5. On standing at pH 1 a violet pigment of unknown structure was formed which absorbed maximally at 503–505 mμ.

6. It has not been possible to separate a second precursor from porphyria urines.

7. The experiments reported, while not providing conclusive evidence, support the view that porphobilinogen itself can form porphyrin, yellow pigment and violet pigment according to the conditions.

We wish to express our thanks to Prof. C. Rimington for helpful discussions, and to the Medical Research Council for a personal grant to one of us (P.E.B.) and for an expenses grant to C.H.G.

Note. Since this paper was accepted for publication, crystalline porphobilinogen has been prepared from human acute porphyria urine (Westall, R. G. (1952), *Nature, Lond.*, **170**, 614). The results described in the present paper have been confirmed in every respect using crystalline material we have prepared by Westall's method, from the urine of rabbits in which porphyria had been induced by the administration of Sedormid (Schmid, R., quoted by Lowry, P. T., Hawkinson, V. & Watson, C. J. (1952), *Metabolism*, **1**, 149).

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The Ammonia and Urea Excretion of Different Species of Amphibia during their Development and Metamorphosis

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Metamorphosis is a short but none the less critical stage in the development of Amphibia during which extensive changes occur, enabling them to transfer from an aquatic to a more or less terrestrial habitat. The anatomical changes associated with the transition have been described by numerous workers, but the underlying chemical and metabolic transformations demanded by such changes are still largely obscure. The present paper deals with one aspect of nitrogen excretion during metamorphosis.

Some years ago the present author observed that metamorphosis in the common frog was characterized by a change in the chemical form in which nitrogen was excreted. The tadpole, up to the time of metamorphosis, excreted about 10 times more of its waste nitrogen as ammonia than as urea, while the frog excreted nitrogen mainly as urea. The change-over was accompanied by a corresponding increase in the concentration of liver arginase (Munro, 1939).

These changes are interesting because they are in harmony with the concept that the form in which waste nitrogen is excreted is governed by the type of environment, aquatic or terrestrial, within which the animal develops and later functions as an adult. Needham (1931) has collected data on the urinary nitrogen partition of a large number of animal

species. They suggest that aquatic animals in general excrete a much higher proportion of their waste nitrogen as ammonia than do terrestrial species. The reason for this may be that ammonia is a highly alkaline substance and is likely to be damaging to the animal's tissues generally, and to the kidney in particular, unless its concentration in the body is kept low by a copious excretion of urine; which of course is most easily accomplished in a freshwater environment.

There exists among the Amphibia a variety of forms showing different degrees of aquatic and terrestrial adaptation. This is associated with corresponding peculiarities of structure, development and metamorphosis. It thus appeared likely that a survey of the form in which nitrogen was excreted during development and metamorphosis in representative members of the group might provide further and more precise evidence of the relation between this characteristic in an animal and its habitat or manner of development.

MATERIAL AND METHODS

The tadpoles of *Rana temporaria* and of *Bufo bufo bufo* in the stock jars were fed on ant pupae, the axolotls (*Siredon mexicanum*) on worms and chopped liver and the adult *Xenopus laevis* on small pieces of liver. The *Rana* tadpoles were prematurely metamorphosed by immersion for 2 days in a thyroid solution prepared by shaking 1 part of desiccated thyroid gland (B.P.) with 400 parts of water and removing the undissolved material by centrifugation (Huxley, 1925). Metamorphosis was stimulated in the axolotls by injecting 1 mg. thyroxine.

Xenopus eggs were obtained by injecting 10 mg. of a crude extract of anterior pituitary-like hormone (A.P.L.) dissolved in 1 ml. of distilled water into the dorsal lymph sacs of a number of adult male and female *Xenopus* toads. The extract was prepared from human pregnancy urine (Langrebe, 1939). Amplexus occurred some hours later and a batch of about 250 eggs was subsequently obtained. Embryonic development was passed in a dish of water. Hatching occurred in 2–3 days. A week later the larvae began to feed and they were transferred to a tank of water to which had been added an infusion of raffia, mud and weed obtained from a pond supporting a large frog population. More than half of the larvae died during the first week. Only ten survived to the stage of metamorphosis.

Hypophysectomized *Xenopus laevis* adults were obtained as a gift from Prof. Langrebe of Cardiff University to whom the author is indebted.

Each type of experiment varied in certain particulars which are referred to in the appropriate sections, but in every instance the method of determining the nitrogen excretion was to place the living material being examined, eggs, larvae or adults, in a known volume of water for a definite time, usually 24 hr., and then to take samples of the fluid for analysis. In order to prevent diffusion of NH_3 from the water into the atmosphere, the fluid was maintained at pH 6·5 with acetic acid and Na_2HPO_4.

Before being used in an experiment, the animals were fasted for 3 days, in order to reduce contamination of the circumambient fluid with formed excreta. An example will indicate the method of transferring the animals from the stock jars to the experimental containers. A group of twenty tadpoles was removed from the stock jar by suction into a wide-mouthed pipette with a rubber teat. They were then washed with water in a Büchner funnel, gently shaken to drain off the surplus water, and tipped into a dish containing 30 ml. of the buffered fluid and left for 24 hr. in a large glass box where the air temperature was maintained at 18·5° ± 1°.

The technique adopted to collect the nitrogen excretion from the head and gill region of the axolotl separately from that of the posterior region, was similar to that adopted by Smith (1929). The axolotl was placed in a metal box of the type shown in Fig. 1. This is in two parts clamped together

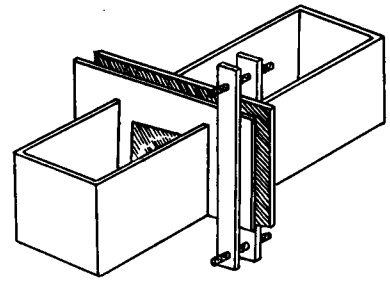

Fig. 1. The two parts of the box used to contain the axolotl. Overall dimensions of box: 18 × 5 × 5 cm.

to provide two compartments. Between them there is fixed a piece of rubber dam with a hole in it sufficiently large to take the body of the animal without unduly compressing it or allowing fluid to leak from the one compartment to the other. Before making an estimation the box was filled with water and the animal placed in the posterior compartment with its head facing the hole in the rubber. A sudden tilt of the box carried the forepart of the animal through the hole and left it invested by the rubber just behind the forelimbs. The water in the box was then drained off, replaced by a measured quantity of buffered fluid (pH 6·5) and the animal left in position undisturbed for some hours.

The NH_3 and urea contents of the samples were determined by the methods described by Conway (1947) using the ordinary Conway diffusion units.

RESULTS

Ammonia and urea excretion during embryonic development

The excretion of ammonia and urea by the developing eggs of *Rana temporaria*, *B. bufo bufo* and *Xenopus laevis* was determined daily until the free-swimming stage. Throughout embryonic development, ammonia formed 80–90 % and urea 10–20 % of the nitrogen excreted. The total amount of ammonia and urea excreted, however, increased progressively during embryonic development. Expressed as nitrogen excreted/100 eggs/day, it was, at the neurula stage 0·172, 0·046 and 0·046 mg. in *Rana*, *Bufo* and *Xenopus*, respectively, whilst on

Table 1. *The ammonia and urea excretion of the terrestrial toad,* Bufo bufo bufo, *and of the aquatic toad* Xenopus laevis *during normal development and metamorphosis*

Amphibian	Stage of development	Ammonia + urea N excreted/g. body wt./day (mg.)	Amount present as 'free' ammonia (%)
Bufo (adult habitat is terrestrial)	Hindlimbs ½ developed	0·147	80
	Hindlimbs functional	0·122	85
	Forelimbs developed under the operculum	0·126	65
	Both forelimbs free. Tailed	0·171	50
	Tail atrophying	0·273	36
	Tail-less	0·251	20
	Adult	0·154	15
Xenopus (adult habitat is aquatic)	No hindlimbs	0·084	85
	Hindlimbs functional	0·087	83
	Both forelimbs free. Tailed	0·084	81
	Tail-less	0·199	77
	Adult	0·091	81

the twelfth day after deposition of the eggs, when embryonic development was complete, it was 0·721, 0·525 and 0·242 mg.

Nitrogen excretion during later larval development and normal metamorphosis

Anurans

Bufo bufo bufo. The proportion of ammonia in the larval excretion remained as high as in the embryonic excretion until the development of the hindlegs. A steady fall then occurred while the hind- and the fore-limbs were growing. Subsequently there was a considerably more rapid fall in the proportion of ammonia excreted, particularly during the stage of tail absorption so that within 5 days it had been reduced from 65 to 20 % (Table 1). At the same time the proportion of urea rose correspondingly. The fully grown toad had only 10–15 % of the waste nitrogen in the form of ammonia.

There was little variation in the sum of the ammonia and urea excreted each day until the stage of intestinal and tail absorption and the freeing of the forelimbs, when it was almost doubled. The rate of nitrogen excretion in the adult toad was, on the other hand, only slightly higher than that of the larva. The changes in the nitrogen excretion during metamorphosis are thus very similar to those occurring in *Rana temporaria* (Munro, 1939).

Xenopus laevis. It was of some interest to compare the nitrogen excretion of an anuran like *Bufo* which leaves the water after metamorphosis with another such as *Xenopus laevis* which undergoes almost as extensive a metamorphosis yet, in spite of its structural adaptation for land life, remains permanently aquatic. Table 1 shows that the nitrogen excretion of this species remains throughout larval development and metamorphosis as ammoniacal as during its embryonic stage. The fully grown adult animal likewise excretes about 80 % of its waste nitrogen as ammonia. Although no change in the proportion of ammonia to urea occurred in the excretion during metamorphosis, the rate at which these two substances were excreted almost doubled during the same period, just as occurred in *Bufo* and *Rana*.

The association of a predominantly ammoniacal excretion and a permanently aquatic habitat in a toad still so well adapted anatomically for land life as *Xenopus*, prompted further investigation, particularly as to the possible occurrence of a seasonal variation in the proportion of ammonia to urea excreted, and to the possibility of inducing experimentally the excretion of a greater proportion of urea. It was not found possible to follow the nitrogen excretion over a whole year, but the ammonia and urea excretion of a group of animals was determined at frequent intervals during 6 months, from February to July. The first part of Table 2 shows that the relative proportions of the two substances did not vary in any consistent way and that the excretion remained predominantly ammoniacal throughout the period.

The possibility of desiccation being able to cause a variation was also considered. Three *Xenopus* toads were exposed to a strong current of dry air for 8 hr. and their body weights thereby reduced 10–20 %. Subsequent determination of their ammonia and urea excretion during the following 3 days failed to show any deviation from the proportions found in untreated animals.

Hypophysectomized Xenopus laevis. The ammonia and urea excretion of three toads, hypophysectomized about 6 months previously, was followed over the same period as for the normal animals referred to above. The proportion of ammonia in the excretion showed no tendency to vary in any consistent way, but it was on the average, considerably lower than that of the normal group (Table 2). From fifteen to

Table 2. *The ammonia and urea excretion of hypophysectomized and unoperated* Xenopus laevis, *determined at intervals over a number of months*

Condition of animals	No. examined	No. of estimations	Ammonia + urea N excreted/g. body wt./day (mg.)	Amount present as 'free' ammonia (%)	Range (%)	Period of observation
Normal	1	23	0·066	82	72–88	Feb.–July
	1	20	0·042	87	70–100	Feb.–July
	1	4	0·084	80	73–86	Feb.–July
	12	10	0·063	80	64–88	Feb.–Apr.
Hypophysectomized 6 months previously	1	25	0·049	54	42–68	Feb.–July
	1	25	0·098	46	30–70	Feb.–July
	1	15	0·114	55	32–70	Feb.–July
Hypophysectomized 3 weeks previously	1	7	0·038	77	67–81	Apr.–July
	1	6	0·045	85	79–94	Apr.–July
	1	11	0·073	76	70–92	Apr.–July
Spayed female	1	11	0·066	74	56–93	Feb.–July

twenty-five determinations of ammonia and urea were made on each toad during the 6 months. In that period the proportion of ammonia averaged 52 % while the corresponding figure for the group of normal animals was 82 %. Determinations were made at the same time of the nitrogen excretion in a spayed female toad and the values found for ammonia and urea were much nearer to the normal than to the hypophysectomized group.

Three more toads were examined at a later date. They differed from the previous animals in having been hypophysectomized for only 3 weeks. Table 2 shows that the proportion of ammonia in the excretion, determined at intervals over a period of 4 months, did not differ much from that found in the group of normal toads. These data diminish the significance of the results with the 'old' hypophysectomized animals. It is difficult, however, to regard the consistently low values for ammonia in the latter group of toads as being within the limits of normal variability. Neither feeding nor starvation produced any marked variation in the proportion of ammonia to urea excreted by this group nor indeed by the other groups, although the total amount of ammonia and urea excreted in these states differed greatly. It is possible that long-standing hypophysectomy may have an influence on the quality of the nitrogen excretion and that the effect is not apparent in more recently operated animals.

Urodeles

Triturus vulgaris *and* Triturus cristatus. The changes in the ammonia and urea excretion during development and metamorphosis in these animals were similar to those occurring in *Rana* and *Bufo* (Table 3). The rate of nitrogen excretion was found to be considerably higher during early larval development than later when body size was much greater.

Axolotl (Siredon mexicanum). Determinations were made of the ammonia and urea excretion of a batch of young axolotls from 1 day after they had hatched until the forelimbs had developed. Another batch of older animals was examined at the same time.

Ammonia formed the greater part of the nitrogen excretion in the earliest stages, but as growth and differentiation proceeded the proportion of waste ammonia fell (Table 4), and during later larval life ammonia and urea were excreted in about equal proportions. One axolotl, the largest, weighing 79 g. was exceptional. It was much darker in colour than the others and the gills, instead of being large and vascular, were small and atrophied. The general appearance was as if metamorphosis had proceeded some way. Table 4 shows that the proportion of ammonia in the waste nitrogen was considerably lower than that of the other axolotls examined.

The rate of nitrogen excretion of the group was distinctly less the heavier the animal up to a body weight of about 10 g. In heavier animals there was no further appreciable decrease.

Nitrogen excretion through the gills of the axolotl. The association of atrophied gills, a larger body weight and a smaller than normal proportion of ammonia in the excretion suggested that the surface area available for excretion might be a factor determining the character of the nitrogen excretion, and that the gills might form part of this excretory surface. Some observations were therefore made on the proportion of ammonia to urea excreted by the gills and head compared with the rest of the body in axolotls with well-developed gills and in others with atrophied gills. Constant watchfulness was required during the course of an experiment for movements of the animal and numerous experiments were vitiated in this way. However, in a number of instances no movements occurred and the results in four such experiments are shown in Table 5.

Table 3. *The ammonia and urea excretion of the newts* Triturus vulgaris *and* T. cristatus *during normal development and metamorphosis*

Amphibian	Stage of development	Body wt. (g.)	Ammonia + urea N excreted/g. body wt./day (mg.)	Amount present as 'free' ammonia (%)
T. vulgaris	Larval	0·08	0·32	75
		0·09	0·21	60
		0·09	0·19	75
		0·12	0·22	74
		0·15	0·15	83
		0·17	0·12	88
		0·20	0·19	63
		0·23	0·07	82
		0·24	0·12	86
	Metamorphosing	0·10	0·23	71
		0·15	0·40	34
		0·20	0·30	40
	Metamorphosed	0·08	0·49	11
	Adult	2·33	0·23	25
		2·50	0·18	11
T. cristatus	Larval	0·42	0·22	69
		0·47	0·27	70
		0·56	0·25	67
	Metamorphosing (gills shrunk)	0·29	0·37	41

Table 4. *The ammonia and urea excretion of axolotls* (Siredon mexicanum) *at different stages during normal development*

Stage of development	Body wt. (g.)	Ammonia + urea N excreted/g. body wt./day (mg.)	Amount present as 'free' ammonia (%)
Hatched 1 day	0·01	0·210	77
Hatched 8 days	0·01	0·367	84
Forelimbs only	0·18	0·169	92
Forelimbs only	0·21	0·210	77
Fore- and hind-limbs	3·70	0·140	70
	4·00	0·074	61
	5·50	0·070	54
	7·50	0·157	36
	10·30	0·066	48
	52·00	0·045	43
	52·00	0·053	60
	55·00	0·035	50
	56·00	0·049	38
Gills shrunken	79·00	0·053	25

The smaller axolotl had well-developed vascular gills, the larger animal had little more than gill stumps remaining. It can be seen from the table that 35 % of the ammonia and urea excreted by the smaller axolotl in 3·5 hr. was lost from the anterior region. On the other hand, only 10 % of the waste nitrogen was excreted by the larger axolotl from the anterior part of its body during a period of 2 hr., in a similar type of experiment. The gills would thus appear, from these observations, to be able to excrete waste nitrogen.

Effect of administering thyroid hormone on the nitrogen excretion

Axolotl. Five axolotls differing in weight from 45 to 70 g. were used for the experiments. Three were injected with 1·0 mg. each of thyroxine and two were used as controls. The ammonia and urea excretion was determined at regular intervals from the time the hormone was administered until after

Table 5. *The ammonia and urea excretion from the head and gills of axolotls determined separately from that of the rest of the body*

Developmental state	Wt. of animal (g.)	Duration of experiment (hr.)	Volume of circumambient fluid (ml.)		Ammonia + urea N excreted (mg.)		Ratio of anterior to posterior excretion
			Anterior	Posterior	Anterior	Posterior	
Larval	10	3·5	25	45	0·054	0·102	0·53
Larval	10	3·5	25	52	0·060	0·105	0·57
Metamorphosing	85	2·0	125	150	0·109	0·602	0·18
Metamorphosing	85	2·0	100	125	0·014	0·437	0·03

metamorphosis was completed, about 25 days later. The results are shown in Fig. 2.

A significant shrinkage in the gills of all the experimental animals could be detected by the tenth day. From about the tenth to the fifteenth day the morphological changes were rapid, and at 25% in the 15 days following the injection of thyroxine, whilst the proportion excreted by the controls showed no consistent change over the same period. About the tenth day after injection the total excretion of ammonia and urea began to increase in the experimental animals and reached

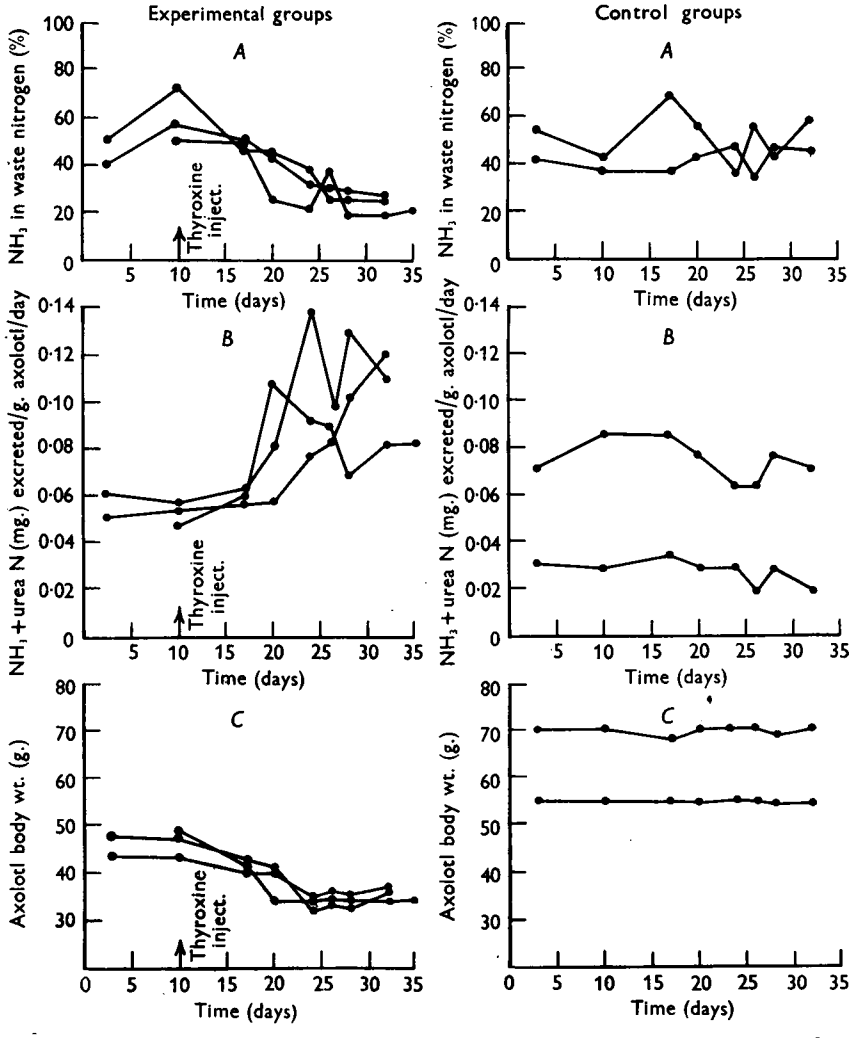

Fig. 2. The changes in axolotls, during thyroxine-induced metamorphosis, in: (*A*) the proportion of ammonia to urea in the waste nitrogen; (*B*) the amount of waste nitrogen (ammonia + urea) excreted/unit wt. of axolotl/day; (*C*) in the body wt. Two control and three experimental animals were used. The latter were injected, each with 1 mg. thyroxine at the time shown by the arrow on the abscissae.

the end of this period the gills were merely stumps and the dorsal fin had been almost completely absorbed. There is a close correspondence between the time of these anatomical changes and of the changes in the character and in the rate of the nitrogen excretion. The proportion of ammonia excreted by the experimental animals fell by about a peak at about the fifteenth day. The body weight, on the other hand, decreased during the same period.

Early larval stages of Rana temporaria. Immersion, in the previously described thyroid solution, of larvae in different stages of development from the tail bud to where the external gills were disappear-

Table 6. *The ammonia and urea excretion of* Rana temporaria *tadpoles at different developmental stages during precocious metamorphosis caused by thyroid extract*

(Tadpoles with hindlimbs ¾ developed were immersed in 1:400 thyroid extract for 2 days.)

Time after initial immersion in thyroid solution (days)	Stage of development	Ammonia + urea N excreted/g. body wt./day (mg.)	Amount present as 'free' ammonia (%)
3	Hindlimbs (¾ developed)	0·14	75
5	Hindlimbs (¾ developed)	0·30	66
7	Tail slightly absorbed	0·26	30
8	Tail half absorbed. Forelimbs covered	0·35	35
10	Tail well absorbed. Forelimbs free	0·32	10

ing, had no effect either on their subsequent survival, their rate of nitrogen excretion or the proportion of ammonia to urea excreted. Some acceleration was noted, however, in the speed at which the embryos developed to hatching in the thyroid solutions as compared with the controls.

Later larval stages of R. temporaria. In a typical experiment, thirty tadpoles varying in weight from 100 to 200 mg. were placed for 2 days in the thyroid solution. They were then removed and placed in water without thyroid extract. On the fifth day after the initial immersion one control and six experimental animals had died. On the twelfth day all of the experimental animals, but only three of the control animals, were dead. The visible morphological changes produced by the hormone suggested a precocious metamorphosis differing markedly from the normal process. The tail, for instance, was well absorbed before the forelimbs were freed, and the whole process appeared to be out of phase in comparison with normal metamorphosis. Table 6 shows that changes in the nitrogen excretion accompanied these bodily changes. After immersion in the thyroid solution for 5 days, the total nitrogen excreted as ammonia and urea had doubled and there was a significant diminution in the amount present as ammonia. By the tenth day the nitrogen excretion was mainly in the form of urea.

DISCUSSION

The foregoing survey shows that, when the adult amphibian environment is terrestrial, the waste nitrogen is excreted mainly as urea, when it is completely aquatic, the waste nitrogen is excreted predominantly as ammonia. Likewise during metamorphosis, ammonia excretion is replaced by urea excretion, but only in those circumstances where terrestrial function is assumed as well as terrestrial form. Thus *Xenopus laevis* undergoes almost as extensive a metamorphosis as *Bufo bufo bufo*, yet the proportion of ammonia in the excretion remains as high in the adult as in the larva of *Xenopus*. It also follows that neither the processes of morphological change for terrestrial life, nor those associated with the degeneration of the aquatic organs, can be regarded as responsible for setting in increased motion at metamorphosis the biochemical mechanisms for urea production and excretion.

Development in the urodeles follows a somewhat different course from that of the anurans. There are three pairs of external gills throughout the larval stage. The forelimbs appear earlier than the hindlimbs. When the latter appear the animal has the complete larval configuration and when metamorphosis occurs the only obvious changes are the disappearance of the gills and of the dorsal fin. Compared with other Amphibia the axolotl is unique in that it remains permanently larval and breeds as such, unless stimulated to metamorphose by dry environmental conditions (Chauvin, 1891) or the administration of thyroid hormone.

Although the axolotl, under the stimulus of thyroxine, develops an almost completely ureotelic nitrogen excretion at metamorphosis, it differs, in its later larval stages, from the other Amphibia examined, in excreting a relatively high proportion of its waste nitrogen as urea. It may be that this tendency to ureotelism during the aquatic phase helps to explain the readiness with which the axolotl, as compared with other Amphibia, will metamorphose in response to environmental stimuli which are adverse to continued existence as an aquatic animal, namely, restriction of the environmental water. The more complete the ureotelism, the better prepared is the animal, in one essential respect, for transformation to land life.

Xenopus larvae in the embryonic stage appear to possess distinct urodelan characteristics. After metamorphosis they show not the slightest tendency to leave the water. The adult characters are a queer mixture of the primitive and the highly specialized. The tongueless condition, for instance, is not an archaic feature but an adaptation for the completely aquatic habit. The presence of the hyoid bone, a metamorphic stage and other signs of terrestrial adaptation in the limbs, strongly suggest that

X. laevis was at one time terrestrial and has since undergone a reversion to aquatic life. Why should the ureotelism which most probably characterized the animal when it was living on the land, have now disappeared almost completely? Needham (1931), discussing a somewhat similar problem in regard to uric acid excretion, points out that the heats of combustion of ammonia, urea and uric acid are 91, 153 and 462 Cal. respectively. He comments that no animal would excrete uric acid as its main nitrogenous end-product unless driven to it by the necessity for survival. It would appear that the Amphibia find it correspondingly advantageous, from the evolutionary standpoint, to discriminate, when the environmental conditions permit, between the lesser energy expenditures involved in ammonia and urea production.

X. laevis thus provides an example of a very extensive biochemical reversion associated with little obvious structural reversion. Neither during metamorphosis nor at any other stage of development is there any recapitulation of the phylogenetically earlier ureotelism. There is some evidence, however, that in certain circumstances a significant increase may occur in the proportion of urea excreted by the adult animal. The increased urea excretion was observed in only three toads, all hypophysectomized, but it was consistently present during many months of observation. Whatever the cause of the increase the fact of its occurrence is in itself interesting, since it demonstrates that the evolutionary loss is not absolute and that improvement in the capacity for urea production is again possible.

Alexander & Bellerby (1938) state: 'After the breeding season in July or August the ponds (vleis or pans) in which *Xenopus* live begin to dry up as a result of evaporation and when this process is completed the toads finally aestivate in the mud at the bottom.' This characteristic of *Xenopus* strengthens the belief that a diversion from ammonia to urea excretion can occur, for it is unlikely that any animal excreting ammonia as the main nitrogenous end-product would be able to survive aestivation. The observations made by Smith (1930) on the lung fish (*Protopterus annectens*) are interesting in this connexion. Half of the waste nitrogen was excreted by the active animal in the form of ammonia, but during aestivation all of the waste nitrogen was retained in the tissues as urea. The lung fish thus appears to be able to excrete one or the other form of waste nitrogen as the environmental conditions necessitate. Under the influence of a hormonal stimulation, possibly, *Xenopus laevis* may be able to do likewise.

SUMMARY

1. The ammonia and urea excretion by different types of Amphibia was followed from the embryonic stage through metamorphosis to the adult fully grown stage. The species examined were *Rana temporaria*, *Bufo bufo bufo*, *Xenopus laevis*, *Triturus vulgaris*, *T. cristatus* and the axolotl (*Siredon mexicanum*).

2. Ammonia was the predominating nitrogenous waste product during the embryonic and larval stages of all the species examined. Urea predominated in the post-metamorphic stages of those species which became terrestrial after metamorphosis, while ammonia predominated in the post-metamorphic excretion of *Xenopus laevis*, the only completely aquatic species examined.

3. The metamorphosis of the axolotl (*Siredon mexicanum*), by the administration of thyroxine, resulted in an increase in the amount of urea excreted relative to ammonia. A similar change resulted during the precocious metamorphosis of *Rana temporaria* by thyroid extract.

4. The question of biochemical reversibility in evolution is discussed in relation to the nitrogenous excretion of *Xenopus laevis* which has made a return to the aquatic environment.

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