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NOTES

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THE LIMBS OF SNAKES

with special reference to the hind limb rudiments of *Trachyboa boulengeri*

By

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Introduction

In most snakes there are no vestiges of the skeleton or muscles of either fore or hind limbs. Dunn and Tihen (1944) suggested that rudiments of a pectoral girdle might be present in one species of snake, *Liotyphlops albirostris,* but it is now thought more probable that the structures in question are parts of the skeleton related to the tongue (Tihen, 1945: Warner, 1946).

Records of the occurrence of two claw-like structures projecting from the small scales on either side of the cloacal shield in boas and pythons appear in the herpetological literature around the beginning of the nineteenth century. Schneider (1799) briefly described in a boa constrictor a small bone, situated between the peritoneum and the ribs in the cloacal region and terminating in a claw. The real nature of this claw, however, was not demonstrated until some years later when Mayer (1825, 1829) showed by dissection that it was actually a part of the rudimentary hind limb. He figured for the first time the limb skeleton and muscles in a boa and showed that comparable rudiments were present in some other snakes such as *Ilysia, "Tortrix"* (probably *Cylindrophis*) and *Typhlops,* as well as in certain snake-like lizards.

These observations were reviewed by Johannes Müller (1831),[1] and more briefly by Schlegel (1837). Mayer's findings were confirmed and extended by d'Alton (1834, 1836) who also described the skeleton and muscles of the limbs in boas and pythons. As far as I know, no original figures of these muscles have been published since this time. Considerably later, Peters (1863) gave the first description of the limb skeleton of *"Stenosoma"* (= *Leptotyphlops*) where the pelvis is better developed than in any other group of snakes.

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[1] Muller's long article on the anatomy and natural history of the "Amphibia" is a valuable survey of the herpetological knowledge of the time, and contains much information which is not to be found in modern text-books. Another contribution of the same period to which I have not yet had access is C. F. Heusinger's "Untersuchungen über die Extremitaten der Ophider" in the *Zeitschrift fur Organische Physik.,* vol. **3.**

Fürbringer's monograph (1870) on the limbs of snake-like lizards contains an account of the limb rudiments also. This is based mainly on the work of Mayer, d'Alton and Peters, but a new and more satisfactory terminology for the different parts of the limb skeleton and for the muscles is introduced. Fürbringer compares the condition in snakes with that in snake-like lizards and emphasises the fact that in the former, in contrast with the latter, the pelvis lies mainly internal to the ribs. Hoffmann's account (1890) is entirely derivative from the early nineteenth century workers; his article is the most recent in which figures of the limb muscles (taken from Mayer and d'Alton) are shown.

Although the condition in snake-like lizards has received considerable attention, only a few original observations (e.g. Boulenger, 1913 : Mahendra, 1936 : Smith, 1939) on the limbs of snakes have been made during the present century; these are entirely confined to the skeleton. The most important recent contribution is that of Essex (1927), who studied interspecific variation in the limb rudiments of *Glauconia* (= *Leptotyphlops*) and *Typhlops*.

This previous work has shown that some vestiges of the hind limbs are present in most, if not all members of the families Leptotyphlopidæ and Boidæ, and in most Anilidæ and Typhlopidæ.[1] No rudiments have yet been found among the Uropeltidæ or Xenopeltidæ, the other families of snakes which are generally regarded as being primitive in various ways.

Schneider originally suggested that the pelvic claws have some sexual function, and this view has been substantiated, at least for Boidæ, by more recent workers. Davis (1936) cites observations to the effect that before coitus the claws of the male are used to stimulate the cloacal region of the female prior to insertion of the hemipenis, and this is confirmed by a personal communication from Mr. R. A. Lanworn of the London Zoological Society's Reptile House. Boulenger (1913) states that the pelvic claws of male boas are used as "claspers" but there is no evidence that they subserve a truly prehensile function and they certainly do not assist in the transmission of the sperm, like the claspers of some elasmobranch fishes.

It is probable that the claws of male snakes are as a rule larger than those of females of the same species, where they presumably have no function. Essex suggests that the pelvic claws of *Leptotyphlops* may show sexual dimorphism but gives no detailed observations on this point. Stickel and Stickel (1946), who examined 21 female and 11 male specimens of the boa *Enygrus carinatus,* found that the claws were absent in some of the females and when present were always smaller than in the males. Some of the earlier workers, however, believed that sex differences in the size of the claws are not always constant, and examination of large samples of different species is desirable.

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[1] The anilid *Anomochilus* (Smith, 1940) and the typhlopids *Anomalepis* and *Liotyphlops* (see Tihen, 1945) are believed to lack all traces of hind limbs.

CONDITIONS IN *Trachyboa* AND OTHER SNAKES

As so long a period has elapsed since the hind limbs of snakes received detailed investigation, it seemed worth while to re-examine these structures, using microscopic methods. Serial sections in the transverse and longitudinal planes respectively were taken through the pelvic region in two late male embryos of the small South American boid *Trachyboa boulengeri* Peracca; [1] for these specimens I am indebted to Mr. Arthur Loveridge of the Museum of Comparative Zoology at Harvard College. Sections of a male embryo of *Python molurus* at a relatively earlier stage in development were also available, but the preservation was inadequate for a study of the muscles. The subadult example of *Trachyboa* from which the external appearance of the limbs was drawn is in the British Museum (Natural History); I am indebted to Dr. H. W. Parker and Mr. J. C. Battersby for allowing me access to it.

Before describing the limbs themselves, it is necessary to give a general account of the skeleton and viscera of the pelvic region. Five vertebræ in *Trachyboa* have been reconstructed and are arbitrarily labelled I—V in antero-posterior sequence. Vertebræ I—III carry ribs of normal shape, the last two being shorter than those in front. The ribs on the vertebræ IV and V and of the following two vertebræ (not shown) are modified, and have forked processes termed lymphapophyses. These partly enclose the large contractile lymph hearts (Fig. 3A, B), which communicate anteriorly with a system of longitudinal lymph vessels related to the ventral aspect of the vertebræ (*see* Chapman and Conklin, 1935). The presence of lymphapophyses is characteristic of all snakes and a few snake-like lizards. The vertebra which bears the first pair of lymphapophyses is sometimes regarded as the first vertebra of the caudal series. Since the pelvic girdle is not (as a rule [2]) attached to the axial skeleton in snakes, identification of the true sacral region of the spine is problematical (*see* Gadow, 1933).

The rectum is continuous with an extensive cloacal chamber which, as in other snakes, is partly subdivided into three compartments, the coprodæum anteriorly, the urodæum, and posteriorly, the proctodæum, which terminates at the cloacal opening. The dilated posterior ends of the ureters run into the upper part of the urodæum, while the vas deferens opens on each side into the lateral aspect of the ureter just posterior to the plane of section in Fig. 3A. The allantoic diverticulum is continuous with the ventral part of the cloaca (Fig. 1B); in embryos of higher vertebrates the allantois, a saccular outgrowth from the hind gut, has the important func-

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[1] The graphic reconstructions figured are almost entirely based on the transverse series. The arrangement of the muscles was difficult to interpret in the longitudinal series and the conditions observed suggested the possibility of some individual variation.

[2] Boulenger (1913, p. 52) states that in Ilysiidæ (= Anilidæ) the ilium (probably the bone termed the anterior process of the pelvis in this article) is attached to the lower branch of the first bifurcate transverse process of the lumbar vertebræ (i.e., the first lymphapophysis). Such an attachment has not been noted in other snakes.

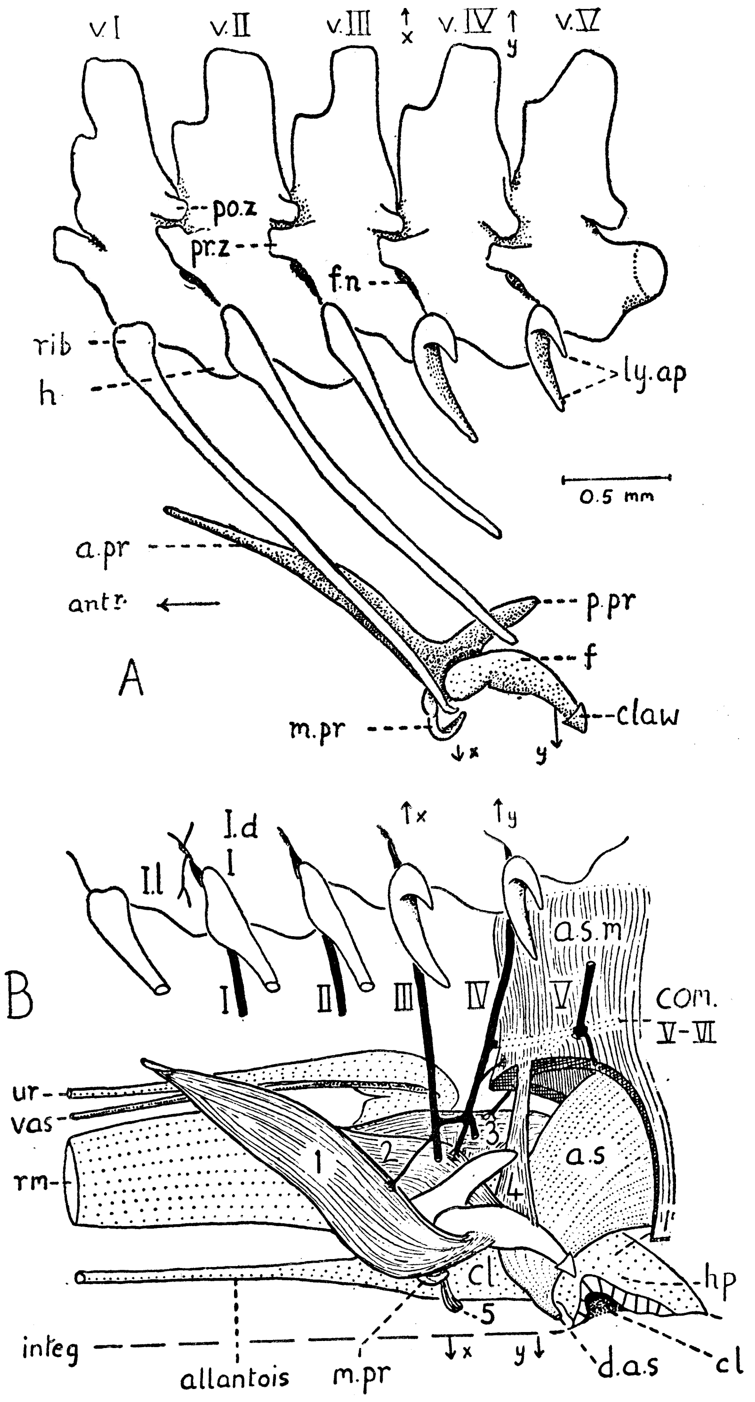

FIG. 1

tions of storage of excretory products and, by virtue of its blood vessels, of respiration. In most lizards a part of the allantoic diverticulum is incorporated into the urinary bladder of the adult. In snakes, however, the bladder is absent and it may be supposed that the diverticulum atrophies during the latest stages of embryonic life.

Other important structures in the pelvic region are the anal sacs (Figs. 1B, 3B.) These organs, which are apparently peculiar to snakes, are a pair of large elongated glands of holocrine type, present in both sexes. They lie on either side of the midline in the base of the tail and each discharges by a single duct just in front of the lateral margin of the cloacal opening. Their secretion is highly odoriferous in some snakes (e.g. *Natrix*), and may have a defensive or a sexual function (*see* Volsøe, 1944). Two sheets of muscle arise from the ventral median processes (hypapophyses) of the vertebræ, and each passes downwards and laterally to split and enclose each anal sac (Figs. 1B, 3B). These muscles (here termed anal sac muscles) are continued ventrally around the hemipenes and are sometimes known as the propulsor muscles of these organs. In the specimens studied the hemipenes were everted, as is usually the case with late embryos of male snakes, and could not be represented in the reconstruction. When retracted, each hemipenis lies beneath the anal sac on each side of the tail; the estimated position of the base of the left organ is shown in Fig. 1B.

The identities of the different components of the limb skeleton in snakes are not at all clear. Mayer and other early workers believed that these structures corresponded with the bones of the leg and foot of other tetrapods, and not with the pelvic girdle. It is now usually accepted, however, that the limb skeleton includes some parts of the pelvis and often one or more of the proximal bones (femur, tibia) of the extremity. Since no consistant terminology for the different parts of the ophidian pelvis have been worked out, and since the establishment of the correct homologies would require extensive morphological and embryological study, I have

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Fig. 1. *Trachyboa boulengeri,* male. Late embryo.

A. Reconstruction of vertebræ, ribs and rudiments of hind limb skeleton of left side seen in lateral view.

B. Reconstruction of muscles and nerves of left hind limb and structures related to it seen from the lateral side. The three anterior ribs are cut short. The communications betwen IV and V, and V and VI spinal nerves, and part of the nerve to muscle 3 lie deep to the anal sac muscle and are shown in dotted lines. Nerves to limb muscles are shown diagrammatically as ending in three small branches on the muscle. Nerves not supplying limb muscles are shown cut short.

Plane of section in Fig. 3A, B, shown by arrows xx and yy respectively. Vertebræ and spinal nerves numbered in roman numerals. Dorsal and lateral rami (I.d, I,1) are shown for nerve I only, and are similar for the other nerves. Limb muscles are numbered in arabic numerals (1-5) in this and subsequent figs. (*see* p. 80).

a.pr.—anterior process of pelvis. a.s.—anal sac. a.s.m.—anal sac muscle. cl.—cloaca. com.—communication between ventral rami of spinal nerves. d.a.s.—duct of anal sac. f.—femur. f.n.—foramen for spinal nerve. h.—hypapophysis of vertebra. hp.—hemipenis (approx. position of base of left organ shown by interrupted lines). int.—line of ventral integument. ly.ap.—lympapophysis. m.pr.—medial process of pelvis. po.z.—post-zygapophysis of vertebra. p.pr.—posterior process of pelvis. pr.z.—pre-zygapophysis. rm.—rectum. ur.—ureter. v.—vertebra.

used a purely descriptive nomenclature for both the skeleton and the attached muscles. The anterior, posterior and medial processes of this account are called by Fürbringer the iliopectineal, ilium and pubis respectively. Other workers have applied the terms ilium, ischium and pubis to the different processes without uniformity.

The pelvis of *Trachyboa* (Fig. 1A) has three processes, the anterior process being much the longest. It lies more or less in the longitudinal plane of the body and passes forwards and obliquely upwards on the deep aspect of the ribs. It is continuous behind with a shorter posterior process which is directed upwards and somewhat laterally, and with a short medial process which passes ventrally and towards the midline. The pelvis in other Boidæ and in Anilidæ (*Cylindrophis, Ilysia*) appears to resemble that in *Trachyboa* more or less closely except that the posterior process may lie more nearly in the dorsi-ventral plane. In the Leptotyphlopidæ the three processes are of more equal length and in some species the medial process may form a symphysis with its fellow of the opposite side. In some species of *Typhlops* the pelvis is also tri-radiate, but in others it consists only of a single longitudinal bar on either side of the cloaca.

The pelvis is usually ossified in adult snakes (although the tips of the processes may remain cartilaginous, especially in Typhlopidæ), and the three processes may be partly demarcated from each other by sutures. In the embryos studied, however, only about the middle two-thirds of the anterior process possessed a thin shell of perichondral ossification. The medial process is partly separate from the rest of the pelvis, and may have chondrified from an independent centre.

In the specimens studied, a stout cartilage (ossified in adult snakes and partly ossified in the *Trachyboa* embryos) articulates with the pelvis at the junction of the three processes, and presumably represents the femur. Despite Fürbringer's statement to the contrary, an acetabulum is present, deeper in the *Trachyboa* embryos (Fig. 3A) than in the python. In some adult Boidæ and Anilidæ the femur has a well developed trochanter-like process about halfway along its ventral aspect which serves for muscle attachment; this feature was not present in our embryos. The femur is absent in Typhlopidæ (*see* Essex, 1927).

In the *Trachyboa* embryo figured, the femur carries the claw on its distal extremity, but in the other specimen of *Trachyboa,* and in the python (Fig. 3E) the claw is borne on a separate cartilaginous nodule. The figures of other workers suggest that this is the more usual arrangement, at least in Boidæ, and this separate nodule has been regarded as a tibia. It is therefore possible that the structure labelled femur in the reconstructions shown includes a tibial contribution fused with it.

The relationships of the claw to the surrounding tissues are of interest. The tip of the femur (or the separate tibial nodule) is directed laterally and forms a cartilaginous core in the base of the claw (Fig. 3C). The tissues internal to the epithelium of the claw which include this cartilage may be regarded as representing the true distal part of the limb. The greater part of the claw, however, is surrounded by an investment of tissue which is apparently an extension from the body wall (Figs. 2B,3C,D.) The claw is separated from this investment by a space lined with epithelium, and its cornified tip projects from this space and becomes visible externally. In the python embryo (Fig. 3E) the epithelium of the claw is not yet differentiated and the extensions from the body wall have not yet grown round to form a complete investment for the claw base. If this interpretation is correct, it would seem that the free part of the limb becomes partly surrounded by a tubular extension from the body wall; this condition is, so far as I know, without exact parallel in vertebrates, though it may possibly occur in some snake-like lizards.

The length of the projecting part of the claw may vary considerably in adult boas, both as between the two sides of the same individual and as between different individuals of the same species. The latter variation probably has a general correlation with the sex of the snake.

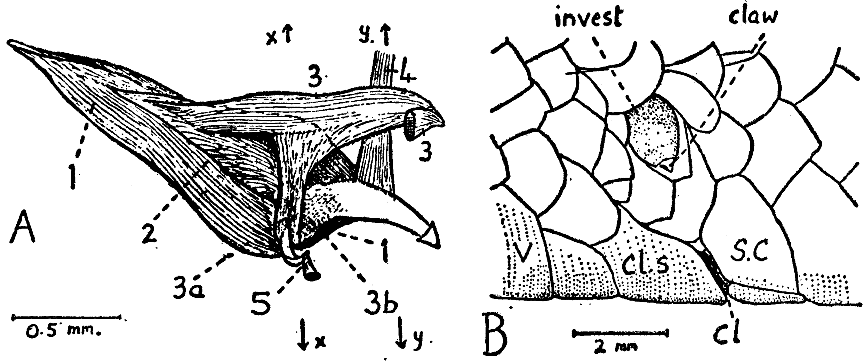

FIG. 2

A. *Trachyboa* embryo. Reconstruction of limb muscles seen from medial side. To facilitate comparison with other Figs., the structures are drawn as though on the left side of the body. Plane of section in Fig. 3A, B, shown by arrows xx and yy respectively.

B. *Trachyboa boulengeri,* probably male. Subadult (British Museum, N.H., No. 1913.11.12, 37-38). External appearances of left claw seen from the side. The body is tilted to show the cloacal shield (cl.s) and ventral scales (v); the 1st subcaudal (s.c) is divided. invest.—claw investment.

The details of the musculature of the hind limb in snakes have previously been studied only in the larger Boidæ. d'Alton recognises seven muscles attached to the pelvis and limb in *Python bivittatus* (probably *P. molurus*) and the accounts of Fürbringer and Hoffmann are principally based on his work. I have only been able to recognise five muscles which are certainly distinct in *Trachyboa*. It is likely that this discrepancy is at least partly due to the splitting of one or more of these muscles into separate structures in the python. Otherwise, d'Alton's description agrees in a general way with my own findings although there are differences in detail, the significance of which cannot be assessed without access to adequate python material. The muscles recognised by me in *Trachyboa* (Figs. 1, 2A, 3) are designated by numbers, as follows:—

1. A large muscle which arises from the whole length of the lateral aspect of the anterior pelvic process, passing on to the ventro-lateral aspect of the latter towards the acetabulum, and then passing ventrally across the head of the femur to be inserted on the ventral part of the "neck" of this bone. The action of this muscle would be to depress the femur and pull the claw outwards away from the body.

2. This muscle arises from the dorso-medial aspect of the posterior two-thirds of the anterior process, passes dorsally over the pelvis in the region of the acetabulum and medially to the posterior process, and is inserted into the dorsal aspect of the head and "neck" of the femur. This muscle would pull the femur dorsally and perhaps somewhat medially.

3. The arrangement of this muscle-complex is not easy to follow. There is a well developed band of longitudinal fibres (labelled 3) which arises posteriorly, in common with its fellow of the opposite side, from a median fibrous raphe just above the cloaca and a little way behind the level of section in Fig. 3B. Over the middle part of its extent the upper border of this band of muscle fibres is free (Figs. 3A, B); anteriorly its fibres are attached to the inner side of the anterior process and have a very close relationship with the fibres of origin of muscle 2 (Fig. 2A). From the middle portion of the lower border of this longitudinal muscle, a narrow band of fibres (3a) passes abruptly downwards and is inserted on to the anterior and posterior edges of the medial process, apparently bridging over its dorsal aspect without being attached to it (Fig. 2A). The posterior fibres of this vertical band terminate ventrally in a very complicated fashion. Some are inserted into the root of the medial process as described; a few (not shown) pass ventrally and are connected by a fibrous tendon with the muscles of the anterior abdominal wall. Others again are directed laterally and have a tendinous connection with the fibres of a small muscle which arises from the lower surface of the root of the medial process (Fig. 3A), and, partly blending with the fibres of muscle 1, is inserted into the inner side of the femoral "neck". This last muscle (here labelled 3b) may have a more distinct entity as a separate muscle in other boids.

Contraction of muscle 3b would seem to pull the femur and claw inwards towards the midline. The action of the other portions of muscle-complex 3 is difficult to estimate, but it is probable that simultaneous contraction of the longitudinal (3) and vertical (3a) bands would tend

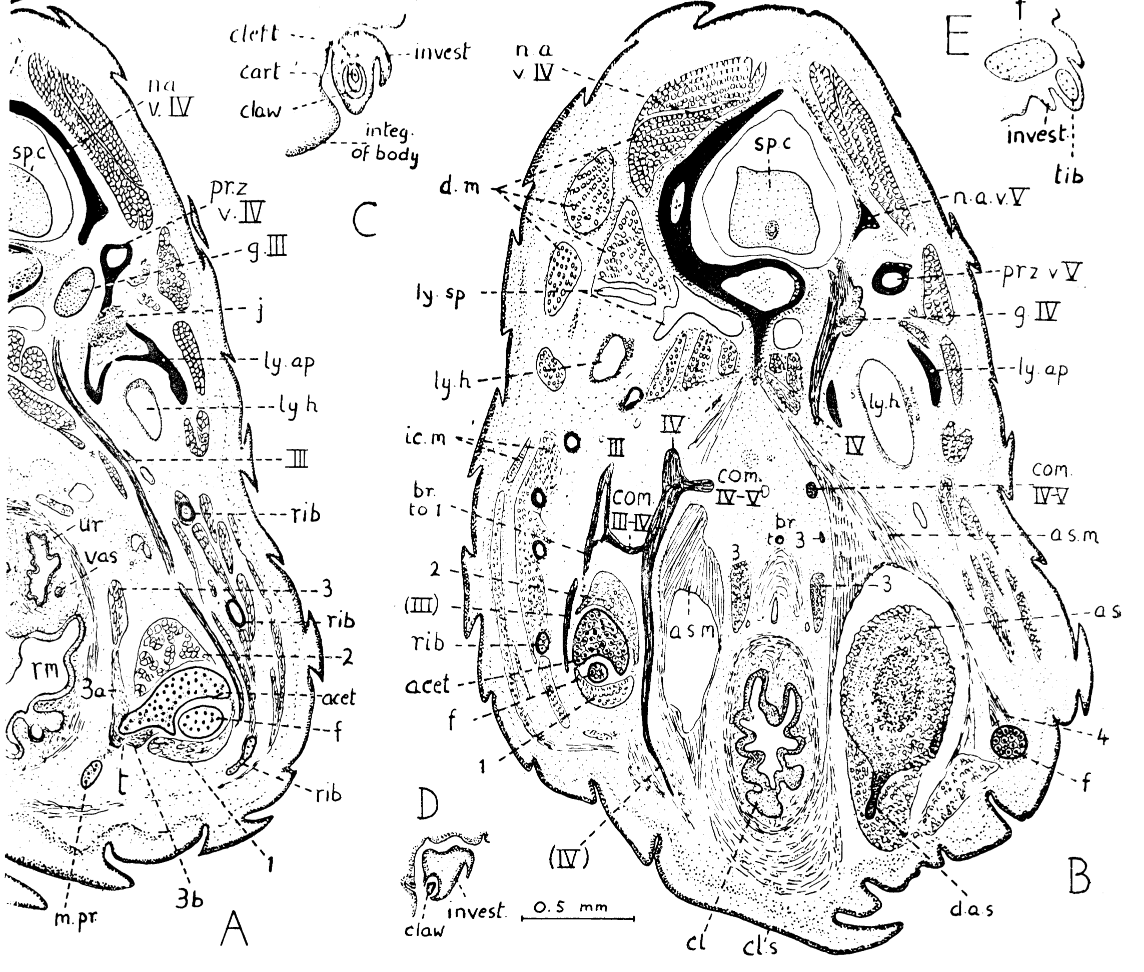

FIG. 3

A—D, *Trachyboa* embryo.

A. Transverse section at level xx in Fig. 1A, B.

B. Section posterior to A, just anterior to cloacal opening. The right side of the section is at the level yy in Fig. 1A, B. The section is somewhat oblique and shows the communications between III and IV, and IV and V spinal nerves.

C. Transverse section through claw base and investment.

D. Transverse section through tip of claw. Level posterior to C.

E. *Python molurus* embryo, at relatively earlier stage than the foetal *Trachyboa*. Transverse section through femur and tibial cartilage. The claw epithelium has not yet differentiated, and the investment is not yet complete.

acet.—acetabulum. cart—cartilage (end of femur, or tibia). cleft—cleft in connective tissue related to end of femur and claw base. d.m.—dorsal muscles. g.—ganglion of nerves III and IV. ic.m.—intercostal and lateral abdominal muscles. invest.—investment of claw base derived from tissues of body wall. integ.—integument. ly.h.—lymph heart. ly.sp.—lymph space. n.a.—neural arch of vertebræ. sp.c.—spinal cord. tib.—tibia. br.—nerve branch. j.—vertebro-costal joint. Other lettering as in Figs. 1, 2. Distal branches of nerves numbered in brackets do not supply limb muscles.

to pull the pelvis as a whole backwards while the medial process is raised and the acetabulum tilted downwards.

4. This muscle, a narrow slip from the outer sheet of the anal sac muscle, is inserted on to the upper side of the shaft of the femur, which it presumably raises.

5. This consists of a few fibres which pass inwards from the tip of the medial process and become continuous with the muscles of the anterior abdominal wall, and probably serves to anchor the pelvis.

The movements described by the free extremity would seem to consist mainly of abduction, adduction, elevation and depression with perhaps some rotation brought about by the tilting of the pelvis. The additional possibility, suggested by Schneider, that the claw might be protruded from and retracted into its investing sheath, after the fashion of a cat's claw, was also considered. The fact that in preserved specimens the extent of the claw visible beyond the investment may vary on the two sides of the same individual seemed to favour this possibility. Examination of the sections shows a definite crescentic cleft in the connective tissue around the dorso-medial aspect of the distal part of the femur and claw base (Fig. 3C). This cleft, which is present in both specimens of *Trachyboa,* is lined by a single layer of flattened cells and has something of the appearance of synovial joint cavity. Although it does not completely surround the base of the claw it might permit some gliding movement between this structure and surrounding tissue. Manipulation of the claws in a living specimen of *Python molurus,* however, provided no evidence that these had any power of retractibility, and it is not easy to see how any of the muscles described could bring about protrusion of the claw in *Trachyboa.*

With the material at my disposal it has only been possible to work out the main features of the limb plexus, which is derived from the ventral rami of nerves I—VI, between all of which longitudinal communications were observed (Fig. 1B). Besides supplying the muscles of the limbs as shown, these nerves send branches to the intercostal and ventral abdominal muscles, and presumably also to the muscles of the hemipenes; the innervation of the latter structures, however, could not be accurately traced. While the ventral rami of the nerves I—III (like those in front of and behind the plexus region) run slightly backwards, nerves IV and V pass in an anterior direction. Owing to the approximation between the ventral rami of III and IV, and the twist of the body of the specimen which gave a marked obliquity to the cutting plane of the microtome knife, the communications between nerves III and IV, and part of that between IV and V, are shown in a single section (Fig. 3B : left hand side). The communications between nerves IV and V, and V and VI, lie deep to the anal sac muscle on either side (Fig. 1B).

DISCUSSION

In many groups of lizards there is a tendency for the body to become elongated and for the limbs to undergo reduction. In some cases (e.g. the slow worm *Anguis*) all external traces of the limbs are lost. The hind limbs and their girdles may be reduced more markedly than the fore limbs (as in some amphisbænids) but the converse is more often the case. In the burrowing scincomorph lizard *Dibamus* external hind limbs are stated by Camp (1923) to be present only in the males where "they appear to be used as claspers", so their function may be similar to that of the pelvic claws of snakes. In a few lizards the shoulder girdle has completely disappeared but no lizard in which the pelvic girdle is entirely absent is known (Camp, 1923). It is possible that the pelvic girdle is less liable than the pectoral girdle to disappear completely after it has ceased to play any part in locomotion owing to its close association with the pelvic viscera and sexual organs.

It is almost certain that the snakes are descended from lizards of some kind. The total loss of both pairs of limbs in most snakes, however, suggests that the group is of greater antiquity than most of the living types of snake-like lizards. Moreover it is clear from the work of Fürbringer that the morphology of the pelvis in those snakes where it is represented differs markedly from that in the snake-like lizards examined. Such conditions (together with other anatomical considerations) suggest that the ophidian stock separated from the lizards at an early period in the history of the latter group, and that none of the existing snake-like lizards are closely related to the ancestors of snakes. It is very probable that the numerous resemblances between such lizards and snakes are the result of parallel evolution.

It is usual to regard the limbs of snakes as primitive, ancestral characters comparable with the human external ear muscles, and, broadly speaking, this view is probably justified. The snakes which possess pelvic limbs (though some of them are highly specialised in different ways), show other primitive features such as certain characters of the skull and muscular system, the presence of premaxillary teeth (in some Boidæ and Anilidæ) and only moderate asymmetry of the viscera (in Boidæ). Nevertheless it is only in the Typhlopidæ that the condition of the hind limbs conforms with the traditional concept of vestigial organs, since, being entirely inside the body in both sexes, they presumably have no active function. In the Boidæ, and probably also in the Anilidæ and Leptotyphlopidæ, however, the limbs in the males, though useless for the purpose of locomotion, have been converted into accessory sexual organs. This change in the rôle of the limbs, which must have occurred before the free extremities were lost, has apparently been effective in preventing the loss of these structures not only in the males, but also in the females of the species.

I am indebted to Mr. J. Lester of the Zoological Society of London for allowing me to examine some of the living snakes in his charge. I am also very grateful to Professor J. D. Boyd for his helpful advice in this investigation.

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THE USE OF AMPHIBIA IN PREGNANCY TESTS: A REVIEW

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All the tests commonly used to demonstrate human pregnancy rely upon the fact that during pregnancy the placenta puts out into the blood stream substances known as gonadotrophins. These are then excreted in the urine and can produce effects upon various species of animals similar to some of those normally produced by their own pituitary gland during the breeding season. The time when the concentration of gonadotrophin in the urine is highest is between the first and third months of pregnancy; and it is often found expedient to concentrate it outside the peak period, using some method such as that of Scott (1940), so that the concentrate may be injected into the animal instead of the relatively dilute urine. This has the added advantage that toxic substances or drugs which might be present in the urine can be eliminated in the process of concentrating the gonadotrophin.

The first animal whose response was investigated was the female South African clawed frog (*Xenopus lævis*), which was shown by Hogben and his co-workers (1931) to respond to the injection of pituitary gonadotrophins by the production of ova, although ovulation and oviposition had never then been noted as occurring under conditions of captivity in this country. Subsequently Bellerby (1934) and Shapiro and Zwarenstein (1934) independently published results showing that *Xenopus lævis* females would respond to the injection of pregnancy urine by the production of eggs some hours later. This was followed up by a multitude of others, who confirmed these results both with pregnancy urine and gonadotrophin concentrates made from this. (For a good bibliography of their papers, see Cowie, 1948.) Frazer and Wohlzogen (1950a) have shown that other species of *Xenopus* (*X. mulleri* and *X. tropicalis*) may be used with similar results.

Bellerby in 1933 tried to produce similar effects by injecting pituitary gonadotrophins into female frogs (*Rana temporaria*). But although ovulation occurred, the ova were only shed from the ovary into the body cavity and not deposited in the water. The work of Noble and Aronson (1942-43), who used the allied *Rana pipiens,* shows that after ovulation the ova are only expelled from the body cavity into the water under the pressure normally applied by the sexual clasp of the male (which can be replaced by the mechanical pressure of a rubber band).

In 1947 the use of males of various species of amphibia was introduced, following the work of Houssay, Giusti and Lascano Gonzales (Houssay, 1947), and of De Robertis, Burgos and Breyter, which showed that the injection of gonadotrophins into the males of the giant South American *Bufo arenarum* results in the liberation of sperms from the testis, and their appearance in the urine in the toad's cloaca. This work is described in a recent review by Houssay (1947), who has pointed out that normally the liberation of sperms from the testis only occurs when the male is in amplexus with the female.

Galli Mainini (1947) was the first to base a pregnancy test upon this phenomenon, and showed that if human pregnancy urine was injected into male *B. arenarum,* sperms could be found in the toad's cloacal urine within a few hours. Since then, many workers have shown the suitability for use of a variety of species of *Bufo,* as well as male amphibia of a number of different orders. The various species used are listed in the Table, and it may be noted that the male *Xenopus* is included among these, as are certain species of frogs. In addition to these, *Discoglossus* has been shown to respond to gonadotrophins (Kehl, 1944).

The British species of toad (*Bufo bufo* and *Bufo calamita*) have both proved suitable for this work, and are much easier to handle under laboratory conditions than the males of *Xenopus* and *Rana* species. In the case of toads, it has been shown that there is no response to the injection of a large variety of substances (other than gonadotrophins) which occur naturally in the body (Houssay, 1947); but Robbins and Parker (1949), in the American *Rana pipiens,* and Hinglais and Hinglais (1949) in the edible frog (*Rana esculenta*) and the common frog (*Rana temporaria*) have shown that these will also give a positive response following the injection of the naturally occurring substance adrenaline. The common toad does *not* react with adrenaline.

It should be realized that in this test a positive response only occurs when there are already sperms developed in the testis, which can be shed thence. In most species of frogs and toads this is the case all the year round, so that these are eminently suitable at all seasons, and Aznar-Ferrares (1949) has suggested that *all* species of frogs or toads would be equally satisfactory for this test. But in the common frog (*Rana temporaria*) it has been found that in October it will not respond even to very large injections of gonadotrophin (Frazer and Wohlzogen, 1950a). It is noteworthy also that one report (Hinglais and Hinglais, 1949), which describes the use of this species, makes the definite statement that the frogs were used during the breeding season, and had not been tried at any other season of the year. The work of Sluiter, Van Oordt and Grasveld (1950) suggests that if frogs have not been well fed during captivity, sperms will not be formed by the testis.

APPENDIX

Our method of using the common British toad, *Bufo bufo*, in the male toad pregnancy test has been briefly described elsewhere (Frazer and Wohlzogen, 1950b). This description is amplified below.

Two male toads of over 15 g. weight each are placed in a 400 c.c. glass

TABLE

List of species used in Galli Mainini test, with experimenters' names

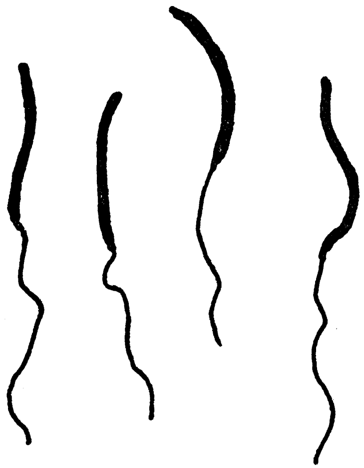
Species	*Authority*
Bufo arenarum	Casas *et al.* (1947) Galli Mainini (1947, 1948) Pinto & Boero (1948)
Bufo marinus	Aldogoal (quoted by Galli Mainini) Mello (1948) Lima & Pereira (1948)
Bufo paracnemis	Mayer *et al.* (quoted by Galli Mainini) Aldogoal (quoted by Galli Mainini)
Bufo d'orbigny	Galli Mainini (1948)
Bufo crucifer	Aldogoal (quoted by Galli Mainini)
Bufo woodhousi	McCallin & Whitehead (1949)
Bufo americanus	McCallin & Whitehead (1949)
Bufo melanostictus	Bhaduri & Bardhan (1949) Jayewardene (1950)
Bufo stomaticus	Bhaduri & Bardhan (1949)
Bufo regularis	Rousselot (1950)
Bufo viridis	Cerquiglini (1948) Bratanov (1949) Wohlzogen (1950)
Bufo bufo	Cerquiglini (1948) Hinglais & Hinglais (1948) Klopper & Frank (1949) Frazer & Wohlzogen (1949, 1950b) Frazer & Wohlzogen (1949, 1950b)
Bufo calamita	Hinglais & Hinglais (1948)
Rana pipiens	Robbins & Parker (1948) Wiltberger & Miller (1948)
Rana tigrina	Bhaduri & Bardhan (1949)
Rana catesbiana	Sandoval *et al.* (1948)
Rana esculenta	Hinglais & Hinglais (1948, 1949) Cerquiglini (1948) Aznar-Ferrares (1948, 1949) Bach & Szmuk (1949) Bieniarz (1950)
Rana ridibunda	Bratanov (1949)
Rana temporaria	Hinglais & Hinglais (1949) Bratanov (1949)
Rana dalmatina	Bratanov (1949)
Rana graeca	Bratanov (1949)
Calyptocephalus gayi	Gunther (quoted by Galli Mainini)
Leptodactylus ocellatus	Galli Mainini (1948) Houssay (quoted by Galli Mainini) Mello (1948)
Xenopus laevis	Robbins, Parker & Bianco (1947) Mello (1948) Gallien (1948)
Odontophrynus cultripe	Mello (1948)
Hyla arborea	Frazer & Wohlzogen (1950a)

beaker, the bottom of which has been covered with moist filter paper. This dampening of the surroundings is necessary if any quantity of urine is to be both formed and drawn off from the toad's cloaca with ease.

Each toad in turn is taken up gently and held in the left hand by the extended hind legs, so as to minimize struggling. Each is injected with 1 c.c. of the fluid (urine or concentrate) from the case of suspected pregnancy. For this injection, a fine hypodermic needle (No. 12) is inserted into the dorsal lymph sac in front of the toad's pelvis and the point of this needle pushed as far back as possible. After the fluid has been injected slowly, the needle is withdrawn gently. These precautions ensure that reflux of fluid through the skin puncture is minimal.

The toads are then replaced in their beaker, after a sample of cloacal urine has been taken from each. Subsequent samples of urine are taken three and six hours later, and if these prove negative a further sample is taken the next day. During this period, the beaker is covered over with a tile and remains on the laboratory bench: it is not kept at any particular temperature or in light of particular intensity, but sunlight is avoided. The toads are thus normally in a semi-shaded position at around 20° C.

The sampling of the toad's cloacal urine is done by gently inserting a fine glass pipette (1 mm. or less external diameter) into the toad's cloaca for a short distance. Frequently urine will track up inside this at once: if it does not, the pipette is gently manipulated back and forth until some urine is obtained. Each drop of urine is decanted on to a glass slide, covered with a cover-slip and examined microscopically at once under the low power ($\frac{2}{3}$ in.—16 mm.). If spermatozoa are present, these are readily identified as they swim across the field, but care must be taken to avoid confusion with cellular debris from the cloaca or protozoan parasites which are frequently seen. On occasion, parasitic worms may also be noted. The figure below represents a drawing of several spermatozoa.



If spermatozoa are found in the sample of urine taken immediately after the injection, the toad concerned is returned to reserve stock and another taken. If, after a negative first sample, spermatozoa are found in any later sample of urine from either toad, the result of the test is taken as positive, no further samples are taken and both toads are returned to reserve stock. If no sperms are seen in 24 hours, the result is assumed to be negative. No toads are re-used for a week, at the end of which time any spermatozoa should have disappeared from the urine. Toads should not be used more than three or four times without a prolonged rest, to allow the testis to replace the "pre-spermatozoa" which have developed into sperms.

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NOTES

FURTHER NOTES ON THE MIDWIFE TOAD (*ALYTES OBSTETRICANS*) IN ENGLAND

In a previous number of this Journal (No. 2, September, 1949) an account was given of the Midwife Toads that were introduced into Bedford nearly 50 years ago and are now living in the garden of Mr. W. S. Brocklehurst. It has been learnt since that there are some toads still in the nursery garden where they were first established, breeding in a pond, thickly overgrown with rushes, that formed a part of the original estate and was not disturbed when building took place. There is also another colony that has established itself in a garden close at hand.

For the following account of a fourth colony I am indebted to Mr. Robert Brocklehurst. It was introduced by him in 1947 into his garden at Woodsets Grange, near Worksop, in the extreme south-west corner of Yorkshire. The house which lies close to the village has an old, matured garden some two acres in extent. It is enclosed by hedges and surrounded by fields. The soil is light and sandy. There are no natural ponds or streams within a mile of the house. On a wall skirting the road which passes the house there is a Bench Mark record stating that it is 233.8 feet above sea level (see Fig.).

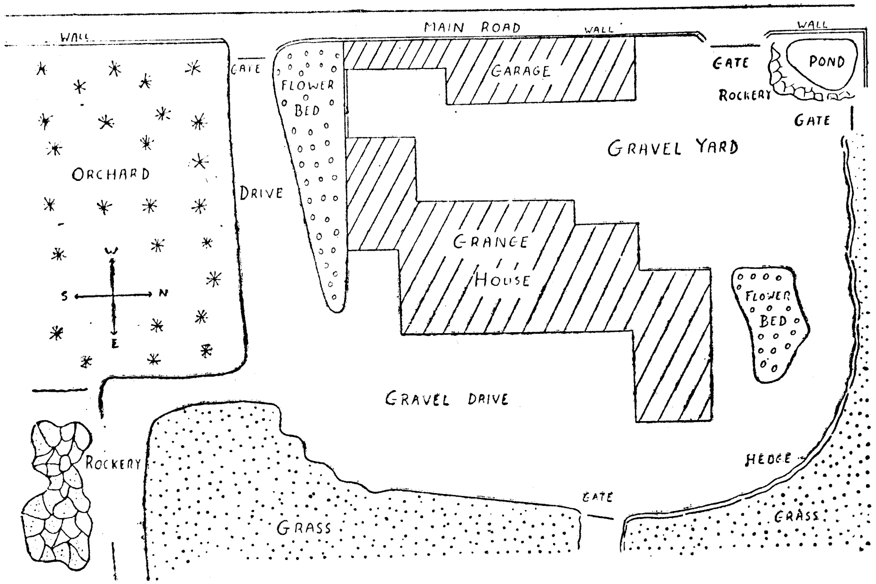
In the autumn of 1946 a concrete pond was built in the gravel covered yard beside the house. It measures approximately 15 feet by 15 feet and is 2 feet 6 inches in depth at its deepest part. The bed of the pond has some 6 to 8 inches of soil and the water level is maintained by rainwater collected from the roof of an outbuilding close at hand. A stone wall extends along two sides of the pond; on the other two is a low rockery made of stones and earth. This now has an abundant growth of wild iris, water mint, valerian and other garden plants. The surface of the pond is thickly covered this year with water lilies.

In the spring of 1947 the pond was stocked with aquatic plants and snails which Mr. Brocklehurst brought from his father's pond at Bedford, and in August of that year five toads were introduced. They were placed on the rockery beside the pond. Nothing more was heard or seen of them that year.

In the spring of 1948 the toads were heard croaking and they continued to call all through the summer. In August about twenty tadpoles from Bedford were introduced. Most of them were well advanced in growth and it is believed that they underwent metamorphosis that year.

On April 15, 1949, a toad was heard croaking, not at the pond site but from a large rockery built by a former owner of the house and standing in another part of the garden some 95 yards away from the pond. Croaking continued throughout the summer, both from the pond site and from the other rockery.

In 1950 two toads were heard on February 18 during a spell of unusually mild weather. In previous years they have not been heard until late in April or early in May. They have been heard calling almost every night this summer. The last time was on September 4 since when they have been silent, although there have been many warm and sunny days. In the latter part of the year their voices have not the crispness or volume of sound that they have earlier in the season.



Plan of Garden

Breeding was observed during 1949 and again this year, beginning early in May. On several occasions males carrying eggs were found buried in dry, warm soil about 6 inches below the surface. Although the toads must take to the water to liberate their tadpoles they do not always stay beside it. Males with eggs have been found 25 yards away from the water.

Unless disturbed they are never seen to enter the water. They are fond of hiding under stones beside the pond and have regular haunts where they can usually be found. They are never seen abroad in the daytime.

The pond now has tadpoles of several sizes representing broods hatched at different times of the year. The largest ones are between 50 and 55 mm. in total length; many have hind legs; some have fore-legs also. The smaller ones are between 20 and 30 mm. in length and are darker in colour. There are also some only 7 or 8 mm. in length. Most of the large tadpoles will no doubt undergo metamorphosis this year. The smaller ones will over-winter and complete it next year. The tadpoles evidently like warmth and constantly haunt the shallow part of the pond where the water is not more than 6 inches deep.

The tadpoles of this toad are evidently distasteful to those animals, such as fish and newts, that are known to eat frog tadpoles. In the pond with them are Golden Carp and Golden Rudd, as well as, in the spring, Smooth and Warty Newts.

Hibernation is on land. Last February when planting up the rockery one was discovered in dry soil some 6 or 8 inches below the surface.

In July last Dr. Ludwig Koch visited the Bedford colony with me to make a recording of the voice of the Midwife Toad for the British Broadcasting Company. It was a warm night, the toads were calling well and excellent recordings were made giving the range in the variation of the voice when a number of individuals are performing at the same time. The main colony was beside the pond but others could be heard in different parts of the garden. The voice of the Midwife Toad is a single, isolated call, repeated at frequent intervals. It has been likened by some to a clear, whistling note; by others to the sound made by a small bell. To me and to those who have been with me and heard it, the call is a soft, piping note, sometimes high-pitched, sometimes deeper in tone. No toad has been observed in the act of croaking. They are extremely shy animals and call only from their hiding places. The chorus at Bedford that night began at dusk and was still in full swing when we left at one o'clock in the morning.

MALCOLM SMITH.

Pyrford, Surrey. *September, 1950*

NEOTENY IN BRITISH NEWTS

Records in literature of neoteny in British Newts are rare. Both the Smooth Newt (*Triturus vulgaris*) and the Palmate Newt (*T. helveticus*) are mentioned, but there is no record of the occurrence of neoteny in the Warty Newt (*T. cristatus*) in this country, although cases are known from Central Europe. All the British specimens recorded were partial albinos, creamy white in colour with black eyes. Usually only single individuals were found, the other newts in the water with them being normal specimens. There is one record of two individuals being found. The discovery, therefore, of a considerable number of specimens living together is of great interest, particularly as the colony contained both albinistic and normally coloured individuals. I am indebted to Mr. Richard Palmer of the Education Officers' Department of the London County Council for the information concerning this colony which was discovered by him in 1927 in the gardens of Southdown House near Millbrook in Cornwall.

The newts were living in two large ponds which were used as reservoirs for a small brick factory beside Southdown House. The ponds were built of concrete with vertical sides and measured about 27 yards by 10 yards in extent and 5 or 6 feet in depth. There was water running in and out of them. In addition to the neotenous individuals the ponds contained a number of normal adult specimens of the Smooth and the Palmate Newt as well as normal sized larvæ of these species.

The neotenous specimens were of two distinct types:

I. An albinistic form, bright yellow or cream in colour with black eyes and red gills. In size they were a little smaller than full grown specimens of the Smooth Newts. About half a dozen of these were seen.

II. Normally coloured individuals slightly larger in size than full grown specimens of the Smooth Newt. About half a dozen of these also were seen.

The difference in the size of the two groups was distinct and there was no overlapping. All the larvæ were provided with well developed external gills and a large tail fin. The head was of the typical tadpole shape, that is much broader behind than in front. Owing to the difficulty in distinguishing the larval form of the Smooth Newt from the Palmate Newt it was impossible to identify with certainty to which species the neotenous specimens belonged.

In the pond with the newts were also a few unusually large frog or toad tadpoles, very dark in colour and at least twice the size of normal frog tadpoles when about to metamorphose.

Unfortunately no further investigation concerning the newts was undertaken. Some of them were caught and kept at the Marine Biological Station at Plymouth where Mr. Palmer was at that time working and later on were taken to the Department of Zoology, University College, London. They lived for about a year, gradually dying off.

Pyrford, Surrey. MALCOLM SMITH.

THE EDIBLE FROG IN SUSSEX

On April 22, 1950, I found a colony of *Rana esculenta* in a pond situated one mile south of Buxted Station, Sussex. The pond has an area of approximately 840 square feet, and a depth of about 6 feet. It stands in midst of a former common, now a ploughed field. The estimated number of frogs, calculated from a series of visits to the pond, is about forty adult specimens and twenty or thirty growing youngsters. Local inhabitants testify to having heard the frogs croaking for at least seven seasons, but no evidence as yet exists as to their origin. They had previously inhabited a much larger pond, 300 yards away, but appear to have forsaken this, probably owing to the presence of ducks and other water-birds.

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