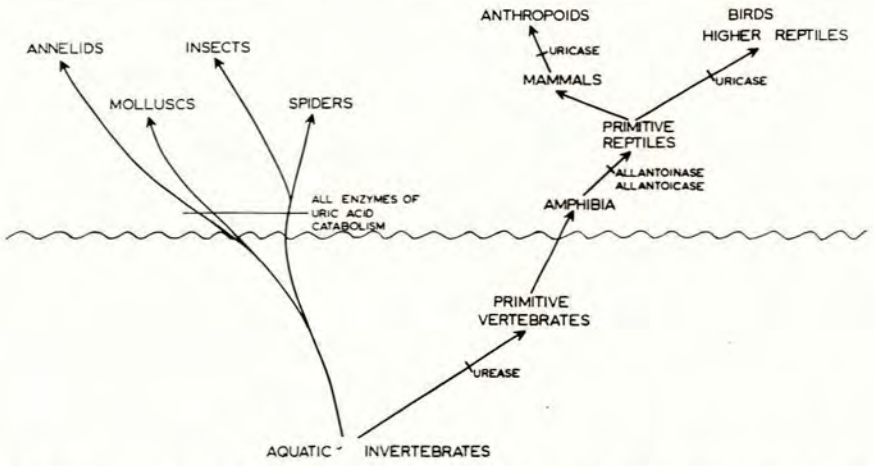

Fig. 6. Evolutionary loss of enzymes of uric acid catabolism. Loss is indicated by a transverse bar.

TABLE I. NITROGEN EXCRETION OF 2 SPECIES OF *CROCODILIA*[2] (MEAN VALUES ARE QUOTED, FOLLOWED BY STANDARD ERRORS)

Species	No. of experiments	Percentage of total non-protein nitrogen excreted as		
		Ammonia	Urea	Uric acid
Crocodylus niloticus	7	66,2 ± 3,5	4,5 ± 1,1	20,8 ± 3,5
Caiman crocodilus	6	52,5 ± 5,2	5,9 ± 1,3	27,3 ± 5,9

TABLE II. ACTIVITY OF XANTHINE OXIDOREDUCTASE IN THE LIVER AND KIDNEY OF DIFFERENT SPECIES (MEAN VALUES ARE QUOTED, FOLLOWED BY STANDARD ERRORS, WITH THE NUMBER OF ANIMALS IN PARENTHESES[12])

Class, order or suborder	Species	Xanthine oxidoreductase activity (μg atoms O_2 absorbed per min per g wet weight)		
		Liver	Kidney	
Lizards	*Homopholis wahlbergii*	0,0	5,7 ± 2,8	(3)
	Pachydactylus bibronii	0,0	5,2	
	Agama atra	0,0	4,6	
	Agama hispida	0,0	6,4 ± 1,7	(3)
	Chamaeleo dilepis	0,0	1,8	
	Mabuia capensis	—	6,8 ± 0,4	(2)
	Mabuia striata	0,0	7,1 ± 1,5	(3)
	Gerrhosaurus flavigularis	0,0	9,2 ± 2,2	(2)
	Cordylus giganteus	—	8,9	
	Cordylus polyzonus	0,0	6,4	
	Cordylus vittifer	0,0	6,4 ± 1,8	(4)
	Pseudocordylus microlepidotus fasciatus	—	9,5	
Snakes	*Boaedon fuliginosus*	1,1	2,4	
	Aparallactus capensis	0,9	1,2	
	Crotaphopeltis hotamboeia	1,9	0,7	
	Atractaspis bibronii	3,0	0,6	
Tortoises	*Geochelone paradalis*	0,20	0,06	
Birds	**Chicken**	3,0[7]		
Mammals	**Rat**	0,96[11]		

TABLE III. NITROGEN EXCRETION OF 5 SPECIES OF *CHELONIA*[13] (MEAN VALUES ARE QUOTED, FOLLOWED BY STANDARD ERRORS)

Species	Habitat	No. of experiments	Percentage of total nonprotein nitrogen excreted as		
			Ammonia	Urea	Uric acid
Pseudemys scripta	**Aquatic**	11	67,1 ± 4,5	5,7 ± 4,5	0,3 ± 0,1
Pelusios sinuatus	**Semi-aquatic**	3	16,0 ± 1,1	62,6 ± 10,6	0
Geochelone angulata	**Terrestrial**	3	3,6 ± 1,1	55,1 ± 4,0	20,4 ± 2,7
Geochelone paradalis	**Terrestrial**	2	6,1 ± 1,5	36,5 ± 2,0	46,8 ± 12,8
Kinixys belliana	**Terrestrial**	1	3,7	23,4	42,5

REFERENCES

1. Schönheimer, R. (1946): *Dynamic State of Body Constituents*. Cambridge, Mass.: Harvard University Press.
2. Cragg, M. M., Balinsky, J. B. and Baldwin, E. (1961): Comp. Biochem. Physiol., **3**, 227.
3. Khalil, F. and Haggag, G. (1960): Z. vergl. Physiol., **43**, 269.
4. Murison, G. L. and Balinsky, J. B. (1961): S. Afr. J. Med. Sci., **26**, 129.
5. Balinsky, J. B., Murison, G. L., Conradie, A. R. and Santo, R. E. (1964): *Ibid.*, **29**, 61.
6. Edson, N. L., Krebs, M. A. and Model, A. (1936): Biochem. J., **30**, 1380.
7. Remy, C. N., Richert, D. A., Doisy, R. J., Wells, I. C. and Westerfeld, W. W. (1955): J. Biol. Chem., **217**, 293.
8. Landon, E. J. and Carter, C. E. (1960): *Ibid.*, **235**, 819.
9. Moyle, V. (1949): Biochem. J., **44**, 581.
10. Balinsky, J. B., Mordant, B. S., Schneiderman, E. R. and Shachman, M. M. (1964): S. Afr. Med. Sci., **29**, 95.
11. Westerfeld, W. W. and Richert, D. A. (1952): J. Biol. Chem., **199**, 383.
12. Murison, G. L. and Balinsky, J. B. Unpublished data.
13. Mordant, B. S. and Balinsky, J. B. Unpublished data.