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DIFFERENTIAL PREDATION BY NEWTS ON ANURAN TADPOLES

By

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(*Received* 5/3/73)

INTRODUCTION

Newts are voracious feeders, frog tadpoles being readily taken (Smith, 1969). Heusser (1971) noted that, in captivity, warty newts *Triturus cristatus* ate tadpoles of the common frog *Rana temporaria* and the common toad *Bufo bufo,* while smooth newts *Triturus vulgaris* ate frog tadpoles, but refused toad tadpoles. Smooth newts, unlike the larger warty newts, could not catch tadpoles more than 32mm. in length. In the United States, Voris and Bacon (1966) stated that fish *Lepomis macrochirus* in captivity preferred chorus frog tadpoles *Pseudacris triseriata* to American toad tadpoles *Bufo americanus,* but their data were not entirely convicing. Out of 8 species of anuran tadpoles tasted by humans, tadpoles of the only bufonid included in the study (*B. marinus*) were found to have the most objectionable taste (Wassersug, 1971). Bufonid ovarian eggs can be unpalatable or toxic to predators (Licht, 1968), while ranid eggs are readily and safely eaten (Licht, 1969). More recently, however, Grubb (1972) reported that, in laboratory predation studies with mosquitofish *Gambusia affinis,* there was no evidence of the spawn of several anuran species, including *Bufo valliceps,* being unpalatable. The fish tended to eat the spawn of species that normally breed in temporary pools and so will not be exposed to such predation in field situations.

This paper reports predation studies extending Heusser's work.

METHODS

Tests were conducted out-of-doors, neither temperature nor light being controlled. Dark grey plastic tanks (35 x 48 x 50cm high) were filled with 15 litres of tap water to a depth of about 9cm. Two flower pots (diameter at mouth, 13cm) were placed on their sides in each tank to serve as shelter for the animals. One newt and 25 tadpoles were added to each tank and the tanks were covered with nylon gauze. The numbers of tadpoles consumed in 48 hours were recorded. During the test period, the tanks were examined frequently to check that no cannibalism occurred. Warty newts (5 males, 4 females; weight, 5.9—15.2g) or smooth newts (11 males; weight, 1.0—3.3g) were used as predators, and common frog or common toad tadpoles at various stages of development served as prey, 5 or 6 newts being tested with tadpoles of each size.

To determine whether tadpoles displayed any obvious reaction to the presence of a newt, 2 glass tanks (20 x 20 x 40cm long) were filled to a depth of 5cm with tap water, and 10 frog tadpoles and one warty newt were placed in each tank. Two other tanks, containing tadpoles only, served as controls. On 22 occasions during a period of 30 hours, the number of tadpoles in each tank in motion at any one time was assessed visually. Tadpoles weighed 400-700mg and were within the development range, stage IX-stage XVII (Taylor and Kollros, 1946). Any tadpoles eaten by the newts were replaced.

RESULTS

Details and results of the predation trials are shown in Table 1. For both warty newts and smooth newts, there was a significant negative relationship between the number of frog tadpoles caught and tadpole weight (for statistical details, see the Appendix). As the tadpoles grew, so they became less vulnerable to predation. The calculated weights above which frog tadpoles should no longer have been taken by these smooth and warty newts were 260 and 1,000mg respectively. Very few toad tadpoles were caught. The smooth newts only took 2 in the whole experiment, and the warty newts also showed a preference for frog tadpoles. For instance, warty newts ate significantly more small frog tadpoles (Trial 1) than small toad tadpoles (Trial 5), which were of similar size ($t=3.27$; d.f.$=8$; $P<0.02$).

No tadpoles were found that had been chewed and then rejected, but some surviving frog tadpoles had the tips of their tails bitten off. There were five such tadpoles in Trial 8 and nine in Trial 9, but none in the other trials.

Unlike newts in a previous study (Cooke and Fulford, 1971) the female warty newts consumed as many tadpoles as the males.

At the beginning of the trials, tadpoles were about 2 weeks old and 20mg in weight. Under the conditions of the trials, a smooth newt kept with frog tadpoles from this stage of the tadpoles' lives until appearance of the front legs, would be expected to eat about 45 tadpoles. If kept with the apparently unpalatable toad tadpoles, a smooth newt would eat on average only 3 tadpoles during this period. A warty newt in a tank with frog tadpoles would eat about 120, while if maintained with toad tadpoles, the number eaten would be about 60.

There were no differences in activity between tadpoles in the presence or absence of a newt. Mean numbers in motion at any one time ($\pm$ S.E.) were 1.6 ± 0.2 and 2.0 ± 0.4 in the glass tanks with the newts and 1.5 ± 0.2 and 2.0 ± 0.3 in the tanks without a newt. Tadpoles were frequently observed swimming between the legs of a newt and on several occasions they rasped at the skin on the newts' backs.

DISCUSSION

The predicted minimum weight at which frog tadpoles would not be taken by smooth newts was 260mg. From my own data, a healthy animal of this weight would probably be 32-33mm in length which agrees with Heusser's (1971) figure of 32mm. Heusser made no mention of the size of the newts that he used. The bitten tails of tadpoles in Trials 8 and 9 were caused by tadpoles tearing themselves free after being caught by the smooth newts. Some of these tadpoles weighed only 50mg, showing that smooth newts can experience considerable difficulty in holding on to tadpoles weighing much less than the 260mg limit. In Trial 9, only 4 tadpoles (initial mean weight, 182mg) were caught, but 9 others had their tails bitten. In stock ponds, tadpoles with two-thirds of their tails bitten off by smooth newts were still sufficiently strong and active to elude capture.

Warty newts, being larger, can catch and swallow larger tadpoles. A tadpole weighing 1.00g, the predicted weight at which frog tadpoles become safe from predation by the newts, would be abnormally large. Its length would be 45-50mm. The results suggest that a dozen warty newts could, during a season, account for every tadpole produced by a pair of frogs. Introductions of frog tadpoles into any small pond with a flourishing newt population would seem to be pointless. Once tadpoles have reached the hind limb paddles stage, they are unlikely to be taken by smooth newts, but even if they survive and breed in the pond, their progeny will be exposed to predation during the earlier part of metamorphosis. It should, of course, be remembered that in a natural situation newts will take prey other than tadpoles (e.g. see Avery, 1968), but then many other predators, both vertebrate and invertebrate, will contribute to the reduction of a tadpole population

The almost total rejection of toad tadpoles by smooth newts and the partial rejection by warty newts was not due to their carcases tasting foul, since no tadpoles were found that had been chewed. Newts hunt mainly by sight and "smell" (Smith, 1969) and it is likely that chemoreception of tadpole secretions is responsible for this rejection.

SUMMARY

Smooth or warty newts have been kept with common frog or common toad tadpoles at different stages of development. For both species of newt there was a significant negative relationship between the number of frog tadpoles caught and tadpole weight. Warty newts of the size used should be able to catch frog tadpoles up to a predicted weight of 1.00 g, while smooth newts should not catch frog tadpoles weighing more than 260 mg. Toad tadpoles were almost totally ignored by smooth newts and were partially rejected by warty newts. The level of activity of frog tadpoles was unaffected by the presence of a newt.

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APPENDIX

Statistical treament of data for warty and smooth newts taking frog tadpoles.

For warty newts taking frog tadpoles, a linear regression line was calculated: $y = 1.884 - 0.628x$, where $y = \log_{10}$ (number of tadpoles eaten + 1) and $x = \log_{10}$ tadpole weight (mg). Analysis demonstrated the regression coefficient to be significantly different from zero ($t = 6.67$; d.f. $= 18$; $P<0.001$) indicating a highly significant negative relationship. The equation can be expressed as: number of tadpoles eaten $= 76.6$. tadpole weight $(mg)^{-0.628} - 1$. Similarly, a highly significant negative relationship was found for smooth newts taking frog tadpoles: $y = 1.931 - 0.800x$ ($t = 5.88$; d.f. $= 15$; $P<0.001$) or: number of tadpoles eaten $= 85.3$. tadpole weight $(mg)^{-0.800} - 1$. Comparing the regression lines for the 2 newt species, the residual of the joint line was significantly greater than the sum of the individual residuals ($F_{2,33} = 128.4$; $P<0.001$) indicating that the lines were different. A comparison of the individual residuals with the common slopes residual showed the two slopes to be significantly different ($F_{1,33} = 12.7$; $P<0.001$). The slope was less negative for the warty newts, because, whereas the mean weight of tadpoles consumed by the smooth newts remained fairly constant irrespective of tadpole size, warty newts in the first 3 trials tended to eat greater weights of tadpoles as tadpole size increased. For the smooth

Trial no.	Predator Species	n	Prey Species	Description at start of trial: Length (mm)	Mean wt. (mg)	Development stage[3]	No. caught in 48 hours (mean ± S.E.)	[illegible]
1	warty newt	5	frog	12.5 – 13	22	Losing external gills	10.0 ± 1.9	[illegible]
2	*Triturus*	5	*Rana*	19 – 21	81	Stages II, III	4.6 ± 1.9	[illegible]
3	*cristatus*	5	*temporaria*	24.5 – 28	176	Stages III-VI	3.6 ± 1.5	[illegible]
4		5		34 – 45	598	Stages XIII-XVII	0.2[1]	4
5	warty newt *Triturus*	5	toad	10.5 – 13.5	25	Internal gills, no hind limbs	2.8 ± 1.1	1
6	*cristatus*	5	*B. bufo*	27 – 31	367	Stages XIII-XVI	1.2 ± 0.8	3
7	smooth newt	6	frog	13	18	Losing external gills	8.7 ± 0.8	0
8	*Triturus*	6	*Rana*	16 – 19	54	Stages < III	2.7 ± 0.6	1
9	*vulgaris*	5	*temporaria*	24 – 28	182	Stages III-V	0.8 ± 0.3	3
10	smooth newt *Triturus*	5	toad *B. bufo*	10 – 11.5	14	Internal gills, no hind limbs	0.4[2]	3
11	*vulgaris*	5		25 – 30	373	Stages XI-XIV	0	5

TABLE 1. Details of predation trials with newts and anuran tadpoles:
[1] single tadpole eaten by 1 newt.
[2] single tadpoles eaten by 2 newts.
[3] stage nos. after Taylor and Kollros (1946).

newts, mean total weights consumed (± S.E.) were: Trial 7, 150 ± 20 mg; Trial 8, 140 ± 40 mg; Trial 9, 150 ± 110 mg; and for the warty newts: Trial 1, 220 ± 40 mg; Trial 2, 370 ± 110 mg; Trial 3, 630 ± 210 mg. The relationship for the warty newts between consumption and tadpole weight did not, however, quite reach statistical significance ($0.06 > P > 0.05$). The intercepts on the x axis for the 2 regression lines, i.e. the tadpole weights above which the predicted tadpole catch would be zero, were: smooth newt, 260 mg; warty newt, 1,000 mg.

THE EFFECTS OF pp'-DDT ON ADULT FROGS (*RANA TEMPORARIA*)

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INTRODUCTION

During the 1960s the common frog (*Rana temporaria*) decreased considerably over much of Britain, and pesticides may have been a contributing factor in some localities (Cooke, 1972a). In the laboratory, spawn and tadpoles have been treated with pesticides so that effects in the field can be recognised and evaluated (Cooke, 1970, 1972b). This paper reports an experiment in which adult frogs were treated with DDT in order to study behavioural and physiological changes. Previous studies (Ellis, Westfall and Ellis, 1944; Tripod, 1947; Isaacson, 1968) were mainly concerned with elucidating LD_{50}s and the site of action of DDT.

METHODS

On day 0, 24 frogs (weight range, 13-31 g; snout-urostyle, 50-67 mm) were randomly distributed into four groups of six. All of the frogs were individually recognisable by sex, size and markings. Each group was maintained in 1.2 litres of tap water in a glass tank measuring 45 x 30 x 30 cm (water depth = approximately 1 cm). The water was renewed daily. In each tank was a hardboard platform 15 x 11 cm, raised 2 cm off the floor, under which the frogs could hide or upon which they could rest out of the water. As food, 2 live mealworms (60-120 mg)/frog/day were placed in a galvanised mesh tray on the platform. The water temperature was 14-22°C and day length was kept at 12 hours. The experiment finished on Day 24.

Frogs were given DDT by subcutaneous injection in olive oil on Days 2, 5, 8, 10, 12, 15, 17, 19 and 22. Treatment solutions were administered from Steriseal disposable syringes, the mean volume (± S.E.) for 12 trial deliveries being 0.169 ± 0.002 ml. Details of the injections were as follows:

Group	Concentration of injection soln. (μg/ml)	pp'-DDT/frog/ injection (μg)	pp'-DDT/ injection (mg/kg frog wt.)
A	0.0	0.00	0.00
B	14	2.4	0.11
C	140	24	1.1
D	1400	240	12

The number of mealworms eaten and the number of faecal sacs produced were recorded daily on a group basis. Hiding scores were determined every morning: for each leg or head not hidden by the platform, the group scored 1 point, and if a frog was completely exposed, the score was 6 points. On Days 1, 9, 16 and 23, the rate of hyoid movement was determined while the frogs remained in the tanks. Frogs were then placed on a moistened Formica table top and jump length was recorded. On each occasion, 10 jumps were measured for each frog, a jump being arbitrarily taken as a single forward movement of > 10 cm. On Days 9, 16 and 23, rates of hyoid and nostril movement were recorded on the table after each frog had finished its jumping session.

Frogs that died during the course of the experiment were dissected within a day of death. Survivors were killed and dissected on Day 24. Liver and thigh muscle samples were analysed for organochlorines by gas-liquid chromatography as described by Cooke (1972b).

RESULTS AND OBSERVATIONS

One frog died in Group B (on Day 14), one in Group C (Day 16) and five in Group D (Days 15, 16 (2), 19 and 23). Frogs in Group D died 2-6 days after showing the first obvious signs of poisoning and the survivor in this group showed sublethal symptoms on Days 23 and 24. The frog that died in Group C had been behaving abnormally for 5 days prior to death, while another frog in this group was moribund on Day 24, 7 days after the first symptoms were noticed. Symptoms of DDT poisoning were restricted to these 8 frogs and varied between individuals. The following symptoms were each displayed by several frogs:

(i) sluggishness, lacking the normal desire to jump or walk when disturbed;
(ii) increased irritability, trembling when touched;
(iii) extension and tremoring of the hind limbs and violent kicking movements;
(iv) adoption of various abnormal postures including lying flat or upside down or tucking a forelimb rigidly under the body;
(v) a greatly increased frequency of croaking; and
(vi) colour change, becoming paler or greener.

In Group D, the residues of pp'-DDT in the liver and muscle samples from the 6 frogs (mean ± S.E.) were 16.9 ± 4.0 ppm and 1.4 ± 0.5 ppm respectively. No metabolites could be found. The liver of the frog that died in Group C contained 8.4 ppm pp'-DDT and that of the moribund frog, 8.0 ppm. Of the livers from the remaining frogs in this group, two did not contain detectable residues, while the others contained 1.4 and 0.10 ppm. Only in the muscle sample from the moribund frog could DDT residues be detected (0.66 ppm). No residues were detected in the liver or muscle samples from frogs in Groups A and B.

Hiding scores are shown in Table 1 for Days 1-12 and 13-24. This is a logical as well as a convenient division in time because sublethal symptoms were just becoming apparent half-way through the experiment. In the second half of the experiment, there was a significant tendency for the frogs not to hide (score increased) in Group C (comparing within group data; χ^2 = 8.2; d.f. = 1; $P<0.01$) and Group D (χ^2 = 96.0; d.f. = 1; $P<0.001$) despite the hiding spaces becoming less crowded as the frogs died. Food consumption and faecal sac production are given in Table 2 for each group during the two halves of the experiment. Compared with Table 1, time is displaced by 1 day, the periods now taken being Days 0-11 and 12-23, because observations made early on Day x on mealworms eaten and faeces excreted relate to events occurring on Day x-1. For the data in Table 2, there were no significant changes within groups between Days 0-11 and 12-23.

Changes in jump length (Table 3) could not be detected prior to obvious signs of poisoning being noted. Only 3 frogs with poisoning symptoms were tested for jumping ability. One in Group C, in a state of advanced poisoning, refused to jump at all. For the survivor in Group D, jump length was significantly reduced on Day 23 (compared with Day 1; Mann-Whitney test; $U_{10,10}$ = 8; $P<0.002$) only the forelimbs being used for propulsion. In the same group, another frog that was uncoordinated on Day 16 jumped just as far as usual despite jumping with splayed legs and landing with hind legs outstretched.

Observations on rates of breathing movements are summarised in Table 4. No obvious changes were apparent that could be attributed to pesticide residues in the frogs. Various trends were noted such as a decrease in the rate of hyoid movement from Group A to Group D when frogs were out of the water, and an increase in the rate of nostril movement from Group A to Group D on Days 9 and 16. However, baseline data for Day 1 were not available and the frogs in Group B might have been expected to show similar responses to those in Group A, since none contained detectable residues. For individual frogs, attempts to relate breathing changes to the extent of the poisoning symptoms also led to the conclusion that differences within or between the groups were not caused by pesticide.

Similarly no significant changes were noted in heart or liver weight (Table 5) and there were no obvious differences in fat body or gonad size between the groups.

DISCUSSION

Sublethal symptoms were similar to those reported for adult or newly metamorphosed ranids treated with DDT (Tripod, 1947; Isaacson, 1968; Cooke, 1970) or cyclodiene insecticides (Kaplan and Overpeck, 1964). The colour changes noted may indicate a decrease in the circulatory level of melanocyte stimulating hormone, perhaps because of increased activity of the hormone release inhibiting factor (Kastin, Schally, Viosca and Miller, 1969; Cooke, 1972b).

Tripod (1947) reported increased respiration in acutely treated frogs, while Kaplan and Overpeck (1964) observed decreased respiration amongst frogs chronically poisoned. In the present experiment no clear changes could be determined in respiratory movements. Lowering and raising of the hyoid is associated with two types of gaseous movement. With the nostrils open and the glottis closed, movement of the buccal floor pumps air from outside into and out of the buccopharynx. With the nostrils closed and the glottis open, air can be sucked out of or pumped into the lungs. Thus simply counting hyoid movements would not detect changes in the relative frequency of these events. However, following both hyoid and nostril movements should detect marked changes in breathing technique, although no conclusions on the volume of gaseous exchange can be made. The supine treated frogs must have relied entirely upon cutaneous respiration whilst in the tanks since their nostrils were permanently beneath the water surface.

After noting increased activity amongst leopard frogs (*Rana pipiens*) treated with DDT, Isaacson (1969) remarked that such behaviour in the field could be disadvantageous for survival. In the present study, treated frogs tended to hide less than normal and became uncoordinated sometimes remaining on their backs for more than a day. Just as DDT-treated, hyperactive tadpoles are selectively taken by newts in the laboratory (Cooke, 1971), so adult frogs suffering from DDT poisoning would probably be more likely to be preyed upon in the field.

Days	Group A 1–12	Group A 13–24	Group B 1–12	Group B 13–24	Group C 1–12	Group C 13–24	Group D 1–12	Group D 13–24
Frog days*	72	72	72	61	72	63	72	36
Total hiding score	9	14	20	12	9	23	18	76
Mean score/frog/day	0.13	0.19	0.28	0.20	0.13	0.37	0.25	2.11

TABLE 1. Hiding scores for each group for Days 1–12 and 13–24. For method of calculating hiding scores, see text.
* Sum of the numbers of frogs alive on each day.

Days	Group A 0–11	Group A 12–23	Group B 0–11	Group B 12–23	Group C 0–11	Group C 12–23	Group D 0–11	Group D 12–23
Frog days*	72	72	72	62	72	64	72	41
Total no. of mealworms eaten	92	119	92	85	83	75	121	55
Mealworms/frog/day	1.28	1.65	1.28	1.37	1.15	1.17	1.68	1.34
Total no. of faecal sacs produced	24	26	20	24	37	23	22	20
Faecal sacs/frog/day	0.33	0.36	0.28	0.39	0.51	0.36	0.31	0.49

TABLE 2. Mealworms eaten and faecal sacs produced by each group for Days 0–11 and 12–23.
* Sum of the numbers of frogs alive on each day.

Frogs behaving in this unusual manner would, likewise, be more conspicuous to human observers. Such incidents have, however, never been reported in Britain, suggesting that they occur rarely if at all. In contrast, in a cotton-growing area in Mississippi, cricket frogs (*Acris crepitans*) have been found with typical symptoms of DDT poisoning (Ferguson and Gilbert, 1967), Vinson, Boyd and Ferguson (1963) having earlier concluded that all frog populations in the Mississippi Delta were likely to be exposed to insecticides.

Frogs in some localities in Britain might accumulate sufficient residues from their food to cause harmful effects. Slugs are probably the main food item of *Rana temporaria* (Savage, 1961) and, in heavily-treated orchards and agricultural fields, residues of DDT and its metabolites in slugs can exceed 10 ppm (Davis, 1968; Davis and French, 1969). On the evidence available at present, the effects of eating such contaminated prey cannot be predicted with any degree of certainty. However, I have recently analysed 14 frogs collected from the field and only one contained detectable residues, again suggesting that poisoning incidents are rare. Moreover, none of the frogs in Groups A and B contained detectable residues, these frogs having been imported from Eire by Gerrard and Haig Limited. The field specimen that contained detectable residues was found dead in a Manchester garden. Its desiccated carcase contained 1.62 μg pp'-DDE (indicative of about 0.2 ppm wet weight at death). This DDE, the DDT metabolite most commonly found in the tissues of other vertebrates, had presumably come from the frog's food since no DDE or other apolar metabolites could be detected in the frogs in Groups C and D. Increases in liver weight after rats and birds have been treated with DDT are well documented. In pigeons, liver weight increases are associated with the presence of the metabolite, pp'-DDE, rather than pp'-DDT (Bailey, Bunyan, Rennison and Taylor, 1969), and lack of conversion in the frogs may have been the reason for no change in liver weights. Out of 12 toads (*Bufo bufo*) that I have so far analysed from the field, 6 have contained detectable residues, the highest level being 1.0 ppm pp'-DDE in a liver sample.

The effects of pesticides and other factors on frog populations in Britain have recently been discussed at length (Cooke, 1972a).

SUMMARY

Adult frogs (*Rana temporaria*) were subcutaneously injected with pp'-DDT for up to 24 days. Poisoning symptoms are described. A marked tendency was noted for the DDT-treated frogs to hide less than normal. Jump length was not reduced prior to obvious poisoning symptoms being displayed. No significant changes were observed in food consumption, faecal sac production, rates of hyoid and nostril movement, liver or heart weight and fat body or gonad size. No fat soluble metabolites of pp'-DDT were detected in liver or muscle samples.

The likelihood is discussed of adult frogs being poisoned by DDT in the field in Britain.

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Group		Day 1	Day 9	Day 16	Day 23
	No. of frogs	6	6	6	6
A	Mean jump length (cm)	17 ± 1	18 ± 1	18 ± 2	18 ± 2
	Mean longest jump (cm)	27 ± 3	31 ± 2	28 ± 4	26 ± 2
	No. of frogs	6	6	5	5
B	Mean jump length (cm)	22 ± 2	24 ± 3	20 ± 3	22 ± 2
	Mean longest jump (cm)	38 ± 3	34 ± 4	30 ± 3	33 ± 2
	No. of frogs	6	6	5	4
C	Mean jump length (cm)	18 ± 1	19 ± 1	20 ± 2	19 ± 2
	Mean longest jump (cm)	27 ± 1	29 ± 2	32 ± 3	29 ± 6
	No. of frogs	6	6	3	1
D	Mean jump length (cm)	22 ± 2	24 ± 2	19 ± 1	13
	Mean longest jump (cm)	39 ± 4	41 ± 5	31 ± 2	16

TABLE 3. Jump lengths of the frogs on Days 1, 9, 16 and 23 (± S.E.)

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Group			Movements/minute Day 1	Day 9	Day 16	Day 23
	No. of frogs		6	6	6	6
A	Hyoid: in water		130 ± 4	126 ± 5	121 ± 2	131 ± 4
	Hyoid:	out of water		157 ± 5	140 ± 4	142 ± 1
	Nostrils:	out of water		54 ± 9	57 ± 3	58 ± 5
	No. of frogs		6	6	5	5
B	Hyoid: in water		132 ± 3	126 ± 2	125 ± 5	127 ± 4
	Hyoid:	out of water		146 ± 7	134 ± 3	134 ± 7
	Nostrils:	out of water		75 ± 14	67 ± 9	64 ± 5
	No. of frogs		6	6	5	5
C	Hyoid: in water		126 ± 3	119 ± 5	120 ± 2	124 ± 9
	Hyoid:	out of water		146 ± 9	132 ± 5	133 ± 3
	Nostrils:	out of water		75 ± 12	68 ± 6	56 ± 6
	No. of frogs		6	6	3	1
D	Hyoid: in water		129 ± 3	124 ± 4	124 ± 7	117
	Hyoid:	out of water		142 ± 4	122 ± 1	122
	Nostrils:	out of water		88 ± 7	79 ± 9	79

TABLE 4. Rate of hyoid and nostril movements of the frogs on Days 1, 9, 16 and 23 (mean ± S.E.).

Group	Initial body wt. (g)	Heart wt. (mg/g initial body wt.)	Liver wt. (mg/g initial body wt.)
A	19.7 ± 2.4	1.84 ± 0.05	40 ± 8
B	21.4 ± 1.8	1.94 ± 0.12	39 ± 5
C	22.3 ± 3.0	1.87 ± 0.10	41 ± 5
D	19.8 ± 1.4	1.58 ± 0.11	36 ± 4

TABLE 5. Initial body weight, heart weight and liver weight of the frogs (mean ± S.E.).

TAIL DEGENERATION IN ANURAN LARVAE

By

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(*Received* 1/6/73)

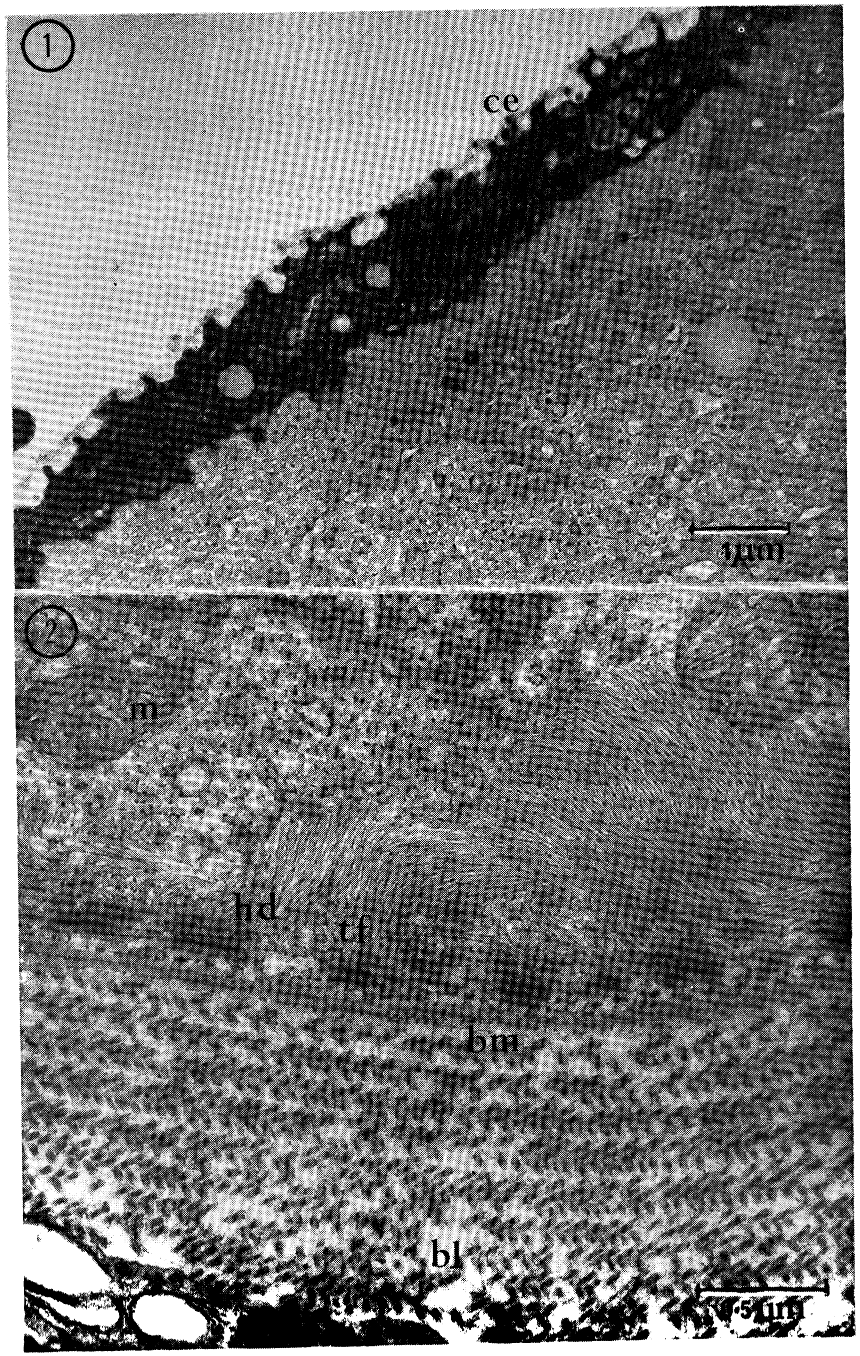
The growth of an anuran larva into a froglet is common knowledge. Doubtless owing to the fact that this feature is (or used to be) so familiar in the wild—or in the classroom for that matter—there is frequently a blasé acceptance of this profound and complex growth process.

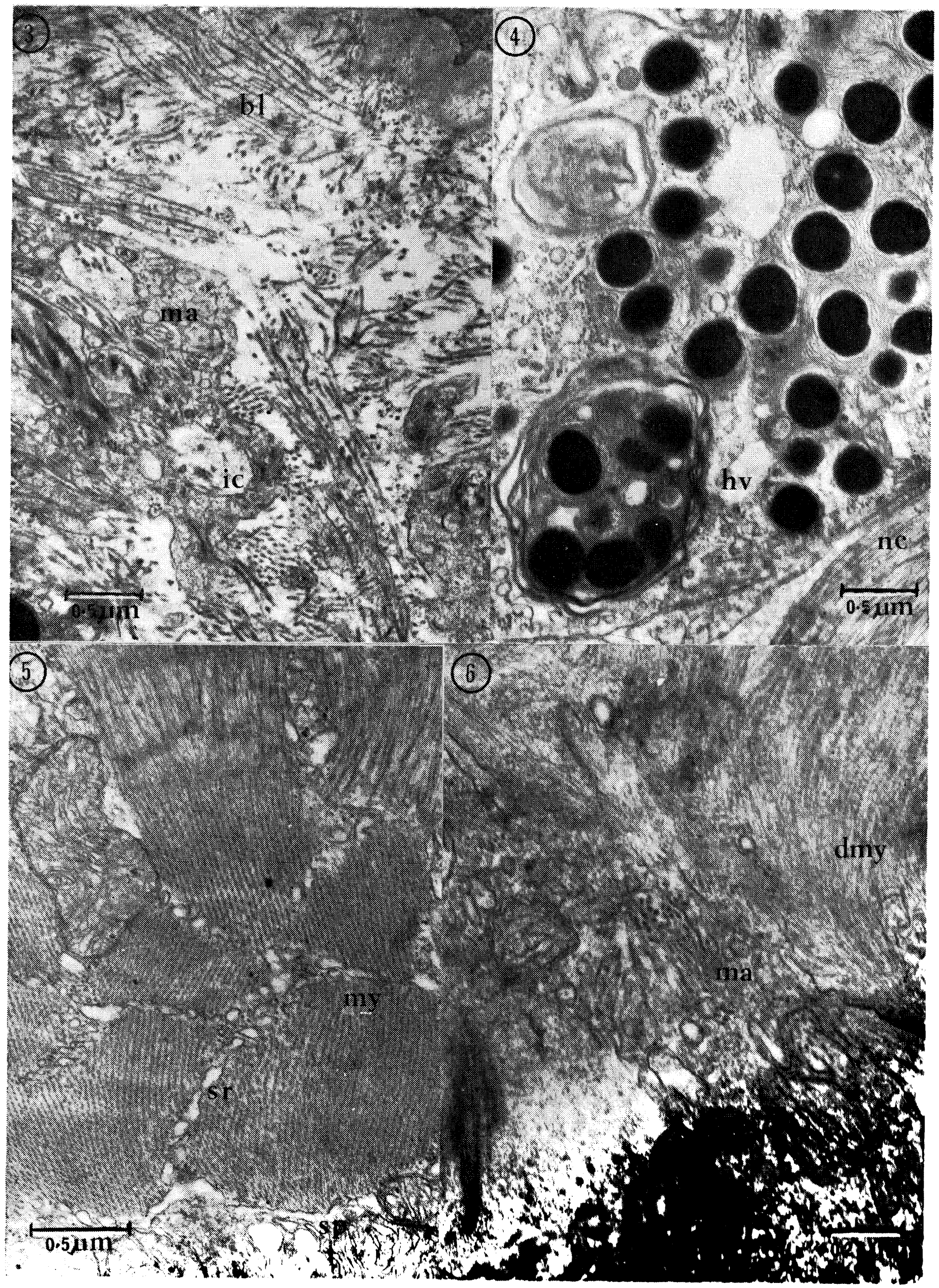
The development of an aquatic living larva into a terrestrial, air-breathing froglet involves its biochemistry, physiology and behaviour and overall morphology (see Bennet & Frieden, 1962; Frieden & Just, 1970). A most elaborate histogenesis results in the loss of some structures and the creation of new ones. In general all organ systems and seemingly all the larval cells are affected for the entire larva is wholly remodelled during the metamorphic process (see for example the intestine of *Xenopus laevis,* Fox, Mahoney & Bailey, 1970; Fox, Bailey & Mahoney, 1972).

A particularly noticeable morphological change at metamorphic climax is the loss of the larval tail. Study of this feature, at an ultrastructural level, would seem to be of value on at least two counts. First, a clearer understanding will add to our knowledge of amphibian ontogeny and could well provide evidence of use in dealing with problems of taxonomy and phylogeny. Second, the anuran larval tail comprises a number of different organ (or tissue) systems which, at climax, suffer swift cellular necrosis. Programmed cell death in a variety of tissues can thus be conveniently examined at a sub-cellular level, and the information gained compared with that available on tissue degeneration in other vertebrate groups including humans in health, ageing and disease. The electron microscope, now a fixture in most University Biology Departments and Research Institutes, is an invaluable and essential tool for investigations of this kind.

Research on different aspects of anuran metamorphosis (especially on *Rana* and *Xenopus*) has shown that: (a) tail degeneration is triggered off by a specific high threshold level of circulatory thyroid hormonal concentration, attained through the complex interactions of the thyroid, hormonal feed-back, pituitary and the hypothalamus (Etkin, 1964, 1970)—exogenous thyroxine, other thyroid hormones and thyroxine analogs *in vitro* act similarly (Kollros . 1961; Kaltenbach, 1968; Hickey, 1971); (b) goitrogens (thyroid inhibitors), thyroidectomy, hypophysectomy and hypothalamectomy abolish the metamorphic progression (see Fox, 1967 and Fox and Turner, 1967 for references); (c) autolysing tail cells increase their content of acid phosphatase and very likely of other lysosomal enzymes too: (d) macrophages substantially increase in numbers in the tail at climax (Lehman, 1953; Salzman & Weber, 1963); (e) they synthesise increased quantities of lysosomal acid hydrolases such as acid phophatase, DNA-ase and cathepsins at this time (Hassan & Autuori, 1964; Weber, 1969; Hickey, 1971; see Fox, 1972a and 1973a for other references); (f) macrophages engulf various degraded substances, either cellular or extra-cellular, of the tail at climax (Fox, 1973c); (g) it is conceivable that some or all tail mesenchymal cells can switch function from synthesising tropocollagen (Jackson, 1968) to that of phagocytosis, though these functions could well proceed simultaneously in the same macrophage (Lapière & Gross, 1963), at least in the initial stages of phagocytosis.

The participation of mesenchymal macrophages in the processes of degeneration and resorption of tissues of the anuran larval tail has been known since the time of Elias Metchnikoff (1883; see Brown, 1946), though some

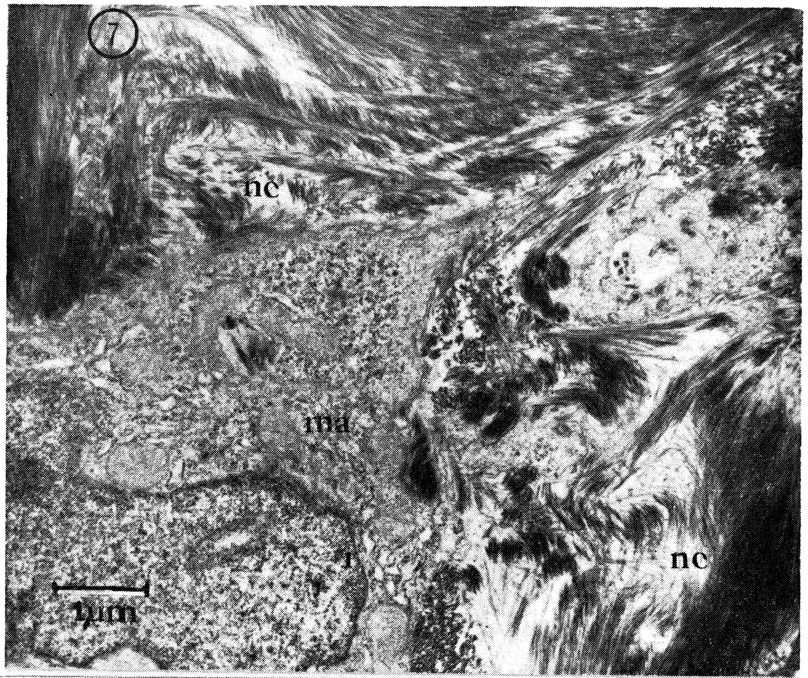


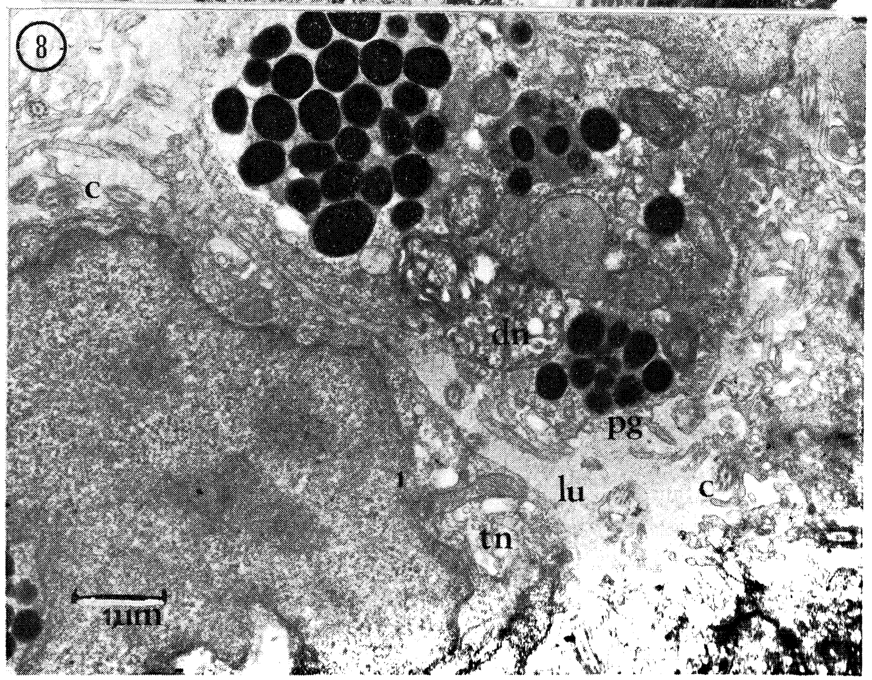


details of their functional behaviour are still not clear. It is possible that in some cases macrophages promote cellular organelle necrosis by an extra-cellular secretion of hydrolysing enzymes, which presumably occurs when extra-cellular collagen fibrils are degraded and thence engulfed by them (Fox, 1972a, b). It is fairly certain that they phagocytose partially or highly degraded cellular substances and thence digest them within heterophagic vacuoles of lysosomal nature. In addition to the macrophagic engulfment of basement lamellar collagen, situated against the floor of the epidermis (Gross, 1964; Usuku & Gross, 1965; Gona, 1969; Fox, 1972a) (Figs 2, 3), they also phagocytose the rest of the tail collagen, including that surrounding the notochordal cells (Fox, 1972b) (Fig. 7), degraded muscle (Weber, 1964; Fox, 1972c) (Figs. 5, 6) and autolysed components of cells of the notochord and nerve cord (Fox, 1973a, b) (Fig. 8). The presence of nervous tissue (including axons) and muscle fibres within phagocytes in the degenerating anuran larval tail, was first reported by Metchnikoff (1883) and Barfurth (1887) (see Brown, 1946).

At metamorphic climax the tail of *Rana temporaria* first becomes necrotic at the tip and the area of degeneration extends proximally; the fins disappear and the tail simultaneously shortens to a blackened stub-like form, before its final disappearance. During this time representative sections under the electronmicroscope show melanocytes (possessing large numbers of pigment granules, or melanosomes, to be present in increasing numbers in the region between the basement lamella of the skin and the notochord and nerve cord (see Eppig, 1970) and later around and amid the disrupted notochordal collagen and the autolysing cells of the notochord and nerve cord. Pigment granules also increase in number in nerve cord cells at climax (Fox, 1973b) (Fig. 8). Likewise macrophages contain pigment granules which appear to be more numerous at climax. Whether they "belong" to the macrophages or were originally derived from necrotic tissue and have been engulfed by them is not clear. Some melanosomes, seen to be existing freely in the cytoplasm, are probably of macrophagic origin; furthermore some may well be present in autophagic vacuoles. However, when at climax substantially increased numbers of them are confined within cytoplasmic membrane-bound vacuoles of actively predaceous macrophages (Fig. 4), then it is likely that some pigment granules at least occur by virtue of macrophages previously phagocytosing autolysed and disintegrated melanocytes and/or other degenerate cells.

The way thyroid hormones direct larval development is not clearly understood. They may, however, elicit the disparate, though integrated, changes through the cell nucleus. It is known that actinomycin D, which inhibits DNA-dependent RNA synthesis, abolishes increase in the synthesis of proteins and of lysosomal enzymes in the anuran tail, which does not then involute at climax (Tata, 1966; Perriard, 1971). The progressively increasing larval circulatory concentration of thyroid hormones may thus differentially and sequentially influence specific gene complexes in different cellular populations, thereby promoting new RNA synthesis (and thence cellular differentiation) or perhaps shutting off others. An increase in the number of tail cell lysosomes and the release of their enzymes, will result in autolysis. Perhaps the lysosomal membranal stability is influenced directly and is labilised by the increased hormonal concentration (Fox, 1973a). It is of interest that prolactin, which in amphibian larvae acts in some ways like a growth hormone, inhibits tail involution in amphibian larvae by antagonising thyroxine action at the reacting tissue level, in part at least by stabilising the lysosomal membrane (Giusta *et al*, 1972; Campantico *et al*, 1972). Likewise the increase in the numbers of macrophages and their lysosomes will provide sufficient and adequately equipped cells for phagocytic activity. One may speculate that the cause of the





behavioural macrophagic change at climax could also be genomal in origin, for only at this time do they seem to attack and engulf the extra-cellular collagenous fibrils of the tail.

In summary: At metamorphic climax and influenced by a high circulatory level of thyroid hormones, cells of the notochord and nerve cord autolyse and lysosomal enzymes are implicated in the process. Some details of muscle autolysis are still obscure (Weber, 1964, 1969; Fox, 1972c) and lysosomal activity has yet to be proven as a prime feature of myofibrillar regression. All the collagen fibrils, at various levels of degradation, certainly and the other tissues probably, are phagocytosed by mesenchymal macrophages and the products digested within heterophagic vacuoles with the aid of lysosomal enzymes. Probably products of cellular necrosis stimulate macrophagic invasion and phagocytic activity. Tail epidermal cells autolyse, cornify and slough (Fig. 1). It seems unlikely that macrophages are involved in their destruction and final disappearance (Fox, 1972a). The fate of the tail blood cells and capillaries is not clear. Probably they too become necrotic and ultimately their products are removed by the macrophages as the capillaries become disarranged, blocked and doubtless damaged during tail involution.

The macrophages—the chief predatory cells of tail involution—may well be the sole survivors of this drastic, all embracing cellular destruction, which proceeds exclusively at climax and, at room temperature, is over in *Rana temporaria* in less than a week.

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DESCRIPTION OF ILLUSTRATIONS

All electronmicrographs are of tail tips of *Rana temporaria* larvae—staged according to the scheme for *Rana dalmatina* by Cambar and Marrot (1954); climax is at stages 49-54 and the tail disappears between stages 51-54—suitably fixed, sectioned and stained by standard electronmicroscopical procedures. Figures 2, 3, 5 and 6 are transverse and 1, 4, 7 and 8 longitudinal sections.

Fig. 1. Stage 51-52. Cornified epidermal cell just preceding sloughing. Note the pronounced difference in structure between the latter and the underlying non-cornified epidermal cells, which as yet show little if any autolysis and cornification.

Fig. 2. Stage 45-47. Part of an innermost epidermal cell and the underlying collagenous basement lamella, of characteristic orthogonal architecture. The typical hemidesmosomes or " bobbins " with tonofibrils leading from them, are prominent. Below again—but not pictured—are mesenchymal cells, which probably participate in the preclimactic formation and certainly in the climactic destruction of the collagen fibrils.

Fig. 3. Stage 52-53. Invasion and ingestion of the degenerating disorganised basement lamellar collagen by mesenchymay macrophages, which digest the collagen within heterophagic vacuoles utilising lysosomal enzymes.

Fig. 4. Stage 51-52. Macrophage situated near the notochordal collagen, with ingested pigment bodies within heterophagic vacuoles.

Fig. 5. Stage 45-47. Myofibrils of tail muscle. Those profiles near the top of the illustration are oblique, verging towards a longitudinal arrangement.

Fig. 6. Stage 52. Macrophage (with collagen inclusions) at the edge of an incipiently degraded muscle; before phagocytosis of the eventual highly necrotic muscle tissue.

Fig. 7. Stage 51-52. Macrophage invading and phagocytosing the disrupted notochordal collagenous sheath.

Fig. 8. Stage 51-53. Autolysis of a nerve cord cell at the lumen margin; a typical large cytolysome. Note the presence of numerous pigment granules, which accumulate in climactic tail nerve cord tissue and the diagnostic ultrastructure at the nerve cord lumen showing the margin of cilia and microvilli.

DESCRIPTION OF ABBREVIATIONS ON THE ILLUSTRATIONS

bm, basement membrane; bl, basement lamella collagen; c, cilia; ce, cornified epidermal cell; dn, degenerate nerve cord tissue; dmy, degraded myofibrils; hd, hemidesmosome; hv, heterophagic vacuole; ic, ingested collagen in heterophagic vacuole; lu, lumen of nerve cord; m, mitochondrion; ma, mesenchymal macrophage; my, myofibrils; nc, notochordal collagen; pg, pigment granules; s, sarcoplasm; sr, sarcoplasmic reticulum; tf, tonofilaments; tn, tail nerve cord.

TADPOLES AND METAMORPHOSED YOUNG OF THE SMOOTH NEWT (*TRITURUS VULGARIS* L.) IN A POND IN GOTHENBURG, SWEDEN

By

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(*Received* 19/12/72)

INTRODUCTION

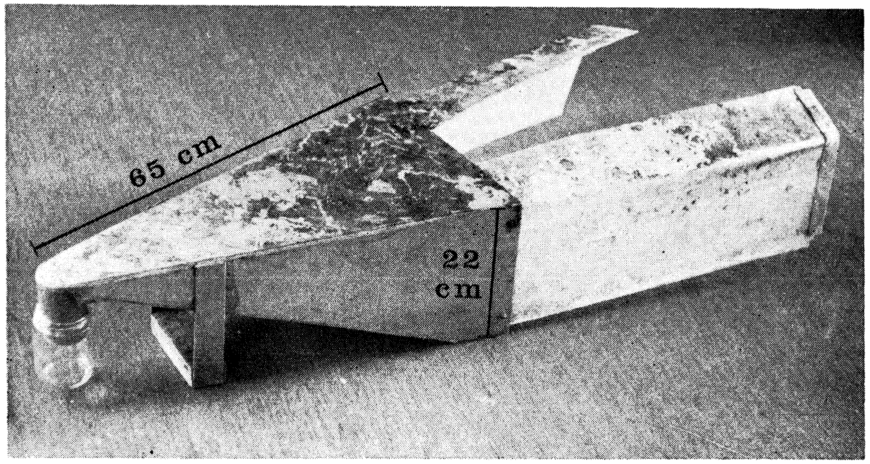
In order to study the dynamics of a newt population it is necessary, on the one hand, to estimate the distribution of adults of different age classes and on the other hand the production of new animals, i.e. the reproduction rate. However, to assess the age groups of adults in natural populations is fraught with difficulties and it is probably impossible, unless long periods of careful observation coupled with elaborate statistical analyses are undertaken. It is much easier to estimate the reproduction rate, especially in a carefully controlled population. Yet there is little information in this field on Scandinavian newt populations and in order to add to our knowledge about them it would seem to be advisable to begin by studying a single small breeding population, which can be carefully controlled. One may add in fairness, however, that such a group of newts in the particular pond studied may not be fully representative of all the breeding populations in the neighbouring territories, but should provide some indication of general nature about them all.

The contribution is a first attempt to estimate reproductive success of a local Gothenburg population of smooth newts, *Triturus vulgaris,* breeding under fairly natural conditions.

DESCRIPTION OF THE EXPERIMENTAL POND

The pond (Fig. 1) is artificial and located in the Rock Garden of the Botanical Garden of Gothenburg in Western Sweden. The Rock Garden is a stony area on a hillside about 85 m above sea level. The vegetation consists of shrubs and herbs but only a few trees. Alpine and high-boreale elements are important parts of the flora. The pond, situated in a cleft, is 7 m long, a maximum of 2 m wide and about 50 cm deep. Newts and their tadpoles are found at the bottom, in an area of 9 m^2, which is smooth and flat, with a few stones but practically no vegetation. The floor consists of blue clay on which there is a thin layer of mud. There are no objects under which newts may hide. As the water is clear, all animals can easily be seen from the edge.

Figs. 1 & 2



The pond is emptied in November and filled with water the following April (newt spawning begins at the end of April or the beginning of May). As the water level can be regulated the pond is never dry except during the winter.

In some years, especially during September, a large number of microscopic green algae are present in the water.

The pH of the water is about neutral.

It is one of several ponds in the neighbourhood, where newts may spawn.

The pond and its surroundings are guarded throughout the year.

METHODS

The pond was visited once a week in the middle of the day during the season. The study program continued during 1970 and 1971. Temperature and water level etc. were checked regularly.

1. *Adult newts.* Every adult in the pond was captured by netting. As soon as they were photographed (for specimen identification by the belly pattern method, see Hagström, 1973), they were returned to the water. The photographs were investigated later in the laboratory.

2. *Tadpoles* (with gills). Tadpole numbers were estimated by counting them in 2-3 areas, randomly selected, each of one m^2, and the average number was multiplied by 9 (= number of square metres of pond floor surface). Size and development-stages (based on gills and limbs) of the tadpoles were recorded.

3. *Newly-metamorphosed animals.* In order to estimate the number a barrier of PVC-plastic was placed at ground edge completely surrounding the pond. It inclined towards the pond so that specimens were restricted and could not leave the water. In some sections of the barrier small holes were covered with a fine net, to permit circulation of water. The plastic sections were so jointed that the joints were absolutely tight. At one end (under a small Salix shrub) a trap of PVC-plastic was placed, its construction based on the pit-fall principle. Newts were caught in a glass-can (see Fig. 2). The trap and barrier were fixed in their place some weeks before metamorphosis began but (even after the adults had left the pond) they were retained as long as there were any young newts in the water. Occasionally newly-metamorphosed animals were found at the opposite end of the pond trying to climb the barrier. They were included in the number of young animals caught in the trap.

RESULTS

1. *Adult T. vulgaris* (Figs. 3, 4; Table 1). During 1970 and 1971 adults were present in the pond at two periods per year. In 1970 breeding occurred during the first period. During the second period several specimens had lost their breeding-dress and the animals seemed to be searching for food in a more active way than during the first period. In 1971, however, both mating-behaviour and egg-laying were observed during both periods.

2. *Tadpoles of T. vulgaris* (Fig. 5). In 1970, all tadpoles were about the same age, hatched about the same time, and were of similar size throughout the season. In 1971 tadpoles hatched during two periods, those of the second group hatched when the first group was metamorphosing.

3. *Metamorphosis of T. vulgaris* (Figs. 6, 7). In 1970, metamorphoses proceeded for a period longer than a month, but in 1971 it was concentrated into two well-marked periods. In 1970 there were 14 metamorphosed specimens, and in 1971 13.

4. *Occurrence of T. cristatus Laur.* (Figs. 3, 4). A few Crested Newts were found in the pond during spawning-time in both years: 6 males and one female in 1970, 4 males and 2 females in 1971. A small number of tadpoles (max. 5) were observed in the pond during both years and in October 1970 one metamorphosed individual was caught in the trap. Such juveniles were not included in any of the data illustrated.

DISCUSSION

The newts in our experimental pond constitute only a small proportion of a larger population, breeding in several ponds in the neighbourhood. Our results therefore reveal little about the composition of the entire local population. Adult newts were recognised in the pond during two periods in each of two years, but in 1970 they did not spawn during the second period. Instead, they were actively feeding. It can not be excluded that newly-hatched tadpoles, present in great number at this time, were the main prey of the adult animals (see Smith, 1969), which may explain why they remained in the water.

The reason why spawning occurred twice in 1971 is not known. All specimens captured during the second spawning period (excl. one male) also occurred in the pond during the first period.

Fig. 3

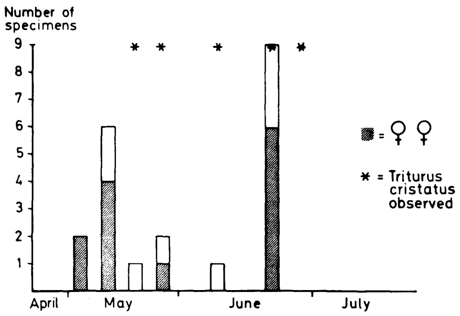


Fig. 4

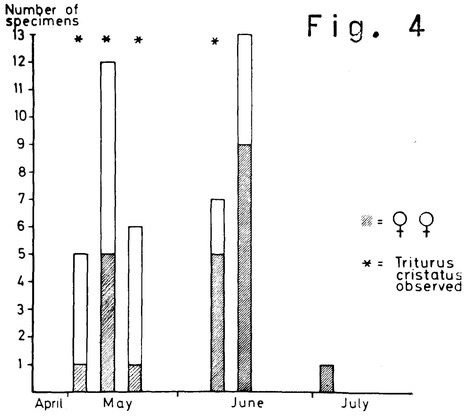
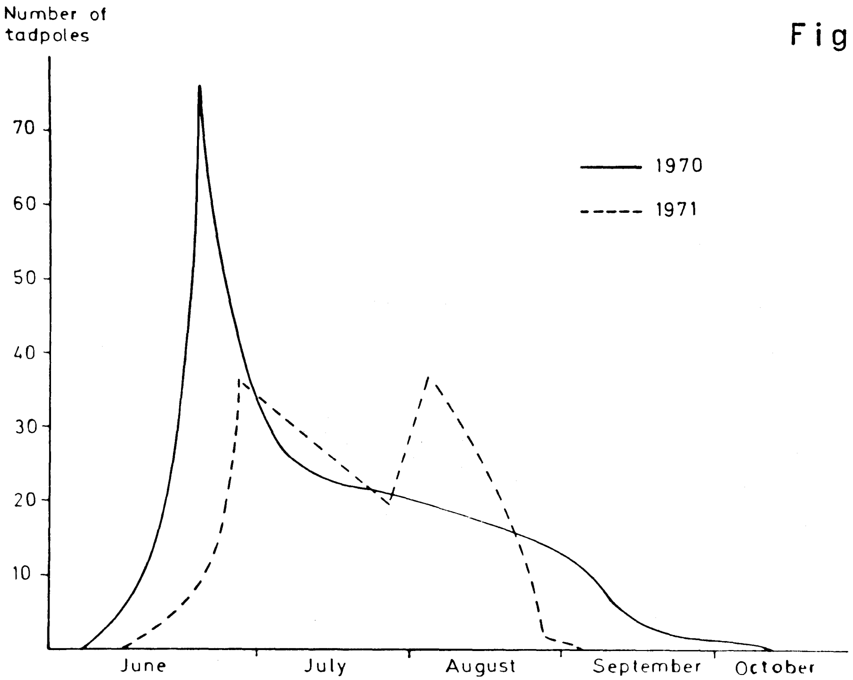


Fig. 5



It must be noted that the reproductive success in our pond is surprisingly low. If this result is representative of the population, the life-span of the mature animals must be high. It is interesting to note, in this context, that newts are known to become very old in captivity (more than 20 years), though this does not in itself imply that the average age of mature animals is high in natural populations.

We do not know which is the most important factor limiting the number of tadpoles in this pond. In a pond in the neighbourhood, with more vegetation, tadpole density was always higher. Presumably the number of eggs laid is high enough. The number of eggs laid per season by one female *T. vulgaris* is 100-250 (Freytag, 1954), 200-300 (Steward, 1969) or even 500-700 (Schiøtz, 1970). The percentage of fertilised eggs, which under normal circumstances hatched in our pond is not known.

In 1971 the two spawning periods were each followed by egg hatching and metamorphosis. The final result, however, is about the same as the year before (Figs. 6, 7). It is concluded that in the experimental pond about two fullgrown tadpoles will be produced per m^2, and of those tadpoles at least about two thirds will metamorphose. This means 2.25 fullgrown tadpoles per female in 1970 and 1.64 in 1971.

After hatching the number of tadpoles decreases, initially at a faster rate than later on.

In 1971 the tadpoles grew faster than in 1970, possibly due to the higher water temperature (Fig. 8) during the summer 1971 (see Gislén & Kauri, 1959). It has been suggested that temperature may influence size at metamorphosis (Creed, 1964), but the size of newly-metamorphosed *T. vulgaris* of the different year groups and of the two groups of young animals in 1971 showed no clear difference.

Fig. 6

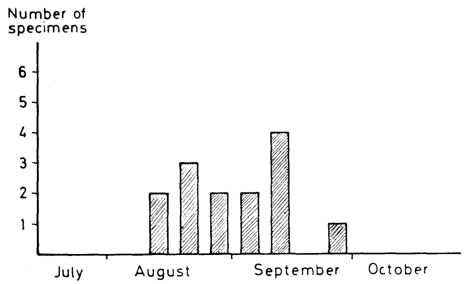


Fig. 7

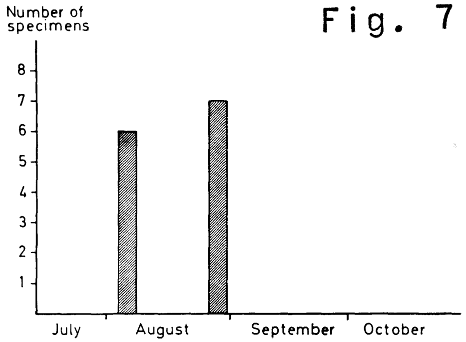
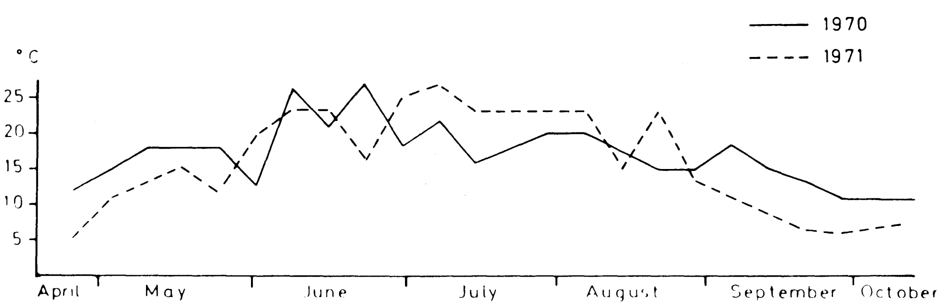


Fig. 8



SUMMARY

Tadpoles and newly-metamorphosed specimens of *Triturus vulgaris* L. in a pond in the Rock Garden of the Botanical Garden of Gothenburg in western Sweden were studied during 1970 and 1971. In both years adults were found in the pond during two periods, one in spring and one in early summer. In 1970 animals spawned only during the first period. In 1971 the two spawning periods were each followed by periods of egg hatching and metamorphosis. The pond accommodated two fullgrown tadpoles per m² (= 2.25 per female in 1970 and 1.64 in 1971), and of these more than two thirds metamorphosed. Tadpoles grew somewhat faster in 1971, possibly owing to higher water temperature. A few *T. cristatus* Laur. were observed in the pond, but their reproduction here was negligible.

ACKNOWLEDGEMENTS

The author is grateful to the staff of the Botanical Garden of Gothenburg for help in several ways during the field work.

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FIGURE 1.
The experimental pond and relevant trapping equipment.

FIGURE 2.
The trap used to capture newly-metamorphosed newts.

FIGURE 3.
Number of adult *Triturus vulgaris* in the pond in 1970.

FIGURE 4.
Number of adult *Triturus vulgaris* in the pond in 1971.

FIGURE 5.
Number of tadpoles of *Triturus vulgaris* in the pond.

FIGURE 6.
Number of newly-metamorphosed *Triturus vulgaris* in 1970.

FIGURE 7.
Number of newly-metamorphosed *Triturus vulgaris* in 1971.

FIGURE 8.
Water temperature of the pond.

	Number of specimens caught in 1970	Number of specimens caught in 1971	Average number of times each specimen was caught in 1970	Average number of times each specimen was caught in 1971	Number of specimens caught in both years
Males	5	13	1.4	1.7	—
Females	8	11	1.6	1.8	2

Table 1

A COMPARISON OF LIPIDS FROM THE FAT BODY AND TAIL OF THE COMMON LIZARD, *LACERTA VIVIPARA*

By

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(*Received* 15/1/73)

INTRODUCTION

It has long been known that the abdominal fat bodies of lizards, like those of most reptiles, are major storage reserves, and that they vary in size from season to season. More recently it has been shown that fat stored in the tail of the Common lizard *Lacerta vivipara* Jacquin, fluctuates in a similar way, and that the quantities of stored material at the two sites are approximately equal (Avery, 1970). In this paper we report an analysis of lipids from the two sites, carried out in order to determine if there were any differences in composition between them.

MATERIAL AND METHODS

Six adult male lizards were captured at Priddy in Somerset between 16 March and 21 March, 1972, i.e. during the period immediately after hibernation. The abdominal fat bodies and the tail fat deposits were removed by dissection and stored in iso-propyl alcohol with 0.0005% butylated hydroxytoluene as antioxidant. The material was pooled for analysis.

Lipids were extracted in isopropanol/chloroform mixtures (Thomas & Stobart, 1970) and fractionated into polar and neutral components (Stobart & Pinfield, 1970) which were estimated gravimetrically. Methyl esters of the neutral lipid fatty acids were prepared by transmethylation and separated by gas chromatography. Glycerides were separated into saturation groups by thin layer chromatography using 0.25 mm Kieselgel plates which were impregnated with 5% $AgNO_3$ and developed in a CCl_4 — $CHCl_3$ — glacial acetic acid—ethanol mixture. Diglycerides were seperated from triglycerides by TLC using Kieselgel plates and a light petroleum 40-60° — diethyl ether — glacial acetic acid mixture (Shewry, Pinfield & Stobart, 1972). After development, TLC plates were sprayed with 50% sulphuric acid and charred for 20 min at 220°C. Quantitative measurements were made by densitometer tracings of the charred areas using a Joyce-Loebl chromoscan. Polar lipids were fractionated into individual phospholipids by TLC with acid and basic solvent mixtures (Nichols, 1964). Free sterols were precipitated as the digitonides which were redissolved in glacial acetic acid and estimated by the Lieberman-Burchard reaction (Moore & Baumann, 1952). Amounts of total free sterols were calculated from a standard cholesterol calibration curve.

RESULTS

COMPONENT ACIDS

95.5% of the lipids from both the fat body and the tail fractionated into neutral components, and 4% into polar components. The polar lipids were predominantly phosphatidyl ethanolamine and phosphatidyl choline.

The major neutral lipid fatty acids which were identified by gas chromatography according to their positions relative to a palmitate standard, are shown in Table 1. The most abundant corresponded to oleic acid, and comprised slightly more than half of the total neutral fatty acid in both tissues. Oleic acid is an unsaturated fatty acid with eighteen carbon atoms and a single double bond in the molecule; it has the structural formula $CH_3(CH_2)_7CH=CH(CH_2)_7COOH$ and is usually represented as $C_{18:1}$ (see Table

1). There were also relatively large quantities of palmitic ($C_{16\,0}$), linoleic ($C_{18\,2}$) and linolenic ($C_{18\,3}$) acids. (There is an excellent account of lipid classification and its biological significance, written for the non-biochemist, in an article on "Fish Nutrition" by C. B. Cowey & J. R. Sargent in *Advances in Marine Biology* vol. 10, 1972). Differences in the percentages of individual acids in the fat body and the tail were small and are almost certainly not significant, although a possible exception is that of linolenic acid, which comprised 10.46% of the neutral fatty acids in the fat body, but only 6.95% of those in the tail.

COMPONENT GLYCERIDES

Chromatographic separation of glycerides by argentation TLC is based on the degree of unsaturation in the molecule; consequently each component does not usually represent an individual glyceride, but a number of glycerides having the same degree of unsaturation.

Eleven saturation groups were found in both tissues; the percentages of each are shown in Table 2. The differences are almost certainly not significant, with the possible exceptions of those in groups 8 and 9. Triglycerides comprised 88% of the fat body neutral lipids and 91% of the tail neutral lipids (Table 3), whilst diglycerides comprised 9% and 4% respectively. The larger amounts of glyceride in groups 8 and 9 in the fat body neutral lipid fraction were possibly related to the high amounts of linolenic acid ($C_{18\,3}$) and diglycerides in this fraction.

Sterols estimated as cholesterol comprised less than 1% of the total neutral lipid in both tissues.

DISCUSSION

There have been very few studies of reptile lipids, and none of those of the tail. Nearly all of the reptile lipids which have been analysed have a high proportion of unsaturated C_{18} acids, with oleic acid predominating (Hilditch & Williams, 1964; Grenot, 1968). Zain & Zain-ul-Abedin (1967) have noted that the fat bodies of the desert lizard *Uromastix hardwickii* contain 90% of esterified fatty acids, and are therefore more equivalent to white adipose tissue than to the brown fat which is associated with mammalian hibernation, since brown fat contains relatively more glycogen, phospholipid and cholesterol, and relatively less neutral lipid. Our resuts are consistent with both of these findings.

Although the abdominal fat bodies and the fat deposits around the tail vertebrae are quite distinct sites, there are no major differences in their composition. Since only one analysis of each tissue has been made, we are not able to determine whether the small differences in the percentages of linolenic acid and diglycerides are significant, but the related differences in the percentages of glyceride groups provide circumstantial evidence that they are. It is interesting in this context that amounts of fat body linolenic acid fluctuated more widely between four North African lizard species than those of six other major fatty acids which were measured (Grenot, 1968).

Since the composition of the deposits in the two tissues is essentially similar, it is perhaps not surprising that their lipids are utilised during the course of hibernation to a similar extent (Avery, 1970). A more detailed account of lipid storage and release in lizards of different age, sex and weight at different times of the year, will be published elsewhere.

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Identity	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	others
Position relative to palmitate	0.31	0.56	1.00	1.24	1.78	2.07	2.80	3.91	4.0
Percentage in fat body	0.91	1.63	13.08	5.85	4.13	51.40	11.88	10.43	2.0
Percentage in tail	1.23	2.28	14.85	6.41	3.91	52.13	11.64	6.95	2.0

Table 1. Neutral fatty acid content of the fat body and the tail. Results are expressed as percentages of the total neutral lipid fatty acid.

Glyceride group number	1	2	3	4	5	6	7	8	9	10	11
Relative front (Rf)	0.89	0.83	0.76	0.65	0.57	0.47	0.37	0.31	0.25	0.19	0.15
Percentage in fat body	3.49	14.90	23.57	11.77	10.12	12.42	5.33	5.65	6.76	3.50	2.49
Percentage in tail	2.42	14.54	27.22	13.09	12.19	12.92	5.67	3.13	3.90	2.70	2.22

Table 2. Glycerides of the fat body and tail. Glyceride group numbers refer to the relative positions of the glycerides on $AgNO_3$-impregnated TLC plates; the results are expressed as percentages of total glyceride.

Spot number	1	2	3
Identity	diglyceride	unknown	triglyceride
Relative front (Rf)	0.30	0.35	0.74
Percentage in fat body	9.10	3.09	87.81
Percentage in tail	4.07	3.51	91.42

Table 3. Total diglyceride and triglyceride in the fat body and tail, expressed as percentages of the total glyceride.

INFLUENCE OF PHOTOPERIOD AND LIGHT INTENSITY ON LIZARD VOLUNTARY TEMPERATURES

By

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(*Received* 23/3/73)

Regal (1967) and Gehrmann (1971) have shown that reptiles kept in temperature gradient chambers exhibit a 24-hour rhythm in their voluntary temperatures (body temperatures associated with normal activity), which is synchronised with a light dark cycle. Although this phenomenon has been considered in detail and although several explanations proffered, the parameter of light intensity has not been discussed in connection with reptile voluntary temperatures.

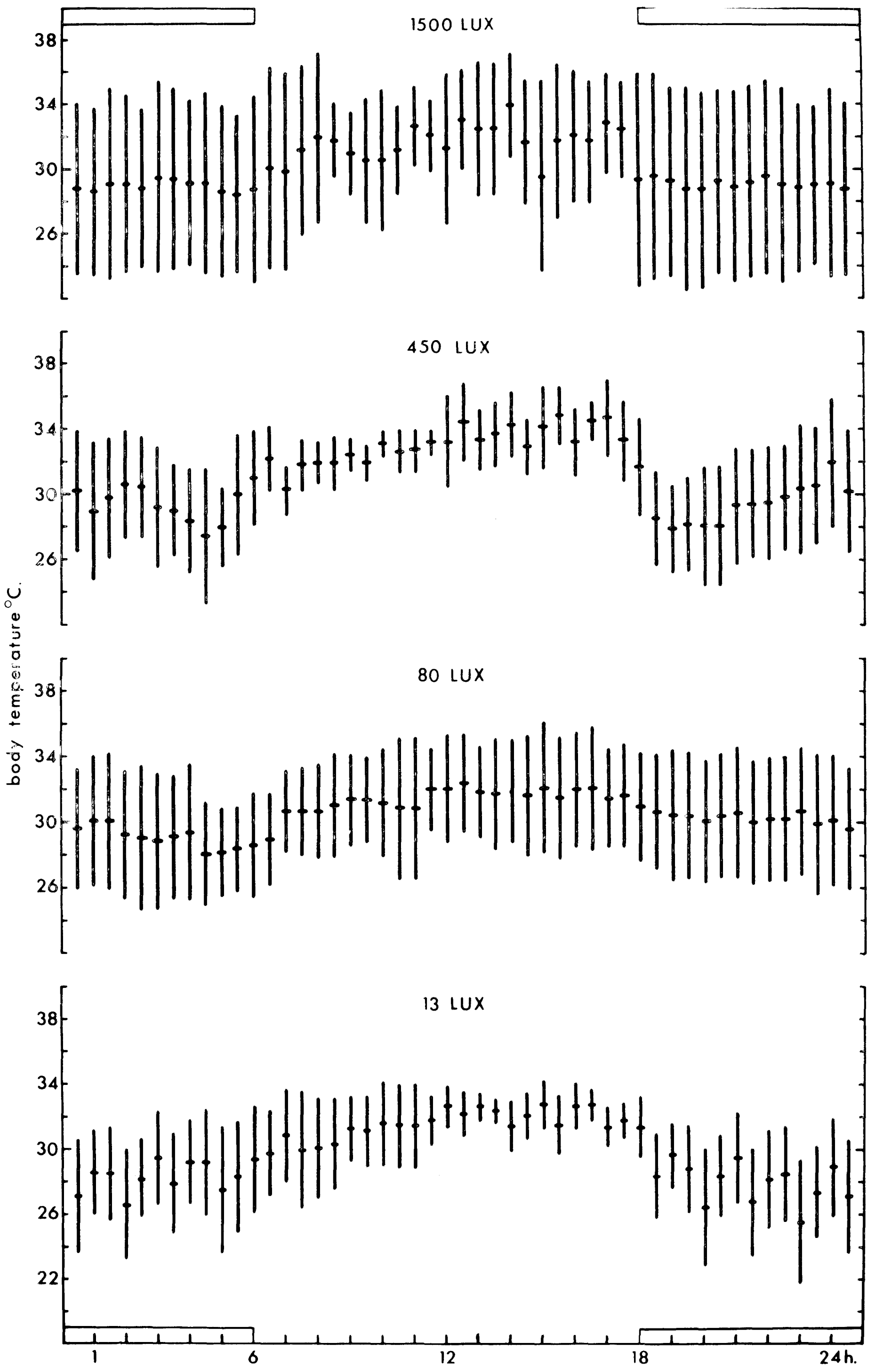

FIG. 1

A complex relationship exists between reptile activity periods and both ambient temperatures and photoperiods. Photoperiods do in part alter activity periods of some reptiles (see Cloudsley-Thompson, 1971 for a review). Further, it has been shown that increased light intensity will decrease spontaneous activity period lengths of *Lacerta sicula* kept in continuous light (Hoffmann, 1960).

As both the nature and the duration of light appear to influence reptile activity periods, and in view of the possible ecological and physiological importance of "voluntary hypothermia" (Regal, 1967), it seemed valuable to investigate the influence of these parameters on lizard voluntary temperatures.

MATERIALS AND METHODS

Lizards from a cool temperate thermal zone (*Lacerta agilis*) were collected in the vicinity of Erling-Andechs, Upper Bavaria and lizards from a warm temperate thermal zone (*Lacerta viridis*) were supplied commercially. The mean weight of *L. agilis* was 13 gms; *L. viridis* was 28 gms. Results from both males and females were combined.

A long box was used as a thermal gradient chamber and this was placed in a room where the room temperature of 5°C set the lowest temperature of the chamber. Heat lamps were placed under the chamber and provided a thermal gradient from 45°C to 5°C. The chamber had two identical compartments so that the temperatures of two lizards were monitored simultaneously. Dimensions of each compartment were: length 200 cm; width 25 cm; depth 33 cm.

Three pairs of water dishes and food dishes were spaced equidistant in each of the compartments. Three cardboard ledges (36 x 5 cm) in each compartment were placed one cm above the base and attached to the central partition. These were located at each end and in the middle of the chamber and they provided submergence localities for the lizards.

One lizard was placed in each compartment and the body temperature (large intestine) of each was recorded continuously with thermocouples. Once the lizard was instrumented it was allowed a 72 hour adjustment period prior to the experiments.

For both species there were four experiments and a different pair of lizards was used for each. The four experiments differed only in the light intensities used. A light-dark cycle (LD) was used for 9-11 days and this was followed by a period of 4-6 days continuous light (LL). Standard room lighting was used and the light source was 120 cm above the chamber. The LD schedule was on a 12 : 12 hour basis with 1,500, 450, 80 or 13 lux in the light phase and 0.01 lux in the dark phase.

Temperatures of a model lizard made from plasticine were used as a control. The model resembled *L. viridis* and was placed at about the centre of the thermal gradient chamber. Temperatures were recorded and treated as if for a real animal.

RESULTS

Mean body temperatures for each half hour interval over a 24-hour period are shown in Figs. 1 and 2. Lizard body temperatures are higher in the light phase than in the dark phase and the difference between light phase and dark phase means (mean of half hour interval means) was 1.6°C-3.7°C for *L. agilis* and 1.9°C-3.7°C for *L. viridis*. (Table 1). During LL the greatest difference between mean temperatures of the equivalent "light phase" and "dark phase" was 0.8°C for *L. agilis* and 1.3°C for *L. viridis* at a light intensity of 450 lux (Table 1). Although temperature records for LL were examined carefully for spontaneous rhythms, no 24-hour cycle was detected, although under natural conditions both *L. agilis* and *L. viridis* exhibit a diurnal monophasic period of activity (Marx & Kayser, 1949; St. Girons, 1971).

A sudden increase in body temperatures at the onset of the light phase is particularly noticeable at 1500 lux and 450 lux for both species while decrease in body temperatures at the termination of the light phase is more gradual. During the light phase and in some half-hour intervals as many as

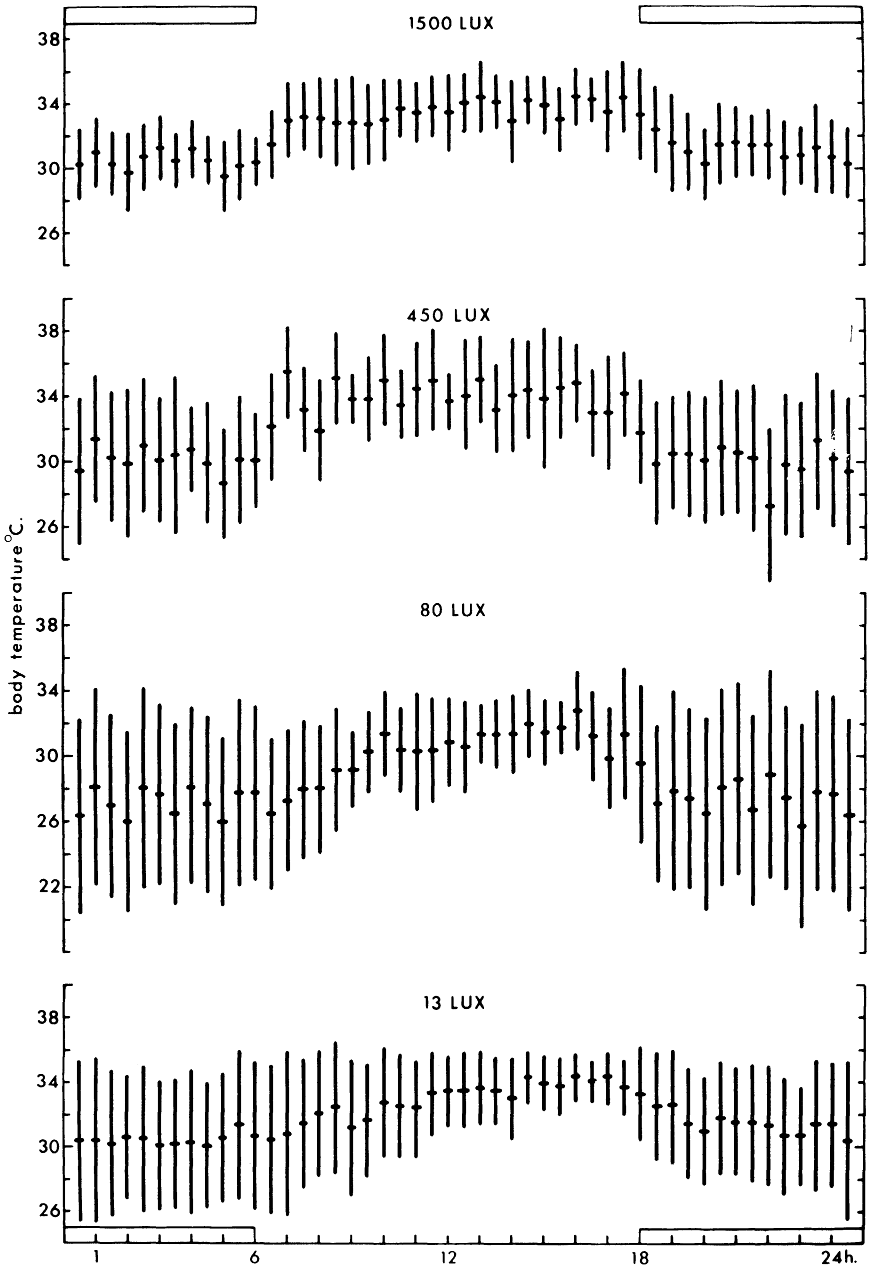

FIG. 2

68.3% of the recorded temperatures might be spread over 13 centigrade degrees (as indicated by one standard deviation) and the spread of body temperature values in each light phase half-hour interval is less than that recorded in each dark phase interval.

Apart from the diminished rate of change in body temperatures at the onset of the light phase with a decreased light intensity, there does not appear to be any change in the voluntary temperature levels over the four light intensities.

The distribution of body temperatures is shown in Figs. 3 and 4 as percentage frequency histograms. A control histogram is shown with data from the model lizard (Fig. 5). Generally the histograms are negatively skewed with some bimodal patterns (smaller peaks at lower body temperatures). The histograms for LL are all very similar but the LD histograms show that there is an increased frequency of higher body temperatures in the light phase of an LD cycle.

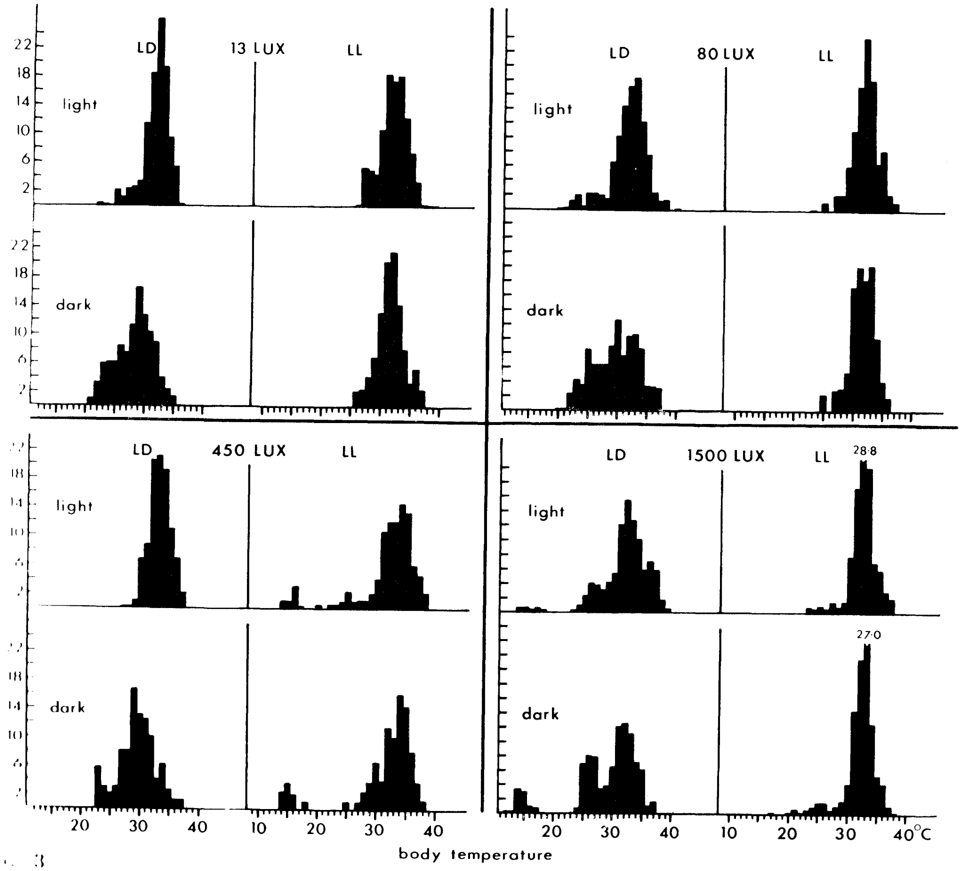

FIG. 3

DISCUSSION

The results show a difference between lizard mean body temperatures recorded in the dark phase and those recorded in the light phase of an LD cycle. This does not necessarily mean that the reptiles show voluntary hypothermia and several possible contributing factors should be noted. At high light intensities in particular there was a sudden change in radiation in the overhead lights and thus a change in the amount of energy available from this source. That is could the lizards utilise the radiation from the light source and could this have an effect on the body temperature levels? This was not investigated but results from the plasticine model lizard suggest that if a lizard remained stationary, then changes in radiation levels would not alter the body temperature. Although there was a considerable range in the temperatures of the model this would have been caused by the fluctuating room temperature.

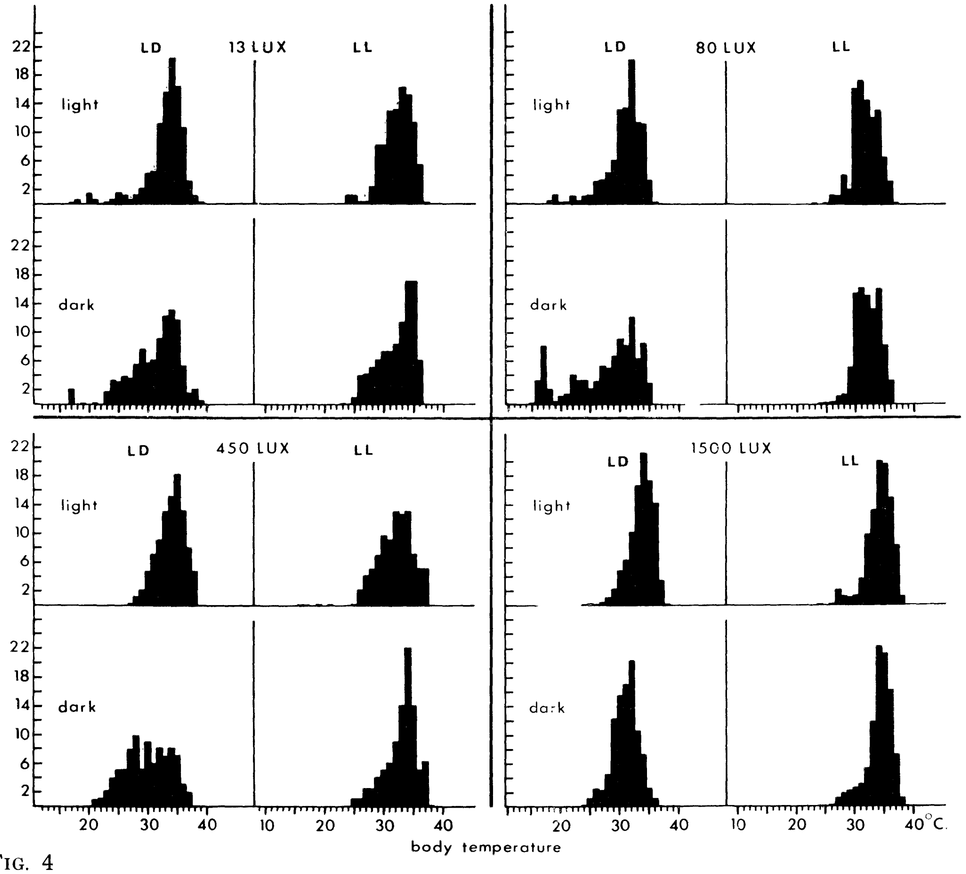

FIG. 4

Changes in the level of metabolic heat production and/or integument conductance, synchronised with the LD cycle is a factor which requires further investigation. It is however only in larger reptiles, with a small surface area in proportion to their mass, that metabolic heat production greatly alters the body temperature, and even in these large animals it is due to the large mass which has a slow loss of heat. In the lizards examined here (13-28 gms) there may be a slight change in metabolic heat production synchronised with the LD cycle but it is unlikely that this could contribute greatly to the present results.

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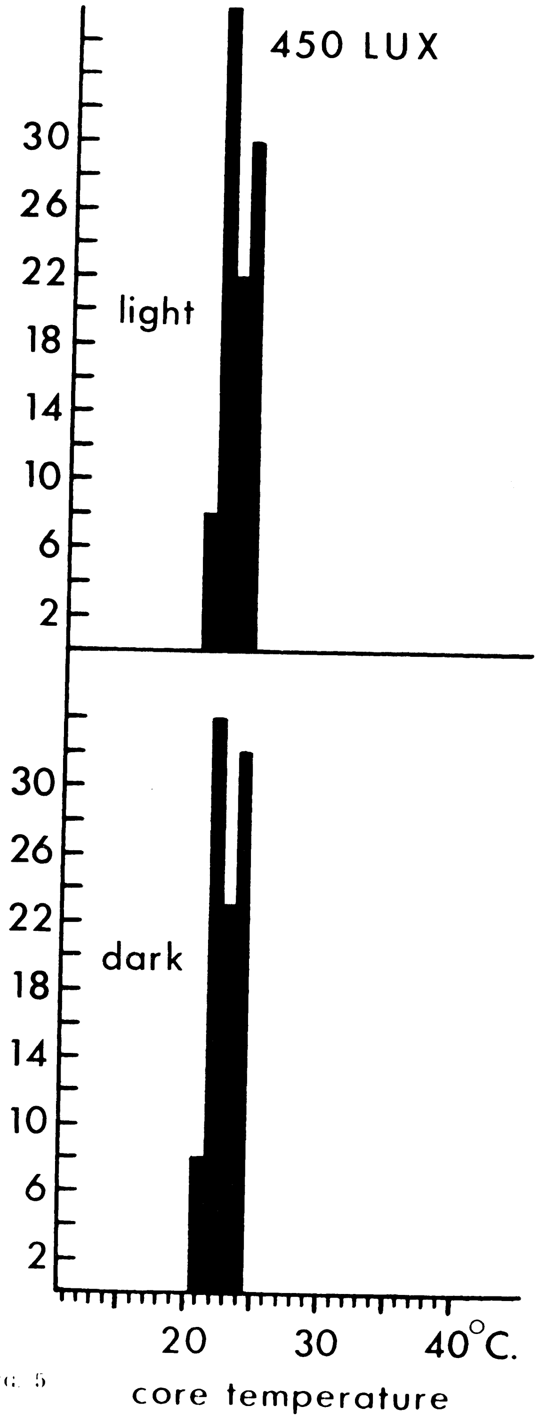

FIG. 5

The difference in body temperature levels could simply reflect a difference in activity. That is during the light phase the lizards are active and will therefore be more likely to move over warmer areas of the chamber more often than during the dark phase when less activity would be expected. The mean half hour temperatures (Fig. 1, 2) do not indicate this, and the small standard deviations in the light phase suggest that the lizards do in fact select out higher temperatures at that time.

It seems plausible that these two species do select lower body temperatures at night. Possibly the termination of light may be a signal for submergence and under natural conditions it is assumed that submergence in these species diminishes chances of predation and exposure to sub-Critical Minimum temperatures. Under natural conditions the lizards submerge during the late afternoon when light intensity has decreased although substrate surface temperatures might still provide sufficient heat energy for the maintenance of voluntary temperatures. It seems therefore that onset of dark acts as a signal to submerge which is coupled with a decrease in body temperature. That is submergence and a decrease in body temperature are synonymous to the animal. In the thermal gradient chamber, the lizard reacts to the onset of the dark phase not by submergence but by a selection of a lower body temperature. In other words in this artificial situation the lizard can not submerge but a lowering of the body temperature is equated with submergence and signals protection from predation and possibly lethal ground surface temperatures.

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	LD			LL		
light intensity (lux)	light phase (°C)	dark phase (°C)	defference	"light phase" (°C)	"dark phase" (°C)	difference
L. agilis						
1500	31.5	29.0	2.5	31.9	31.9	0.0
450	33.0	29.3	3.7	31.6	30.8	0.8
80	31.4	29.8	1.6	32.3	31.6	0.7
13	31.5	28.3	3.2	31.8	31.6	0.2
L. viridis						
1500	33.4	30.8	2.6	33.6	33.9	0.3
450	33.8	30.1	3.7	31.6	32.9	1.3
80	30.2	27.3	2.9	31.7	31.9	0.2
13	32.9	31.0	1.9	32.0	31.9	0.1

Table 1. Mean body temperatures in a thermal gradient.

EXPLANATION OF TEXT FIGURES

FIGURE 1.
Lacerta agilis mean body temperatures for each half hour interval in a light dark cycle. Light phase commences at 0600 hours. The horizontal bar is the mean and the vertical line is ± one standard deviation. Data for each light intensity are the combined results from two animals over 9-11 days.

FIGURE 2.
Lacerta viridis and as for Figure 1.

FIGURE 3.
Lacerta agilis percent frequency histograms of body temperature in LD and in LL. Data for the light and dark phase in LD are the same as used in Figure 1. Data for LL are the combined results from two animals over 4-6 days. No 24-hour rhythm was detected in LL and so temperatures for the equivalent "light" phase histogram represents 06.00 hours to 18.00 hours.

FIGURE 4.
Lacerta viridis and as for Figure 3.

FIGURE 5.
Model plasticine lizard percent frequency histogram of temperatures recorded for ten days in LD.

A NOTE ON A TAILLESS EMBRYO OF THE LIZARD, *CALOTES VERSICOLOR*

By

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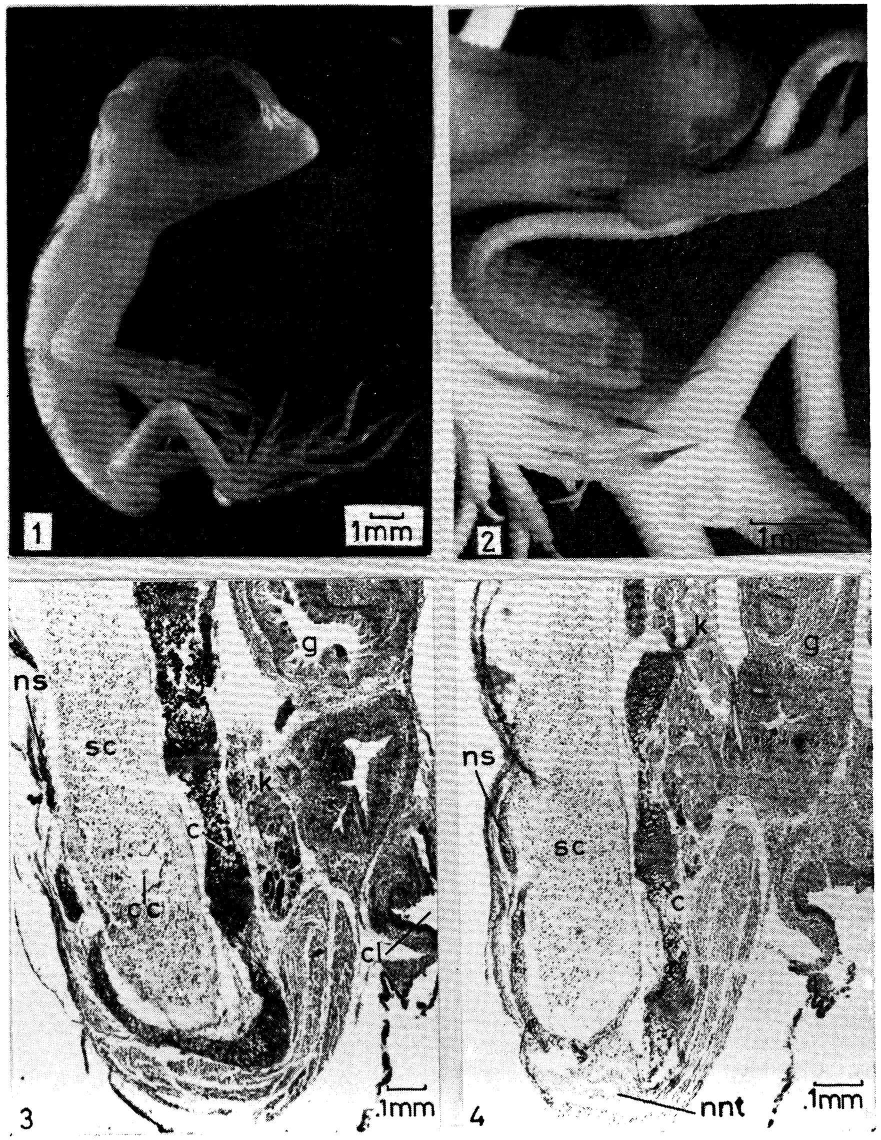
(*Received* 13/1/73)

During our studies of the embryology of *Calotes versicolor* we examined well over 500 embryos at different stages of development. The embryos were obtained from eggs collected from the field or recovered from gravid females. All the eggs were successfully incubated up to hatching at room temperature (22.2° to 31.7°C) on water flooded cotton wool.

A clutch of 16 eggs (usual clutch size 11 to 20) was recovered from a gravid female on 14th August, 1971. Of these eggs 12 were set aside for incubation and the other four were used for biochemical studies. Of the 12 eggs set aside for incubation 8 were opened during the course of incubation and 4 were incubated up to their hatching on 7th October, 1971. Except for a single embryo recovered by opening an egg on 20th September all the other embryos and hatchlings of this clutch were found to be normal.

The abnormal embryo was 16 mm long and was at stage 40 of development (Muthukkaruppan *et al.*, 1970). It showed an abnormality hitherto unreported in any lizard: complete absence of a tail (Fig. 1). The only other morphological deviation in the embryo concerned the location of the cloaca. Normally the cloaca is located a little posterior to the junction of the hind limbs with the body but in this embryo it was located at the level of the junction itself (Fig. 2). A postanal stump, 1 mm x 2 mm, was formed due to the forward shifting of the cloaca. For histological studies the embryo was fixed in 4% buffered formalin (pH 7.2) and sectioned at 4 μm in paraffin wax. The sections were stained with methyl green-pyronin or alcian blue-eosin.

The embryo was normal with respect to the disposition and development of the alimentary canal, kidneys, hind limbs and the limb girdles as well as the spinal cord. The posterior sacral vertebra of the embryo, on the other hand, had deveoped a 65 μm thick cartilage which effectively blocked the distal end of the neural canal (Fig. 3) except for two small lateral openings. Consequently the spinal cord abruptly terminated anterior to this cartilage; but was apposed with the non-nervous tissue passing in through the lateral openings (Fig. 4). The caudal vertebrae were represented by a single cartilaginous nodule 100 μm high and 25 μm thick.



It is extremely hazardous to comment on the causes of this malformation. However, after the study of the embryos in this and other clutches the authors are left with an impression that the malformation may have been due to genetic rather than environmental factors. Regarding the embryological history of taillessness the authors visualise the following two possibilities. The neural tube did not develop past the level of the last sacral vertebra. And this primary event may have prevented the induction of the caudal vertebrae and other tail structures (Holtzer and Detwiler, 1953). Alternatively, though less probably, the development of a cartilage at the caudal end of the neural tube of the last sacral vertebra may have been the primary event. This may have obstructed the posterior growth of the spinal cord and the formation of a tail. Anyway, it seems unlikely that the taillessness may have resulted due to any obstruction to the blood supply of the tail (Bellairs, 1965).

The authors wish to express their gratitude to Professor L. Mulherkar for encouragement and laboratory facilities.

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EXPLANATION OF TEXT FIGURES

FIGURE 1.

Lateral view of the tailless embryo of *Calotes versicolor*.

FIGURE 2.

Ventral view of a normal (lower) and the tailless (upper) embryo. The cloaca is at the level of the junction of pelvic limbs in the tailless embryo but is a little posterior to the junction in the normal embryo.

FIGURE 3.

Posterior part of a median sagittal section through the tailless embryo. c—centrum of vertebra, cc—central canal, cl—cloaca, g—gut, k—kidney, ns—neural spine, sc—spinal cord. Note the well-formed cartilage closing the posterior end of the neural canal.

FIGURE 4.

Posterior part of a sagittal, but lateral, section through the tailless embryo. Note the direct apposition of the non-nervous tissue (nn) and the spinal cord through the lateral openings on the posterior end of the neural canal. Abbreviations as on Figure 3.

SCALE REGENERATION IN *THAMNOPHIS SIRTALIS*

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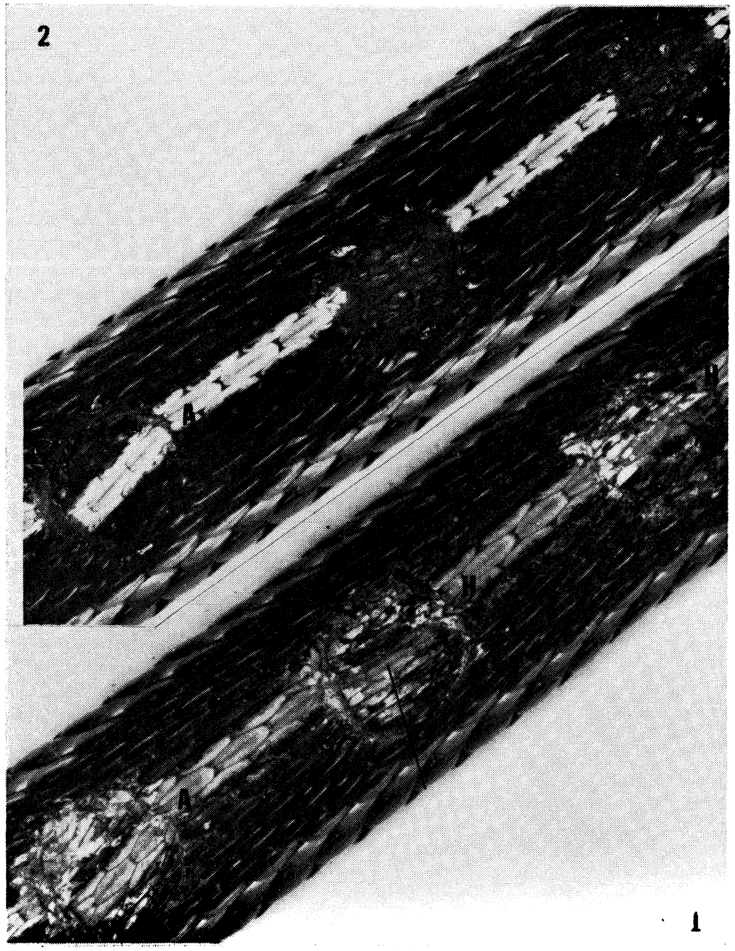
The comparative aspects of wound healing have focused interest on the regeneration of epidermal appendages such as hair and scales. New scales have been observed during tail regeneration in many lizards (Noble & Bradley, 1933). Also, Weber (1905) noted scale rudiments when one of the large shield-like scales was removed from the head of *Lacerta*. Noble and Bradley (1933) confirmed Weber by observing scale regeneration after excising full-thickness skin from the back of *Tarentola*. May (1923) also reported scale regeneration after the rejection of skin homografts in *Anolis carolinensis*. To my knowledge the Ophidia have not been studied.



Although the preceding studies have shown scale regeneration in lizards which regenerate tails, I have not been able to find examples of scale regeneration during wound healing in lizards which do not regenerate a tail. Consequently, a second part to this study asked if new scales would appear in a reptilian species which could not regenerate a tail. This would test whether scale regeneration is necessarily linked to the capacity of some squamates to regenerate a tail. Could scale regeneration evolve as a separate mechanism or did lizards evolve scale regeneration only because they also evolved the capacity to regenerate a tail?

The garter snakes were obtained from E. G. Steinhilber & Co., Oshkosh, Wisconsin. The animals were said to be from Manitoba and were identified as *Thamnophis sirtalis parietalis* by Wright and Wright (1957). The care, feeding and anaesthetic used during the experiments have been described (Terebey, 1972).

Scale regeneration was studied in animals after the rejection of skin homografts. During the course of two seasons, homografts were successfully exchanged between 85 animals (Fig. 1). All homografts were rejected except on one animal where homografts continued to appear healthy after six months of observation. Autografts healed in and remained unchanged (Figs. 1 and 2). Autografts were usually rotated but did not show a rearrangement of pigment and scale pattern to the pattern of surrounding host skin (Fig. 2). The immunological aspects of this study have already been presented elsewhere (Terebey, 1972).

Following a course of homograft rejection, two animals were set aside to observe the long term changes which would take place during wound healing. The site of skin homografts was re-surfaced by new epithelium and by a layer of dermal melanophores since the area assumed the black pigmentation of host skin. After about 70 days after grafting, new scales rudiments were observed. These scales were at first thought to be dermal remains of the homografts which had been covered by new epithelium. However, the rudiments continued to grow and elongate and assume the gross morphology of scales (Fig. 2).

A control operation involving an open wound without the transfer of a homograft was not done as it did not occur to me at the time. However, several of these operations have been reported in the lizards, arguing against the influence of skin homografts in the appearance of new scale rudiments (Noble & Bradley, 1933; Whimster, 1965). Also, the scales always assumed the antero-posterior orientation of host skin and not the rotated orientation of the skin homografts.

Woodland (1920) found that autotomy was a universal finding in lizards which regenerate a tail. The absence of autotomy in the garter snake and in Ophidia in general establishes that scale regeneration can be maintained in species which do not regenerate a tail. This was confirmed by failure to find new tail growth after the amputation of the old tail in male and female *Thamnophis sirtalis*.

Scale regeneration has not been reported in lizards which do not regenerate a tail although this may be simply due to the fact that such studies have not been done. According to Woodland (1920), the absence of autotomy in these lizards is an asset since the tail is used for prehension (gecko *Ceratolophus auriculatus* and chamelaeons), swimming (aquatic monitors, Iguanidae, *Amblyrhynchus*, *Lophurus* and *Physignathus*), steering in water (*Basilicus*) and steering in air (*Ptychozoon*) and balancing in air (*Draco*). Studies in these species would confirm whether scale formation is a fundamental property of the squamate integument. Clearly tail regeneration is not.

Finally, the immediate value of this study is that it gives additional evidence for the functional similarity of the integument in snakes and lizards. In the past this has always been assumed but rarely shown by experimental studies.

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EXPLANATION OF TEXT FIGURES

FIGURE 1.

A skin autograft (A) and two homografts (H) on day 50 after grafting. Homografts show signs of fading along the margins of the scales.

FIGURE 2.

The same animal on day 90 after grafting. A shedding took place between day 50 and 70 and removed the remains of the faded homografts. Scale regeneration is found at the graft site and was evident by day 70 after grafting. The autograft (A) looked unchanged.

WATER RELATIONS OF THE AFRICAN TOAD, *BUFO MAURITANICUS Schl.*

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INTRODUCTION

One of the few African toads, *Bufo mauritanicus* Schl. is restricted to the north-western region of the continent—Algeria, Tunisia and Morocco. In the rest of Africa, from Egypt to the Cape, and Senegambia to Ethiopia, it is replaced by *Bufo regularis* Reuss. *B. mauritanicus* reaches a length of 7.5-10.0 cm: *B. regularis* averages 5 cm in West Africa and is largest at the Cape where its length is about 12.5-15.0 cm (Gadow, 1901). This may suggest some relationship between size, surface area and environment. Around Khartoum, *B. regularis* has an average body length of about 5.5-7.0 cm (Cloudsley-Thompson, 1967a), but a similar range of sizes is indicated in the Rukwa Valley of Tanzania by the work of Chapman & Chapman (1958). The sexes do not differ greatly in size.

The water relations of *B. regularis* have been investigated in some detail (Cloudsley-Thompson, 1967b), but no comparable work has been carried out on *B. mauritanicus*. A welcome gift, from Mr S. Gorzula, of half a dozen specimens collected by him in Tunisia, has enabled me to make a comparison between the two species in this respect.

METHOD AND RESULTS

In order to test the effect of ambient air temperature on the rate of evaporation and body temperature, individual toads were placed in desiccators over calcium chloride. The desiccators were placed in an incubator maintained at temperatures between 21° and 25°C, and the toads exposed for periods of 1 hr. Water loss was ascertained by weighing before and after each experiment, and body temperatures were taken orally with a thermocouple and potentiometer.

The results obtained are given in Table 1 alongside comparable figures for *B. regularis* (from Cloudsley-Thompson, 1967b). They show a greater rate of water loss from *B. mauritanicus* than from *B. regularis*, and a greater degree of evaporative cooling in the former species.

The mean total water content of *B. mauritanicus* (obtained by completely desiccating, in an oven at 50°C, any toads that died during the course of the work) was found to average 55-60%. This compares with a mean of 77% in *B. regularis* (Cloudsley-Thompson, 1967b).

TABLE 1.

Water loss (% body wt/hr ± Standard Error) and mean oral temperatures (N = 5) of *B. mauritanicus* in dry air at various ambient temperatures, compared with similar figures for *B. regularis* (from Cloudsley-Thompson, 1967b).

	B. mauritanicus		*B. regularis*	
ambient temperature (°C)	water loss (%)	body temperature (°C)	water loss (%)	body temperature (°C)
21	2.4 ± 0.44	20.2	—	—
30	4.3 ± 1.05	25.8	0.5	28
35	5.9 ± 1.48	26.4	2.4	31
40	10.2 ± 1.63	29.3	3.8	34
45	11.2 ± 1.56	31.2	5.8	37

DISCUSSION

B. mauritanicus loses water more rapidly in dry air at various ambient temperatures than does *B. regularis*, and is able to maintain a greater temperature differential with the environment. Clearly, neither species is able to conserve water to a significant degree. Indeed, the rate of water loss from *B. regularis* is similar to that from the European toad *B. bufo* (L.), and it is obvious that *B. regularis* has not become adapted to tropical conditions in the ability to conserve water through the reduction of transpiration (Cloudsley-Thompson, 1967b). It seems doubtful, therefore, whether the difference in the abilities of *B. mauritanicus* and *B. regularis* to conserve water can in itself have much adaptive significance, since neither species survives for long in a dry atmosphere. On the other hand, the maintenance of a low body temperature for short periods in dry air may be useful in hotter habitats, and this may explain the differences observed. Moreover, *B. regularis* may well have evolved in the more equable regions of central Africa and have invaded Egypt and the Sudan by way of the Nile Valley, as suggested by Gadow (1901).

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NOTES ON SOME BRISBANE FROGS

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It is inevitable that this brief account would be incomplete in view of the fact that my stay in Australia on the outskirts of Brisbane, Queensland, was only from the end of June to early August 1972, the Australian winter. By day the ground surface was dry, warmed by a temperature of 70-90°F. under a clear sky but darkness suddenly fell about 17.30h., accompanied by a drop in temperature to about 40°F. and heavy dew, which evaporated rapidly as the temperature rose at about 07.00h. The dry bush was intersected by many creeks of varying depth and some rivers affording evidence of tremendous seasonal flooding. Such are the winter conditions of the environment of the local frogs. There are no native toads.

My first "find" was an immature female Toothed Frog (*Adelotus brevis*), located under a wooden bungalow at the base of a timber support, and on being brought out into the light it immediately put on its "death act" (my term), which agreed in every way with that occasionally exhibited by *R. temporaria.* The frog stretched out its legs so rigidly that its back actually became concave; this was accompanied by violent shaking and thereafter complete stillness but there was no substance exuded as is done by our *Rana temporaria.* The thigh patches appeared to enlarge and certainly became brighter red in colour so that one gained an impression of bleeding. After 12 minutes slow recovery became evident. The right foreleg moved first, followed by positioning of the other legs so that the frog assumed a sitting posture but when touched, it immediately turned over onto its back (as *Rana* does) displaying its inflated black and white patched underside. No throat movements were visible. Eventually it turned over and hopped away. Although several other speciments of this species were found in the bush, none repeated the above performance.

The next species to be located were seen towards the top of an India rubber plant growing in a garden at Redcliffe (near the coast). *Hyla bicolor* rested in the axils between stem and petioles, on the shade side of the plant, whereas *Hyla gracilenta,* neatly packaged, rested along the midribs of more mature leaves. These were the only *H. gracilenta* specimens I saw but *H. bicolor* was active early in the day among damp grass at the edge of a billabong; its camouflage was perfect.

The very lanky *Hyla lesueuri,* which leaps tremendous distances, appeared to me to have the widest distribution wherever there was suitable dampness, especially on farmland drainage.

Young frogs were seldom found in the company of mature animals with the exception of the burrowing frog (*Lymnodynastes ornatus*), where a mixed party was resident in the deepest layers (next to the soil) of a nurseryman's sand heap. The sand was quite damp where they rested. When brought to the surface they literally sank backwards into the sand again, scraping with their hind feet and pushing backwards with their forearms so that they were out of sight in virtually no time at all.

The River Condamine was reduced to very small size at this time of the year but in the damp muddy pockets of its great bed much driftwood etc. had been deposited and under the thicker pieces young frogs were resting. The best "find" here was a collection of 25 immature frogs, all huddled tightly together, of four different species, *Hyla peroni, H. lesueuri, Lymnodynastes ornatus* and another one still unidentified. But the wood used as hide-outs had to be thick enough to prevent daytime evaporation of the moisture from below; split logs were favoured most of all.

The "creek" frogs rested among stones either near the water's edge or on raised beds where the water was more shallow. Here were found *Hyla verreauxii, Crinia signifera* complex, *Pseudophryne bibroni* and another immature frog, beautifully striped but not yet identified.

Perhaps the frog whose hide-outs were the most interesting was *Hyla caerulea,* which seemed to favour damp places near habitations, especially in the vicinity of water tanks. The first to be found was using a weathered fence post near a marsh. It was sitting at a depth of 12 ins. level with the burnt holes through which wire passed; when prodded by a stick it barked very loudly. It was surprising how such a large frog could squeeze itself up and down so narrow a passage among the weathered columns of wood. The last specimen encountered will always be remembered as it lived in a cistern at Woodvale Park and Koala Sanctuary near Oakey and was flushed out each time the toilet was used!

Cane toads (*Bufo marinus*) were everywhere where the soil was sandy and they burrowed into the banks as *B. calamita* used to do at Ainsdale. The Cane Toads were imported from Hawaii in 1939 to clear an infestation by the Cane Beetle (another alien!) but as is often the case, once the intended job has been done, the introduced species looks elsewhere for food; in this case, among other animals, the much more desirable native frogs. I was told that these enormous toads actually sit outside beehives snapping up the bees as they leave—until they themselves are shot by the beekeeper in desperation. Perhaps there is a moral here?

Call notes of different species of frogs were heard intermittently especially from vegetation at the edge of small mountain creeks and billabongs. A very loud sustained chorus commenced at dusk and continued far into the night, by moonlight. Cane Toads seem to have a distinct communication as individuals will answer one another from different sides of a pond, and the inflection of the voice varies.

The only tadpoles seen were in a very small deep drainage pool. They were of various sizes ranging from very young ones to those about to produce their hind legs. My samples were said to be "probably" of *H. caerulea.*

Most of the specimens found were released immediately after they were photographed, though obviously every individual of the same species was not photographed. A few others were taken to the Brisbane Museum for confirmation and thence they were returned to their natural habitat. One specimen was to have been released by the departmental Curator in the Museum Park. The Brisbane Museum was given my list of localities to add to their records. A recent book by Densey Clyne (1969) titled "The Australian Frogs", Periwinkle Nature Guides in Colour, Melbourne, was of use (and of much interest) for my expeditions.

EDITOR'S NOTE

After vol. 5, No. 1 of the "British Journal of Herpetology" had been printed and despatched, it was realised that the page numbers continued on from vol. 4, No. 12, instead of beginning afresh with page number 1. The error was the Editor's alone, who offers his sincere apologies for the mistake. It has been decided, however, that under the circumstances the best thing to do is to continue the pagination for vol. 5 as now followed in its first number, a procedure which will reduce any confusion to the absolute minimum.

LETTERS TO THE EDITOR

. . . and this they call conservation

Volume 171 pt. 2, pp. 153-165 of the *J. Zool. Lond.* has a paper by W. W. K. Houston reporting that with the help and under the auspices of the " Nature Conservancy and the Staff at Moor House ", he has caught and killed 427 frogs to prove the earthshaking fact that a frog eats anything alive it can catch commensurate to its size. There can hardly be even a teenage zoologist in the world to whom this fact was not well known anyway. Why did the Durham Zoology Department encourage an investigation so wasteful of our ever dwindling wild life and what, in the name of Logic, has all this to do with Conservation?

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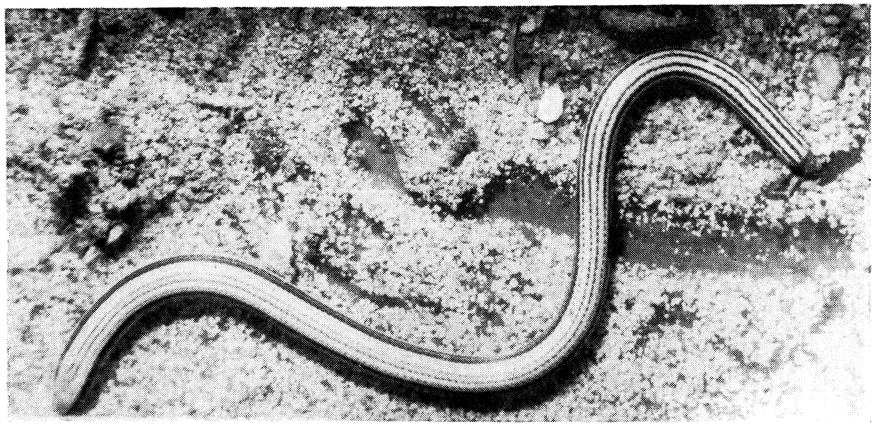
A RARE ARRIVAL

An enthusiastic Israeli herpetologist recently brought me the creature shown in the accompanying photograph. If I had not known that it came from Israel I would, at first sight, have taken it for a slowworm, but *Anguis fragilis* does not occur in the Middle East. The reptile shown was dug up in the Negev desert. Its name is *Ophimorus latastei* and it is, in fact, a legless skink which, when caught, had already lost the tip of its tail. The first specimen ever caught was found in the region of the sea of Galilee. It is commonly known as the Asian Sand Skink and, since it lives mainly underground, is very rarely seen. Unfortunately, we do not know the nature of its staple food, but it can be kept alive on a diet of housefly larvae. The total length of the skink at the time of writing is 15cm.



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BOOK REVIEW

Blair, W. F. (1972) (ed.) *Evolution in the genus* Bufo. pp. viii + 459. U of Texas, Austin and London. Price $17.50.

For some years a select band of American workers has been considering problems of the relationship between the toad species, using a variety of different techniques. The present work is the outcome—one almost said " culmination ", but there is obviously still work to come—of all this research. The book has been written by a team of eighteen, mainly from the United States, but also including experts from South America, Poland and Italy. One author has worked for some years in Australia. In this company, it would be invidious to mention names. The basic material comprised living toads of a wide variety of species from most parts of the world, together with preserved material made available from the collections in the major museums.

The difficulties may be glimpsed from the start, when Professor Blair records that one can only state that there are roughly 200 species in the genus *Bufo,* and that it will not be possible in the foreseeable future to give the exact number. The book tries to assess the origin, both in time and geographical location, of the genus, with its evolutionary history. This has entailed consideration of the fossil record, anatomical and morphological detail, present-day distribution and behaviour, the results of hybridisation experiments, mating calls, breeding pattern and general ecology, as well as chromosome numbers and type, and the secretions of the parotoid glands. A mass of the data is given in a series of appendices.

The final conclusion bases the place of origin of the genus as probably South America, where the earliest known fossil possibly of the genus is from the Lower Oligocene, though the earliest undoubted *Bufo* is from the Lower Miocene of Florida. The genus evolved from the Leptodactylidae and has developed along narrow and broad-skulled lines, already distinguished from each other as long ago as the Miocene. A number of problems of dispersal have been evaluated, but a whole series of other problems still remain.

This is a large book and does not make easy reading for those who are not already possessed of some knowledge of the techniques used, or who are not prepared to study each chapter with care. But they will find it amply repays their efforts. There is a mass of black and white illustrations, as well as six colour plates, each showing a dozen or more species of *Bufo,* including half a dozen species of other genera. These plates attracted me at first, but close inspection made me pause. Some of the photos are excellent, but others are the reverse. In some cases, this is due to poor photography, and in others colour reproduction is too poor. But this can be set aside by the workmanship of the text. Although the price may sound high, at present money values it is absurdly low in return for a mass of useful fact crammed into a well-produced volume.

J.F.D.F.

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