to Dr R. L. M. Synge, F.R.S., for gifts of gramicidin S and α -(L-valyl)-L-ornithine. One of us (I.M.L.) is indebted to the Medical Research Council for a Scholarship.

REFERENCES

- Barry, G. T., Gregory, J. D. & Craig, L. C. (1948). *J. biol. Chem.* **175**, 485.
- Battersby, A. R. & Craig, L. C. (1951). *J. Amer. chem. Soc.* **73**, 1887.
- Blackburn, S. & Lowther, A. G. (1951). *Biochem. J.* **48**, 126.
- Brockmann, H., Grubhofer, N., Kass, W. & Kalbe, H. (1951). *Ber. dtsh. chem. Ges.* **84**, 260.
- Burton, H. S. (1954). *Chem. & Ind.* p. 576.
- Chargaff, E., Levine, C. & Green, C. (1948). *J. biol. Chem.* **175**, 67.
- Chibnall, A. C. & Rees, M. W. (1953). In *Chemical Structure of Proteins*, p. 79. London: J. and A. Churchill Ltd.
- Consden, R., Gordon, A. H. & Martin, A. J. P. (1947). *Biochem. J.* **41**, 590.
- Craig, L. C., Hausmann, W. & Weisiger, J. R. (1953). *J. biol. Chem.* **200**, 765.
- Craig, L. C., Hausmann, W. & Weisiger, J. R. (1954). *J. Amer. chem. Soc.* **76**, 2839.
- Dermer, O. C. & Hutcheson, J. W. (1943). *Proc. Okla. Acad. Sci.* **23**, 60.
- Ehrlich, F. (1907). *Ber. dtsh. chem. Ges.* **40**, 2538.
- Grassmann, W., Hörmann, H. & Endres, H. (1953). *Ber. dtsh. chem. Ges.* **86**, 1477.
- Harfenist, E. J. (1953). *J. Amer. chem. Soc.* **75**, 5528.
- Hausmann, W., Weisiger, J. R. & Craig, L. C. (1955a). *J. Amer. chem. Soc.* **77**, 723.
- Hausmann, W., Weisiger, J. R. & Craig, L. C. (1955b). *J. Amer. chem. Soc.* **77**, 721.
- Hirs, C. H. W., Stein, W. H. & Moore, S. (1954). *J. biol. Chem.* **211**, 941.
- Ingram, V. M. (1953). *J. biol. Chem.* **202**, 193.
- Kaczka, E. & Folkers, K. (1949). In *The Chemistry of Penicillin*, p. 244. Princeton University Press.
- Karrer, P., Portmann, P. & Suter, M. (1948). *Helv. chim. acta*, **31**, 1617.
- Kuhn, R. & Drawert, F. (1954). *Liebigs Ann.* **590**, 55.
- Lockhart, I. M. & Abraham, E. P. (1954a). *Biochem. J.* **58**, 633.
- Lockhart, I. M. & Abraham, E. P. (1954b). *Biochem. J.* **58**, xlvii.
- Lockhart, I. M., Newton, G. G. F. & Abraham, E. P. (1954). *Nature, Lond.*, **173**, 536.
- Lowther, A. G. (1951). *Nature, Lond.*, **167**, 767.
- Markham, R. (1942). *Biochem. J.* **36**, 790.
- Mills, G. L. (1950). *Nature, Lond.*, **165**, 403.
- Mills, G. L. (1952). *Biochem. J.* **50**, 707.
- Moore, S. & Stein, W. H. (1948). *J. biol. Chem.* **176**, 367.
- Moore, S. & Stein, W. H. (1951). *J. biol. Chem.* **192**, 663.
- Moore, S. & Stein, W. H. (1954). *J. biol. Chem.* **211**, 907.
- Neuberger, A. (1948). *Advanc. Protein Chem.* **4**, 297.
- Newton, G. G. F. & Abraham, E. P. (1953a). *Biochem. J.* **53**, 597.
- Newton, G. G. F. & Abraham, E. P. (1953b). *Biochem. J.* **53**, 604.
- Newton, G. G. F. & Abraham, E. P. (1954). *Biochem. J.* **58**, 103.
- Piez, K. A. (1954). *J. biol. Chem.* **207**, 77.
- Porath, J. (1953). *Nature, Lond.*, **172**, 871.
- Porath, J. (1954). *Acta chem. scand.* **8**, 1813.
- Richter, D. (1937). *Biochem. J.* **31**, 2022.
- Schroeder, W. A. & LeGettê, J. (1953). *J. Amer. chem. Soc.* **75**, 4612.
- Synge, R. L. M. (1945). *Biochem. J.* **39**, 363.
- Weisiger, J. R., Hausmann, W. & Craig, L. C. (1955). *J. Amer. chem. Soc.* **77**, 731.
- Woiwod, A. J. (1949). *J. gen. Microbiol.* **3**, 312.
- Work, E. (1949). *Biochim. biophys. Acta*, **3**, 400.

Nitrogen Excretion in the Tadpoles of *Xenopus laevis* Daudin

BY ELUNED E. UNDERHAY* AND E. BALDWIN

Department of Biochemistry, University College, London

(Received 13 May 1955)

As was pointed out by Delaunay (1931), and emphasized by Needham (1931), the excretion of ammonia as the predominant end-product of protein metabolism is characteristic of aquatic animals as a whole but is supplanted by that of urea (amphibia; chelonian reptiles; mammals) or uric acid (saurian reptiles; birds) in amphibious and terrestrial animals. Needham (1931) collected numerous data in support of this general proposition.

The amphibia are of particular interest from this point of view since they are, in general, wholly

aquatic during the larval period and go on the land only after metamorphosis. Munro (1939) found that the tadpoles of *Rana temporaria* are wholly ammoniotelic, and that the transition to ureotelism takes place in the course of metamorphosis. Thus this animal recapitulates its chemical as well as its evolutionary past in the course of its larval development.

In *Xenopus laevis* we have an amphibian which passes both its larval and adult lives without leaving the water. Unlike *Rana* and the other frogs and toads that have been studied, the adult *Xenopus* continues to excrete the bulk of its waste nitrogen as ammonia (Munro, 1953; Cragg, 1953).

* Present address: Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, London, W. 1.

Ammonia-nitrogen accounts for 60–80% of the total nitrogen, but urea is nevertheless present in the excreta and accounts for some 20% of the total nitrogen. These facts harmonize with the generally accepted opinion that *Xenopus* is descended from truly amphibious and therefore presumably ureotelic ancestors, but has made a secondary return to the water. In so doing it has reverted largely to the aquatic and ammoniotelic type, though retaining still a small measure of ureotelism as evidence of its evolutionary past.

A somewhat similar case was discovered by Moyle (1949) among chelonian reptiles; here typically amphibious species excrete some 40–60% of the total nitrogen in the form of urea and only some 5% as ammonia, whereas secondarily aquatic forms produce only about 20–25% of the total nitrogen as urea and about 20% in the form of ammonia.

Interesting changes in the nitrogen excretion of *Xenopus* tadpoles are evidently to be expected, though Munro (1939, 1953) could find no evidence that they and the tadpoles of *Rana* behave at all similarly; *Rana*, but not *Xenopus*, changed over to ureotelism at metamorphosis.

Cragg (1953) found that adult specimens of *Xenopus*, if kept in moist surroundings, but out of water, excreted very little nitrogen but appeared rather to store it as urea, for, on their return to the water, a massive excretion of urea took place. Little ammonia was excreted, and only after some days was the normal pattern of excretion re-established. In this respect *Xenopus* resembles the lungfish *Protopterus* during and immediately after aestivation (Smith, 1930). Alexander & Bellerby (1938) report that the adult *Xenopus* commonly aestivates during the dry season and it seems probable that it survives by storing urea, as does *Protopterus*, until the rains come.

Clearly, then, in spite of its ammoniotelic habits, *Xenopus* has the ability to produce urea from the bulk of its waste nitrogen. It seemed to us desirable to discover, if we could, at what stage in development this ability makes its appearance. Tadpoles of *Xenopus* were accordingly examined at intervals up to the end of metamorphosis. Ammonia and urea were determined in the environment at a number of stages and the ratios of ammonia-nitrogen to urea-nitrogen so obtained were compared at successive developmental stages.

MATERIALS AND METHODS

Care of animals. Tadpoles of *Xenopus laevis* were obtained 2 days after hatching and immediately pipetted into tall glass jars, each containing about 4.5 l. of tap water. From 45 to 50 tadpoles were placed in each jar and kept under conditions similar to those described by Deanesly & Parkes (1945). No special precautions were taken to keep

the temperature of the water constant; daily observations of the maximum and minimum air temperature showed that it fluctuated between 13° and 24°.

The tadpoles were fed entirely on freeze-dried liver powder from which the larger particles had been removed by sieving. From about the age of 1–7 weeks they were fed twice weekly and then three times weekly until metamorphosis. After metamorphosis small pieces of rat liver or earthworm were substituted for the liver powder.

As the time of metamorphosis approached the animals were allowed a greater volume of water. At metamorphosis, which began about 11 weeks after hatching, some individuals were placed in separate small vessels for observation, but because of the large numbers this was not possible in all cases.

Collection of excretory products. For the collection of excretory products, animals were kept in a known volume of water for 24 hr., the number of animals in each experiment depending upon their size and the volume of water used. Each group was so selected that its members were all at a similar stage of development. An immediate initial determination of any NH_3 that might have been transferred with the animals was made. No food was given during the test period, but the animals were fed normally up to the start of the 24 hr. collection period. At the end of this time the tadpoles were removed from the water, drained on filter paper and weighed in a bottle containing a few millilitres of clean water. The samples were filtered to remove any faecal matter and then analysed.

No animals died during the collection periods, although a small number, especially in the early stages, died as a result of being weighed.

Ammonia was determined colorimetrically by the phenol-hypochlorite reaction. The method was essentially that used by Russell (1944), except that bleaching powder was replaced by a 40% (v/v) solution of Deosan (Deosan Ltd., 345 Gray's Inn Road, London, W.C. 1). All determinations were carried out with a Gallenkamp colorimeter fitted with an Ilford 204 filter (orange-red). Ammonium sulphate solutions were used as standards for calibration.

With all solutions containing tap water, i.e. urine specimens and standards, a slight brown gelatinous precipitate formed when the colour developed during heating in the water bath, and this was removed by filtration. Control experiments with standard solutions in both tap and distilled water showed that filtration did not affect the colorimeter readings.

The laboratory atmosphere was kept as free as possible of ammonia. To discover whether any absorption of atmospheric ammonia took place, tubes containing standard solutions of ammonium sulphate were left, at various stages during the colour development, for 5 min. on the bench near an unstoppered bottle containing dilute NH_3 soln. No absorption could be detected.

Urea was determined by treating the specimens with soya-bean urease in a Conway unit, the NH_3 produced being allowed to distil into boric acid and then estimated together with the preformed NH_3 (i.e. the fraction excreted as free NH_3) by the procedure described above. Preformed NH_3 itself was also determined after distillation in a Conway unit to check possible losses, which proved, however, to be negligible.

RESULTS

The growing tadpoles, even when kept under identical conditions, do not reach any given stage of development at the same age. However, when animals of different age but at the same developmental stage were studied, it was found that the changes in the percentage of nitrogen excreted as ammonia corresponded to the developmental stage and to weight but not to age. This is shown by the results given in Table 1.

Table 1. *Effect of age, weight and development on ammonia production*

The weight groups are selected from those listed in Table 2 and the number of animals in each group is given there. A, Animals of the same age vary in size and also in excretory product; B, animals of different age but similar size show similar excretory behaviour.

Age (days)	Weight (mg.)	Total $\mu\text{g.}$ of N excreted/g. body wt./day	Percentage of total N as free NH_3
A 38	46	302	78
40	224	253	52
A 52	142	352	50
54	34	141	75
59	366	229	39
B 24	39	278	79
54	34	141	75

To follow the changes that occur in the excretion of ammonia and urea up to and through the period of metamorphosis, the animals were therefore divided into groups with similar characteristics of development and thus of similar weight.

As Table 2 shows, very young tadpoles excrete nitrogen mainly in the form of ammonia. As they gain in weight the relative percentage of ammonia decreases and reaches a minimum at the time when the hindlimbs are developing but increases again when the forelimbs emerge and the head and tail shrink. After completion of metamorphosis the excretion of ammonia is still higher and exceeds by far that during metamorphosis itself.

The results listed in Table 2 also show that the total nitrogen excreted per day per gram body weight increases abruptly when head and tail are shrinking. A similar but less marked change has been reported by Munro (1953).

DISCUSSION

These results show that the larval *Xenopus laevis* passes under normal conditions through a stage during which a significantly higher percentage of nitrogen is excreted in the form of urea than is found in either very young larvae or in the adult. These findings are at variance with those of Munro (1953), but the discrepancy may well be due to the

Table 2. *Nitrogen partition in excreta of developing Xenopus tadpoles*

Developmental stage	Average wt. of one animal (mg.)	No. of animals	Total vol. of water (ml.)	$\mu\text{g.}$ of N excreted/tadpole/24 hr.		Ammonia-N Urea-N	Total $\mu\text{g.}$ N excreted/day/g. of animal
				NH_3	$\text{NH}_3 + \text{urea}$		
Hindlimbs absent	9-25*	18	25	2.9	3.3	7.3	—
	16-24*	20	25	5.1	5.7	8.5	—
	22	12	30	2.1	2.9	2.6	130
	23	10	25	6.1	7.8	3.6	337
	34	15	30	3.6	4.8	3.0	141
	39	10	30	8.6	10.9	3.7	278
	46	10	30	10.9	13.9	3.6	302
	75	10	50	11.0	17.0	1.8	227
	97	6	50	19.3	31.2	1.6	322
Hindlimbs developing	142	5	50	25.0	50.0	1.0	352
	224	4	112	31.5	60.0	1.1	253
	230	4	98	22.9	49.9	0.85	217
	258	4	100	44.0	65.5	2.0	254
	366	4	98	32.3	83.5	0.63	229
	446	4	93	27.9	71.8	0.64	161
	488	3	99	52.8	138.6	0.62	284
	516	3	99	81.8	135.7	1.5	283
	584	3	95	54.3	82.8	1.9	142
Forelimbs emerged, head and tail unchanged	602	2	99	122.8	186.6	1.9	310
	763	2	99	62.0	149.8	0.7	196
Head shrunk and tail shrinking	323	3	100	122.0	188.7	1.8	584
Tailless: metamorphosis completed about 10 days previously	496	3	99	231.7	311.2	2.9	627

* Upper and lower weight limits calculated by extrapolation from (ammonia + urea)-N/g. animal/24 hr in all the other experiments apart from those during metamorphosis.

fact that Munro's experiments were carried out under less satisfactory conditions; he himself states that more than half the tadpoles died during the first week, and that from the original 250 eggs only 10 survived to metamorphosis. Our own animals were well fed on a high-protein diet and had a high survival rate. Munro, on the other hand, does not state that his animals were fed but only that they were kept in water 'to which had been added an infusion of raffia, mud and weed obtained from a pond supporting a large frog population'.

According to our findings the changes during metamorphosis are similar to those found in other amphibians for which the ratio of ammonia to urea during development has been investigated. These are *Rana temporaria*, *Bufo bufo bufo*, *Triturus vulgaris*, *cristatus* and *Axolotl* (Munro, 1939, 1953). The only difference between *Xenopus* and these other species is that whereas *Xenopus* returns to a predominant excretion of ammonia after displaying a tendency to ureotelism during its larval life, the other species become and remain ureotelic during adult life. It appears in fact that a progressive tendency towards ureotelism is a feature of the larval life of amphibia in general, but, whereas the completion of metamorphosis is commonly attended by the development of total ureotelism, *Xenopus* shows only a transient move towards ureotelism and reverts towards ammoniotelism again even before the forelimbs begin to emerge.

Taken together with the observations of Cragg (1953) and Munro (1939, 1953), our results suggest that if *Xenopus* were to live a terrestrial or semi-terrestrial instead of an aquatic life, it would be capable of meeting the problems of nitrogen disposal in the manner usual among amphibia, i.e. by

ureotelism. Indeed, it seems likely that its survival during the dry season depends upon precisely this device. Certainly the dependence of *Xenopus* on an aquatic habitat is not to be attributed to failure to develop the machinery necessary for ureogenesis.

SUMMARY

1. The excretion of nitrogen in the form of ammonia and urea by *Xenopus* tadpoles has been studied up to the end of metamorphosis.

2. There is a marked increase in the relative proportion of urea nitrogen in the early stages of metamorphosis followed by a fall as metamorphosis proceeds.

3. The total nitrogen excreted/gram body weight/24 hr. shows a sharp increase in the late stages of metamorphosis.

Our thanks are due to Dr D. R. Newth, who kindly provided the tadpoles used in this work, to Dr Mavis Cragg for permission to quote from her unpublished results, and to Dr W. H. Hampton, who first drew our attention to the fact that *Xenopus* tadpoles excrete urea.

REFERENCES

- Alexander, S. S. & Bellerby, C. W. (1938). *J. exp. Biol.* **15**, 74.
 Cragg, M. M. (1953). Ph.D. Thesis, London University.
 Deanesly, R. & Parkes, A. S. (1945). *J. Endocrin.* **4**, 324.
 Delaunay, H. (1931). *Biol. Rev.* **6**, 265.
 Moyle, V. (1949). *Biochem. J.* **44**, 581.
 Munro, A. F. (1939). *Biochem. J.* **33**, 1957.
 Munro, A. F. (1953). *Biochem. J.* **54**, 29.
 Needham, J. (1931). *Chemical Embryology*, vol. 2, p. 1137. Cambridge University Press.
 Russell, J. A. (1944). *J. biol. Chem.* **156**, 457.
 Smith, H. W. (1930). *J. biol. Chem.* **88**, 97.

The Branched-Chain Fatty Acids of Ox Fat

3. THE ISOLATION OF 14-METHYLPENTADECANOIC ACID (ISOPALMITIC ACID) FROM OX PERINEPHRIC FAT*

By R. P. HANSEN, F. B. SHORLAND AND N. JUNE COOKE

Fats Research Laboratory, Department of Scientific and Industrial Research, Wellington, New Zealand

(Received 8 June 1955)

Ox perinephric fat has been shown to contain trace quantities of the branched-chain fatty acids (+)-14-methylhexadecanoic acid (Hansen, Shorland & Cooke, 1952) and 15-methylhexadecanoic acid (Hansen, Shorland & Cooke, 1955b); in this paper is reported the occurrence of the C_{18} iso-acid 14-methylpentadecanoic acid.

* Part 2: Hansen *et al.* (1955b).

EXPERIMENTAL

As reported earlier (Hansen *et al.* 1955b) fractionation of the 'liquid' methyl esters obtained from hydrogenated ox perinephric fat yielded 22 fractions, the following four of which pertain to this paper: J/21, L12, wt. 38.06 g., saponification equiv. 272.1, m.p. 22.8–23.8°; J/21, L13, wt. 41.59 g., saponification equiv. 272.3, m.p. 23.9–25.9°; J/21, L14, wt. 41.72 g., saponification equiv. 273.1, m.p.