

# ON THE CLOACAL REGION OF ANURA in Particular of Larval *Ascaphus*

by

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(With 44 text-figures)

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## ABSTRACT

The ontogeny of the cloacal region of *Ascaphus* is described from limited larval material (beginning approximately at the stage of hind-limb bud development). A comprehensive series of *Bufo angusticeps* larvae and late pre-larval embryos were used for comparison. The adult and/or late larval conditions of the cloacal region in *Ascaphus*, *Bufo*, *Bombina*, *Leiopelma*, *Rana* and *Xenopus* are compared.

The rods of Noble supporting the cloaca in *Ascaphus* and the tendinous sheet connecting these with the epipubis are shown to be modifications of an interfemoral ligament present, with thickened lateral margins, in all the *Anura* studied. The cloacal lips differentiate early in metamorphosis in *Ascaphus* and *Bufo* and bear similar relations to the interfemoral ligament in these and other *Anura*, so that they are apparently homologous. The posterior part of the urodaeum is lengthened in the adult male *Ascaphus* to form the "tail" (phallus).

The hind-limb anlagen of *Ascaphus* appear directly beneath the spinal myomeres and immediately behind the posterior tips of the abdominal muscle cords. In *Ascaphus*, *Bufo* and *Bombina* the abdominal muscles (metamerically disposed in *Ascaphus* and *Bufo*) are initially attached posteriorly to the spinal myomeres but are separated from them anteriorly. It is probable that the mm. compressores cloacae are derived from the hind-limb anlagen. In all *Anura* examined, including members of all the South African families, the a. ischiadica and n. ischiadicus have a small muscle (designated m. circumflexor arteriae) associated with them; it is presumably capable of compressing the artery against the nerve.

The cloacal region of *Ascaphus* appears to be less specialized than that of *Rana* and *Xenopus*, contrary to what is generally believed for the last two genera. *Bufo*, particularly, and *Bombina* have undergone less specialization.

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## INTRODUCTION

This investigation was undertaken at the suggestion of my promotor, Prof. C. G. S. de Villiers, and to his encouragement, particularly in the initial stages of the work, and his inspiration, its completion is largely due.

During the course of an investigation of the anatomy of the cloacal region of the adult male *Ascaphus*, including some details of the adult female and larvae, it was decided to attempt to determine the ontogeny of the cloacal structures from the limited *Ascaphus* larval material available. The present paper represents the results of this attempt.

As suggested by the title of the paper (van Dijk, 1955) in which the work on the adult male was recorded — "The 'Tail' of *Ascaphus*: A Historical Résumé and new Histological-Anatomical Details" — an attempt was made to review the literature on the cloacal region of *Ascaphus*.

As the paper was completed (and accepted for the M.Sc. degree) in March 1953, Bhaduri's work on the urinogenital system of the *Anura* (Bhaduri, 1953) which included an investigation of *Ascaphus*, and Ritland's studies (1955a and 1955b) on the post-cranial skeleton, nervous and muscular systems of *Ascaphus* were thus not discussed.

## ENUNCIATION OF THE PROBLEMS

According to classic investigations the muscular layers of the mesenteron of *Chordata* are derived from the splanchnic mesoderm, which constitutes the mesial wall of the splanchnotome of each side, and the trunk and abdominal musculature develops in the somatic mesoderm, which constitutes the lateral wall of the splanchnotome of each side. By an investigation of the development of the (visceral) muscle layers of the coprodaeum at the coprodaeal-proctodaeal junction, and of the (somatic) abdominal muscle layers in the region of the proctodaeal aperture, the origin of the muscle-lining of the proctodaeum might be determined. The development of the tail musculature and of the muscles of the hind-limb might be relevant to this problem in view of their proximity to the proctodaeum. The relation of the coelom, posterior lymph sacs, pelvic girdle and urostyle, and of the blood vessels and nerves of the cloacal region, to the cloaca during development might provide important information on the development of the proctodaeum.

In *Ascaphus* the development of the cavernous tissue of the cloaca, of the (post-pubic) rods of Noble supporting the cloaca, and the muscle layers apparently dorsally striated and ventrally unstriated and associated with these rods (cf. van Dijk, 1955), merit investigation. The developmental origin of the mm. caudalipuboischiotibiales and (mm. pyriformes) and of the epipubis and mm. epipubici, might be relevant in view of the association of the former with the tail and hind-limb in the region of cloaca, and in view of the epipubis forming the posterior attachment of the abdominal muscles antero-ventral to the cloacal aperture (though separated from the latter by the pelvic girdle).

The distribution, forms and functions of the anterior slips of the mm. compressores cloacae extending from these muscles around the ischiadic

nerves and arteries in *Rana*, *Ascaphus* and *Xenopus*, present problems which require investigation, particularly as *Leiopelma* has no such slip arising from the m. compressores cloacae (van Dijk, 1955).

With a knowledge of the development of the proctodaeum of the *Anura* the evolution of the cloaca and copulatory organs of the *Amniota* might be capable of solution.

#### ACKNOWLEDGEMENTS

At the completion of my studies for the degree of Doctor of Science I would like to express my indebtedness to all, zoologists and non-zoologists alike, from whom I have received inspiration, learnt techniques and gained knowledge.

The inspiration of working both under Dr. de Villiers, and also with the research students he has taught, has been very great. The staff of the Zoological Institute, Stellenbosch (Dr. C. G. S. de Villiers, Dr. C. A. du Toit, Dr. C. S. Grobelaar and Dr. M. E. Malan) have all helped to make my years of work there of the happiest.

The facilities of the Zoology Department, University of Cape Town were used during 1956; those of the University of Natal, Pietermaritzburg during 1957 and 1958. The use of these facilities is much appreciated.

#### MATERIAL

The larval *Ascaphus* material examined was available at the Zoological Institute, Stellenbosch, largely in the form of serial sections or remnants imbedded in paraffin-wax, these all being derived from studies by de Vos during 1938 (on *Leiopelma*) and van Eeden (1951). The six larvae used by de Vos were Haemalum bulk-stained and sectioned transversely. Those sections which were counterstained by her had usually been so treated with Eosin, occasionally with Light-Green, Bismarck Brown or van Gieson's Picrofuchsin; those not done by her were counterstained with Eosin during this study. The thicknesses of the sections in each series were not recorded by de Vos and were not always constant. The larvae of van Eeden, sixteen in number, were bulk-stained with Borax-Carmine and the sections (transverse except 1 sagittal series) were counterstained with Azan. The thickness of the sections cut by van Eeden, and those cut in the present study, were recorded and were usually uniform for each series. In addition to the material sectioned or imbedded by de Vos and van Eeden four *Ascaphus* embryos from Mason County, Washington (A. M. N. H. Nos. 50583 and 50586, 1930) were treated in the same manner as van Eeden's specimens, giving 3 frontal and 1 transverse series cut at 10  $\mu$ .

Thus of *Ascaphus* 4 embryos, 22 larvae, and (from the studies of de Villiers, 1933, and van Dijk, 1955) 3 adult males and 2 adult females were available as serial sections.

For comparative purposes 17 *Cacosternum capense* larvae, 9 *Bombina pachypus* larvae, 1 *Plethodon* sp. adult, 1 *Siphonops annulatus* adult, 1 *Ambystoma maculatum* (= *Amblystoma*) adult, 1 *Salmo fario* juvenile, 5 *Periophthalmus*

*koelreuteri* small adults, 1 *Homo sapiens* embryo (late indifferent/female? stage), and 200 specimens of *Bufo angusticeps* (representing all the recognizable stages) were sectioned. There were also available 1 series of a *Leiopelma hochstetteri* adult male (from Wagner, 1934a and 1934b), 1 series each of *Rana grayi* adult male and female, 2 of *Xenopus laevis* larvae and 1 juvenile *Xenopus laevis*, 1 of a *Bufo gariiepensis* juvenile, 3 of *Breviceps* juveniles and a postmetamorphic *Arthroleptela* series.

Material utilized for dissection included numerous *Xenopus laevis* adults, and one adult of each of *Rana angolensis*, *Pyxicephalus natalensis*, *Phrynobatrachus* sp., *Heleophryne* sp., *Phrynomerus* sp., *Bufo regularis* and *Chiromantis xerampelina*.

## TECHNIQUES

The techniques used frequently differed from those in general practice. They are therefore described at some length.

### Ascaphus, Leiopelma, Xenopus and Rana sections

The average thickness of the sections in each of de Vos's series was determined by means of the micrometer fine-focus of a microscope (checked against other microscopes). It was also done by assuming dermal glands to be spherical, measuring their diameters (by means of an eye-piece micrometer) and comparing these diameters with the number of sections which the glands occupied or in which they appeared, the terminal sections in which the glands appeared usually being assumed to be half-occupied. The second of these methods was found to be reliable, giving consistent results.

Drawings on thin paper at enlargements of 50 diameters, were made (usually of every 4th section) by means of a vertical slide projector, Zeiss Luminar lenses being used as they gave wide, flat and bright images. The drawings were rendered transparent with xylene, each was adjusted in position to correspond with those before (and sometimes after) it, and base-lines for reconstruction were then marked by drilling orientation holes through the piles of papers. Graphic reconstructions were then made (see Pusey, 1939) of left lateral aspects (denoted *norma lateralis sinistra*), and sometimes dorsal aspects (denoted *norma dorsalis*). Use was also made of liver base-lines in doing lateral reconstructions from van Eeden's series and from those cut from his wax blocks (which contained liver slices). These base-lines were found to be of little use for adjusting the orientation of successive sections, but useful in establishing a general trend in direction over long intervals. Liver base-lines were most useful in orientating where a skeletal structure appeared or disappeared in a series of sections, thereby affecting the extent to which each section stretched during mounting. Sections through the pelvic girdle often stretched less than those in front of, or behind, them, and the cloaca tended to stretch away from the tail in those sections in which it was not held by skin to it. (For liver base-line technique see van Eeden, 1951, and Schepers, 1938.)

For truer dorsal reconstructions the degree of skewness (sagittalness) of the transverse sections was estimated by measuring the distance between

distinct bilateral structures (such as the ilia) and the distance by which one of these preceded the other in the series of sections (number of sections occupied x section thickness). The distances so measured gave the tangent (or cotangent) of the angle of skewness, so that the true mid-line of the reconstruction could be drawn and the mid-line of each drawing could be aligned to this. For some distorted sections two mid-lines were drawn through each drawing and the structures on which each mid-line was based (ventral and dorsal respectively) were then reconstructed separately.

Photomicrographs were made of significant sections in all the series reconstructed, the levels of the sections being indicated on the reconstructions. Photomicrographs were also made of instructive sections from series not reconstructed, these illustrating, for instance, statements not figured in the 1955 work, the scope of which was reduced for publication. Use was also made of photomicrographs instead of drawings for reconstructions, and proved to be time-saving and otherwise vastly superior where orientation difficulties were not prohibitive. Such difficulties with photomicrographs arose from the need to use the cheaper, relatively opaque, photographic paper, instead of film plates or film-type papers which would have made possible simultaneous comparison of a greater number of photographs.

Where orientation difficulties were not great (e.g. where the notochord could be assumed to be straight, or in dorsal views) reconstructions were also done successfully by projecting the image of each section onto the reconstructing board, adjusting it to a base-line (e.g. notochord for lateral, and mid-line for dorsal, views), and to a vertical or horizontal line; then proceeding as usual with the image substituted for the usual drawing or photomicrograph.

Certain of the sections of Prof. de Villiers's specimens of adult *Ascaphus* male and female, which had been stained with Haemalum and van Gieson's Picrofuchsin, were restained with 1% aq. Light Green or van Gieson's Picrofuchsin followed by 1% aq. Light Green. The same treatment was given to sections through the cloacal region of the other adult female *Ascaphus*, which had been stained with Borax-Carmine-Azan, and to sections of the *Xenopus* adult after removal of the Eosin which had been used as counterstain to Haemalum. The Acid Fuchsin (of the van Gieson's Picrofuchsin) stained the collagenous connective tissue red, while the Light Green was taken up by non-collagenous connective tissue and by muscle. The greater affinity for Picric Acid (of the van Gieson's Picrofuchsin) of striated muscle, and, to a lesser extent, of unstriated muscle, resulted in a grass-green tinge (Light Green + Picric Acid) in the muscles as compared with a blue-green colour in the non-collagenous connective tissue. The Light Green was employed for  $\frac{1}{2}$  to 1 minute and differentiated in water to give the correct overall colour on the section (approximately 15 secs. to 1 min.). Van Gieson — Light Green, Picro-Indigo-Carmine, Picro-Nigrosin and Retterer's Alum Carmine were employed on sections through the cloacal region of the male adult *Ascaphus* and on the female adult *Ascaphus* sectioned for that work.

The developmental stages of *Ascaphus* of which reconstructions were made have been numbered according to the criteria used for *Xenopus laevis* in the Hubrecht Laboratory publication "Normal Table of *Xenopus laevis* (Daudin)" (Nieuwkoop and Faber, 1956). For the stages up to stage 57 reference was made to the hind-limb development, for the later stages the

conditions of the opercula and fore-limbs were used. (The hind-limbs of the later stages of de Vos's material were incomplete distally.)

**Bufo angusticeps material (and Bombina, Cacosternum, Ambystoma, Plethodon, Siphonops, Salmo, Periophthalmus and Homo)**

The *Bombina*, *Salmo* and *Periophthalmus* specimens after fixation in Bouin's fluid, and the rest, except *Bufo*, after formalin fixation, were all treated much as described below for *Bufo*.

The *Bufo angusticeps* material was identified by reference to de Villiers (1929) and Noble (1926) initially; subsequently by the time of the year at which oviposition took place, the form of the egg-strings, and also by the dark pigmentation of the eggs and larvae (apparently darker than that of any other South African anuran). The material was killed and fixed in Lenhossek's Picric Acid — Mercuric Chloride — Acetic Acid — Alcohol solution, washed after 24 hours, decalcified (in some cases) in 5% Nitric Acid in 70% alcohol, stained in Grenacher's Borax-Carmine (except alternate early stages, which were Haemalum bulk-stained), dehydrated (sometimes only to 96% alcohol), cleared in Methyl Benzoate-Celloidin, impregnated for a half-hour in Benzol Wax at about 30°C, impregnated in vacuo in 52°C paraffin wax and imbedded and sectioned in fresh 52°C wax. The animals, whole or with only a part of the tail missing, were sectioned frontally most often, transversely least often, the relative numbers (excluding the earliest stages) being 74 frontally, 54 sagittally, 39 transversely, i.e. approximately 4 Frontal: 3 Sagittal: 2 Transverse series of *Bufo*.

Counterstaining of the *Bufo angusticeps* material was done with Heidenhain's Azan (except the Haemalum-stained early stages, for which Eosin was used). In addition 1% aq. Light Green was used for most of the older, and some of the younger, specimens. It was found that it facilitated recognition of striated muscle, non-collagenous connective tissue and nerve fibres. Striated muscle stains a characteristic orange-brown (similar to Bismarck Brown in hue) because of its affinity for both Orange G (from the Azan) and Light Green; non-collagenous connective tissue is stained blue-green, and nerve fibres a characteristic grey-green. The blue of collagenous connective tissue (Aniline Blue from the Azan) and the pink of unstriated muscle fibres (Borax-Carmine) are little affected by Light Green unless this is employed for too long. The use of Light Green derived from a suggestion of Prof. de Villiers that it be used as a counterstain to Haemalum. Safranin (= "Safranelin"?) was tried as a stain for non-collagenous fibres (Maskor, 1953), but seemed unsuitable for immature anuran tissue.

Limited use was made of base-lines, these being produced by boring holes at right angles to the plane of sectioning in wax blocks and filling the resulting holes in the sections with Indian ink. Wax models such as those described in the earlier paper (van Dijk, 1955), and photomicrographic stereograms similar to the graphic stereogram used in that paper (op. cit., fig. 29), were used to visualize the milieu of the cloaca and associated structures. The photomicrographic stereograms were produced by distorting photomicrographs by means of specially constructed anamorphote lens systems (which have since become familiar in connexion with "cinemascope").

## Dissections of members of each of the families of South African Anura

After a number of different approaches to the cloacal region of *Xenopus* had been attempted, median incisions through the skin were made, extending half the way along the back above the cloacal aperture in all the specimens (*Xenopus* and others) dissected. This was followed by a median incision through the membrane over the urostyle. It was then usually possible to separate the fat containing the coccygeal lymph hearts and the septum iliaceum mediale of one side, intact; this separation being achieved by carefully loosening these from the m. coccygeo-iliacus, and hooking them laterally whilst the urostyle and m. coccygeo-iliacus were hooked over to the opposite side. This dissection exposed the cloaca in dorsal and lateral view. Using a hooked needle, it was possible, in numerous *Xenopus*, to locate the dorsal aorta and pull it sideways so that the fluorescein injection technique used by de Graaff (1957) could be applied, the injection taking place after the dorsal aorta had been returned to its natural position with the needle in it. Thus blood-flow in the aa. iliaceae could be watched under ultraviolet light.

### PROCEDURE

As complete a series of *Ascaphus* as could be obtained from the available material, was made. Similarly, as complete a series of *Bufo angusticeps* was prepared as was possible from the specimens sampled at intervals during development. Extra specimens of *Bufo* at each sampling were preserved in wax ready for sectioning, should it be necessary. The comparative material listed above was then prepared.

Reconstructions of all the discernible features of as much of the pelvic region as possible, were made of all the *Ascaphus* stages that could be reconstructed. Of the *Bufo angusticeps* material, reconstructions of the salient features of the entire animal were made at the stages which marked the first appearance, and which illustrated the development of, the proctodaeum, the permanent gut lumen (unoccluded by yolk), the pronephric ducts, the abdominal muscles, the limb-buds, and the cloacal muscles.

Conclusions on the *Ascaphus* material were arrived at, illustrated and noted. These conclusions were checked on the *Bufo angusticeps* material and illustrated with further notes. Only then was the relevant literature thoroughly examined. This was done in an attempt at avoiding "anticipatory set", which produces subjectivity of approach, particularly in perception. Finally the material was re-examined together with the other animals prepared.

In presenting the results an attempt has been made to reproduce the evidence on which descriptive statements were based. Photomicrographs serve both to illustrate features and to check on the necessarily somewhat subjective reconstructions.

It must be noted that, while the *Ascaphus* material has been described at length and the *Bufo* material cursorily, the preparation and examination of *Bufo angusticeps* stages represented more than half of the work involved in this study. Without recourse to *Bufo angusticeps*, representing the typical anuran condition, the *Ascaphus* material could not have been adequately interpreted or described.

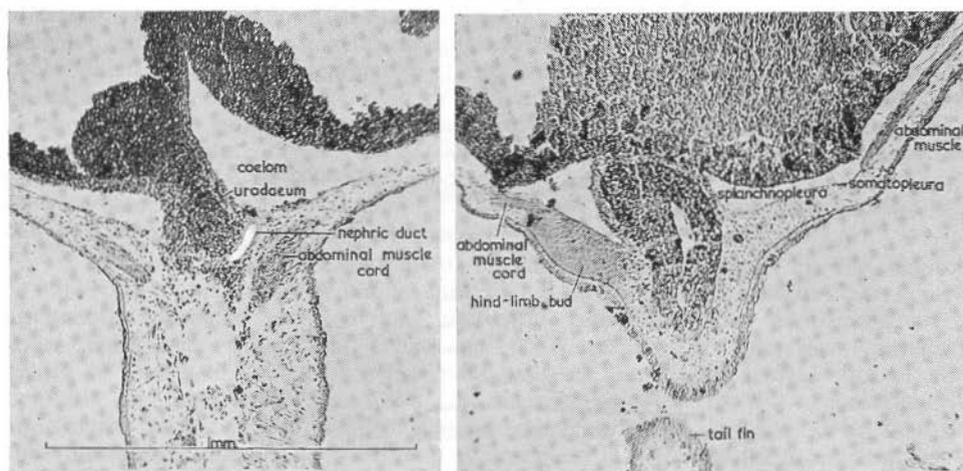
### Note on Terminology

Gadow's (1887) terminology for the cloaca of *Amniota* has been adopted. The cloaca is thus considered to consist of a terminal ectodermal *proctodaeum*, a *urodaeum* into which the urinary and genital ducts and the bladder open, and a *coprodaeum* receiving the intestine. The use of this terminology implies that the urodaeum and coprodaeum are accepted as being of endodermal origin, for these parts of the cloaca are distinguishable in *Amniota* internal to the cloacal membrane (which represents the limit of the ectodermal proctodaeum) before this membrane perforates. That the urodaeum and coprodaeum are of endodermal origin in the *Anura*, appears to be established by evidence, presented below, that the urinogenital ducts open into an endodermal part of the cloaca.

### DESCRIPTION OF THE STAGES IN *ASCAPHUS*

#### Stage 47 (figs. 1 and 2)

The nephric ducts open into the urodaeum at the posterodorsal margin of the coelom (fig. 1). Behind the urodaeum the proctodaeum is largely isolated from the inner, splanchnic, wall of the splanchnotome. The coelom extends furthest posteriorly along the gut laterally (fig. 2), ventrally not reaching as far posterior as the openings of the nephric ducts. From their ventral situation in the body-wall anteriorly the abdominal muscles pass dorsally caudad, separating the hind-limb buds partially from the coelom anteriorly (fig. 2, left), and completely from the myomeres of the tail anterodorsally (fig. 1), attaching to these myomeres at the level of the nephric aperture.



Figs. 1 and 2.

*Ascaphus* embryo, Stage 47. Cloacal region (norma dorsalis).  
Photomicrographs of frontal sections x 50.

Stage  $\pm$  50 (figs. 3 and 4)

The relations of the segments of the abdominal muscles to the myomeres of the tail and trunk are well shown in the specimen at this stage. The posterior-most segment of the abdominal muscle is applied to the ventrolateral face of the myomere corresponding to the 9th spinal nerve (here referred to as the 9th *spinal myomere*). The second abdominal muscle segment from the posterior end is lateral to the coelom and ventrolateral to the 8th spinal myomere. The posterior-most abdominal segment is anteriorly mediad to that in front of it, but is lateral to it further posteriorly. The 9th and subsequent spinal myomeres curve ventrally round those behind them and extend dorsally on the inner faces of the latter. A few muscle fibres occur mediad to the posterior tip of the posterior-most abdominal myomere.

The coelom extends to near the posterior edge of the 9th spinal myomere posteriorly; medially it extends between the 9th spinal myomeres further dorsally than the level of the top of the last abdominal myomere. Laterally the coelom extends posteriorly almost to the posterolateral margin of the 8th spinal myomere dorsally, extending beyond this margin further ventrally.

Figs. 3 and 4.

*Ascapus* larva, Stage  $\pm$  50 (both figs. from one specimen).  
Posterior abdominal region x 25.

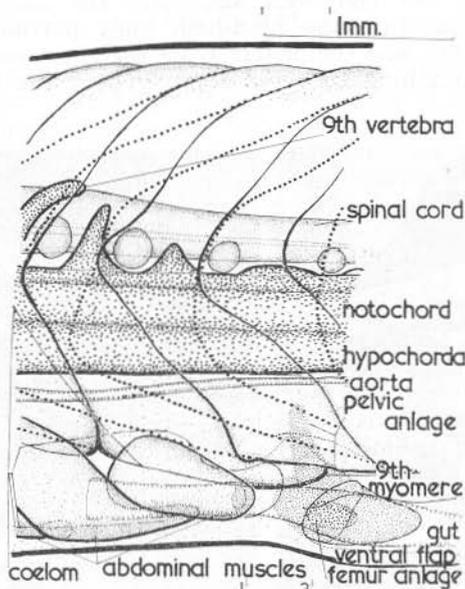


Fig. 3.

Norma lateralis sinistra.  
Graphic reconstruction from  
transverse sections.

The 8th spinal and abdominal myomeres are thus separated by coelom at this stage.

Hind-limb buds are visible at this stage and a femur anlage is present near the base of each. The concentrations of cells of each limb anlage extend anteroventrally to just anterior to the posterior limit of the coelom. Laterally each anlage extends anterodorsally over the posterior tip of the 9th spinal myomere onto the lateral face of the 10th spinal myomere. From this part of each limb anlage the ilium and associated structures will differentiate.

Mediodorsal and medioventral to the hind-limb buds are cell aggregations, the latter clearly constituting the anlagen of the rods of Noble.

The hypochord in the specimen is much thicker than in all other larvae examined, and the difference may not be developmental, but specific.

The myomeres and nerves of the specimen were numbered from the vertebral arches, the most anterior shown in fig. 3 being found, by counting, to be the 9th. At this stage the 9th arch is well-developed, but dorsally incomplete.

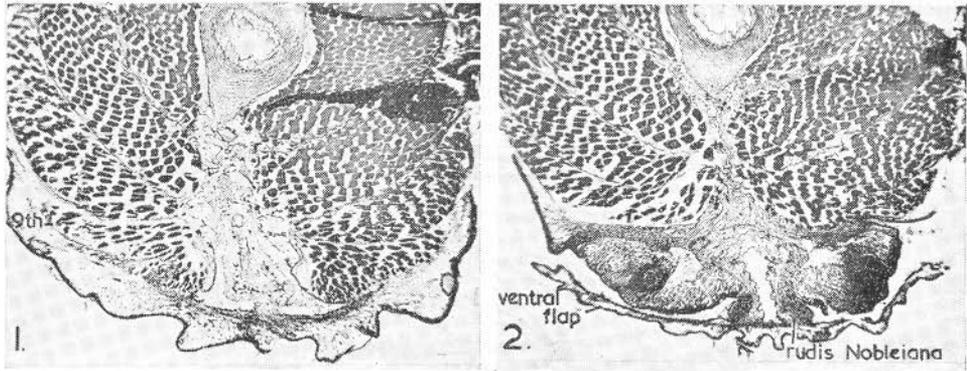


Fig. 4.

Norma posterioris. Photomicrographs of transverse sections.  
(The numbers refer to levels in fig. 3.)

#### Stage 51 (figs. 5 and 6)

The proctodaeal aperture is situated on the dorsal surface of a flap of skin which covers the hind-limb buds ventrally and protrudes beyond them posteriorly. The aperture is roughly triangular, with the base near the posterior tip of the ventral flap, and the apex anterodorsal to this. Just anterior to the apex of the proctodaeal aperture a ventral projection from the tail attaches to the dorsal surface of the proctodaeum; anterior to this point the proctodaeum, and the flap of skin ventral to it, are suspended from the tail. Anteriorly the furthest limits of the proctodaeum are marked dorsally by the opening of the nephric ducts, ventrally by a thickening which represents the bladder. The coelomic cavity does not extend ventrally beyond the bladder, but dorsally the median urinogenital aperture is now a short distance

from the end of the coelom, which extends to the level of the 12th spinal nerve. The circular muscle layer present around the coprodaeum is continued in the urodaeal region; but it is interrupted dorsally where the nephric ducts join and enter the urodaeum, and ventrally at the bladder. Posterior to the nephric ducts the dorsal wall of the gut slopes down steeply, the circular muscle layer again becoming continuous dorsally where this slope decreases, which is still within the coelomic cavity laterally and dorsally. Dorsally the circular muscle layer extends caudad to the end of the coelomic cavity and a short distance beyond, reaching approximately the posterior limit of the bases of the hind-limb buds. The circular muscle layer also becomes continuous posterior to the bladder anlage which is beyond the ventral limit of the coelomic cavity; but it does not extend as far into the extra-coelomic tissue ventrally as dorsally. Longitudinal muscles are not clearly distinguishable, and would thus seem to be less extensive than the circular layers.

A dorsal median ridge of the gut epithelium, extending from the urinogenital aperture to the anterior limit of the bases of the hind-limb buds, is present and constitutes a characteristic feature observable in adults, in which it extends to the cloacal aperture.

Figs. 5 and 6.

*Ascaphus* larva, Stage 51 (both figs. from one specimen). Cloacal region x 25.

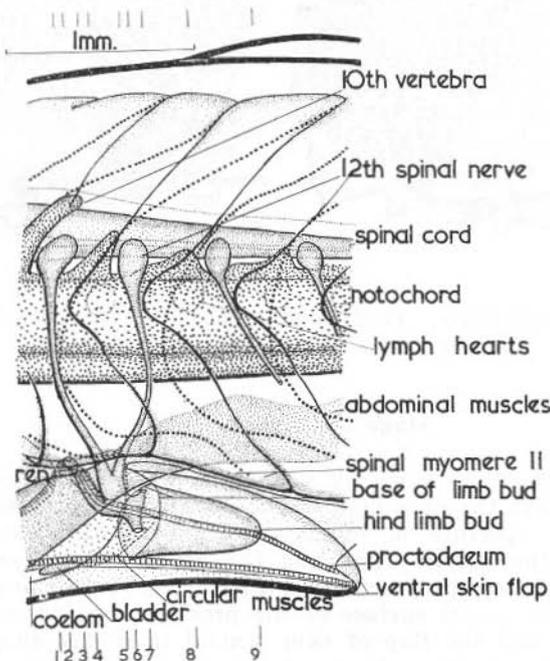


Fig. 5.

Norma lateralis sinistra.

Graphic reconstruction from transverse sections.

The plexus ischio-coccygeus, arising from nerves 9—12, passes to the hind-limb buds along the extreme posterior edge of the lateral wall of the coelom. The 10th spinal arch, but not the 11th, extends above the spinal cord.

The abdominal muscle cord of each side passes caudad and mediad under the spinal myomeres at the level of the posterodorsal limit of the coelom, and lies in contact with the medial face of the 11th spinal myomere and subsequent myomeres, its posterior limits not being distinct. A slight fold in the outer aspects of the 10th and 11th myomeres may be observed accommodating the abdominal muscle cord, which passes between these myomeres and the base of the hind-limb bud of that side.

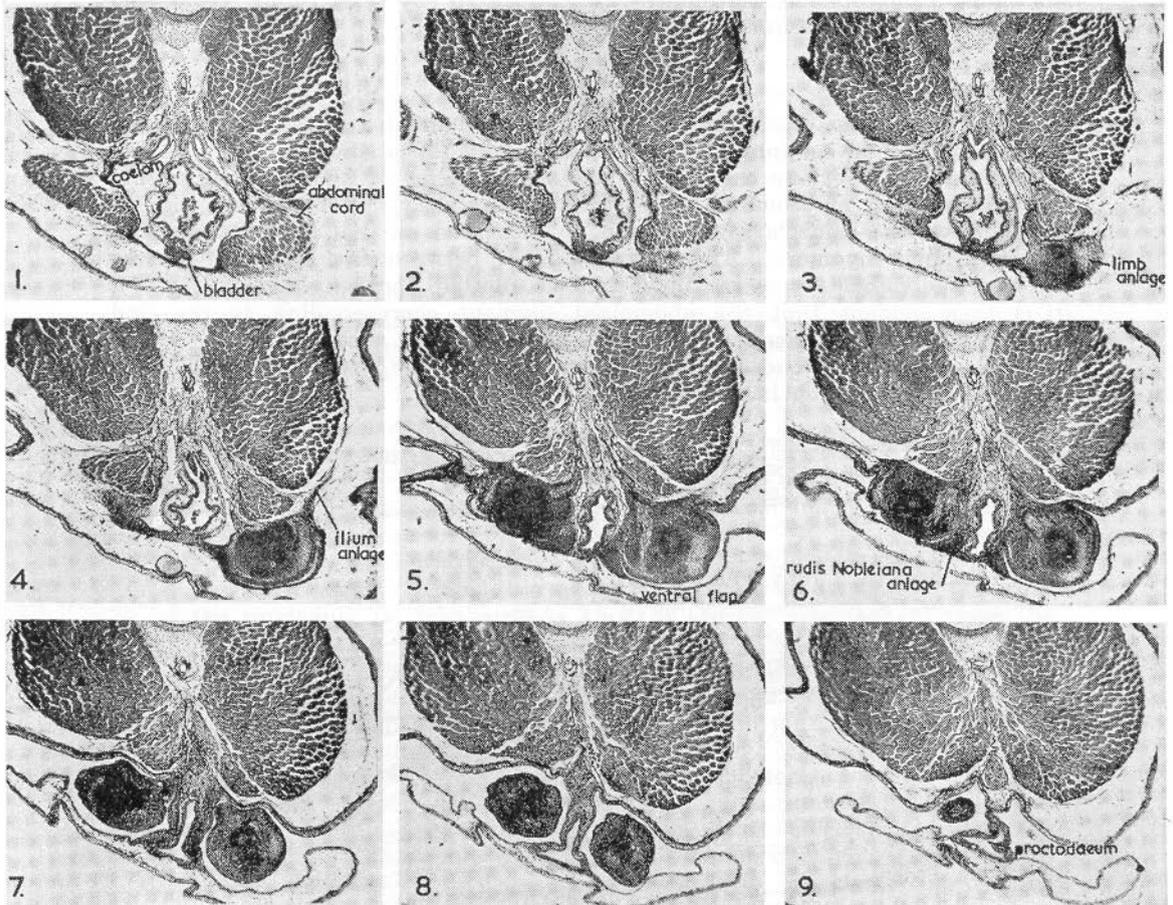


Fig. 6.

Norma posterioris. Photomicrographs of transverse sections.  
(The numbers refer to levels in fig. 5.)

The kidneys and their ducts extend to the level of the 10th spinal myomere.

At the level of the urostyle 4 coccygeal lymph hearts are present laterally near the anterior edges of the 11th to 14th spinal myomeres.

At the ventral limits of the bases of the hind-limb buds, and along the medial borders of these bases, are thickenings; these ventrally representing the (post-pubic) rods of Noble, and dorsally the fibrous connective tissue which extends to the urostyle from the posterior tips of these rods in the adult, particularly the female adult.

The anlagen of the muscles of the hind limbs are becoming distinguishable around pro-cartilage in the limb-buds; the anlagen of the mm. pyriformes and mm. caudalipuboischiotibiales are indicated by extensions from the myogenic tissue of the limb-buds towards the urostyle.

### Stage 52 (fig. 7)

At this stage the segmentation of the abdominal muscles can be made out in longitudinal sections. What is presumably the n. iliohypogastricus can be observed to innervate the second last of the series of abdominal segments near its posterior myocomma. Since the nerve is clearly derived from the 8th spinal nerve, this muscle segment can be identified as that corresponding to the 8th spinal myomere. The correspondence between the spinal myomeres and the abdominal myomeres may thus be plotted (broken lines in fig. 7). It is then evident that the abdominal segment corresponding to the 2nd spinal myomere is rudimentary and the one before it is absent.

The hind-limb bud is slightly shorter than the ventral flap of skin; the 10th vertebral arch is well developed.

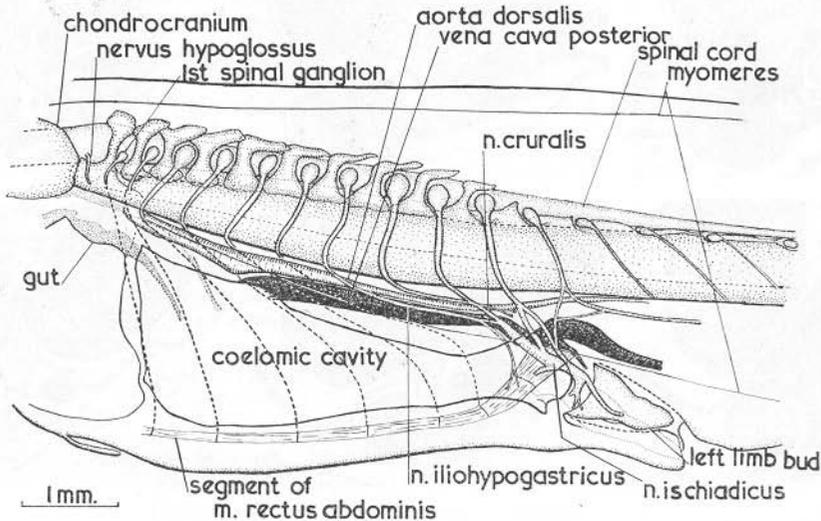


Fig. 7.

*Ascaphus* larva, Stage 52. Trunk region (norma lateralis sinistra) x 12.5  
Graphic reconstruction from sagittal sections.

### Stage 56 (figs. 8—10)

The proctodaeal aperture has the same form as in Stages 51 and 52, but is not as near to the tip of the ventral flap. The ventral projection from the tail attaching to the dorsal wall of the proctodaeum is notched so that its edge slopes cranioventrad then caudoventrad. The flap of skin and the proctodaeum are thus suspended more freely than in Stage 51. At the level of the bases of the hind-limbs the proctodaeum is more dorsal than further posteriorly, and there is a distinct slope between the two levels. The appearance of the proctodaeum at the slope is that of the more anterior part dorsally and of the more posterior part ventrally, this being particularly noticeable in the breadth of these portions — dorsally the proctodaeum narrows gradually caudad, ventrally it narrows more rapidly cephalad (fig. 9).

The bladder has separated from the urodaeum, and partially from the floor of the coelom, anteriorly. It is anteriorly notched medially and thus bluntly bifid. The lumen is distinct, anteriorly bifid and anterolaterally it projects dorsally. There is a distinct common portion of the nephric ducts which opens into a fairly easily distinguishable urinogenital sinus directly dorsal to the bladder.

The floor of the coelomic cavity is raised to the level of the bladder just anterior to the bases of the hind limbs. The coelomic cavity extends behind the bladder, and above its level, on either side of the gut, projecting more than half-way into the region of the bases of the hind limbs. As the plexus ischio-coccygeus is now also further forward, being situated approximately at the level of the nephric aperture, the coelomic cavity extends considerably posterior to the plexus. The coelomic cavity reaches the level of the 13th spinal ganglion posteriorly; dorsally it reaches about two fifths of the distance to the vertebral column medial to the spinal myomeres. As the coelom stretches further dorsally as well as posteriorly than in earlier stages, the abdominal muscle cord still passes across its posterodorsal limit.

The abdominal muscle cord of each side lies under the 10th spinal myomere, being internal to the 10th, 11th and 12th myomeres posteriorly and forming a broad ventral sheet, isolated from the spinal myomeres, anterior to the pelvic girdle. The ventral sheets are medially quite close to each other, the connective tissue between them constituting the *linea alba*. Signs of segmentation are slight in the ventral sheet, but posteriorly there are two distinct segments on each side. A nerve corresponding to the 8th spinal myomere, and a branch from the 9th spinal nerve, together form the nerve — hence presumably the *n. iliohypogastricus* — which supplies the abdominal segments on each side at the myocomma between the ventral sheet and the next segment. (The nerve corresponding to the 8th spinal myomere passes to the lateral surface of that myomere, traverses the lateral surface of the 9th spinal myomere, and meets the branch from the 9th spinal nerve ventral to the myomeres somewhat anterior to the ventrolateral edge of the 10th spinal myomere.) The nerve in the myocomma gives fibres to both the segment behind it and the broad ventral sheet in front of it, these therefore presumably corresponding to the 9th and 8th spinal myomeres respectively. The last segment of each side constitutes the portion on the inner faces of the spinal myomeres and presumably corresponds to the 10th spinal myomere. The

positions of the last two segments on each side is such that they could be considered posterior extensions of the corresponding spinal myomeres, as may be well seen in dorsal view (fig. 9).

The pelvic girdle is present in the form of two cartilages anterior to the bases of the hind limbs; the hind limbs are clearly divisible into femur, crus

Figs. 8—10.

*Ascaphus* larvae, Stage 56 (figs. 8 and 9 from one specimen). Pelvic region x 25.

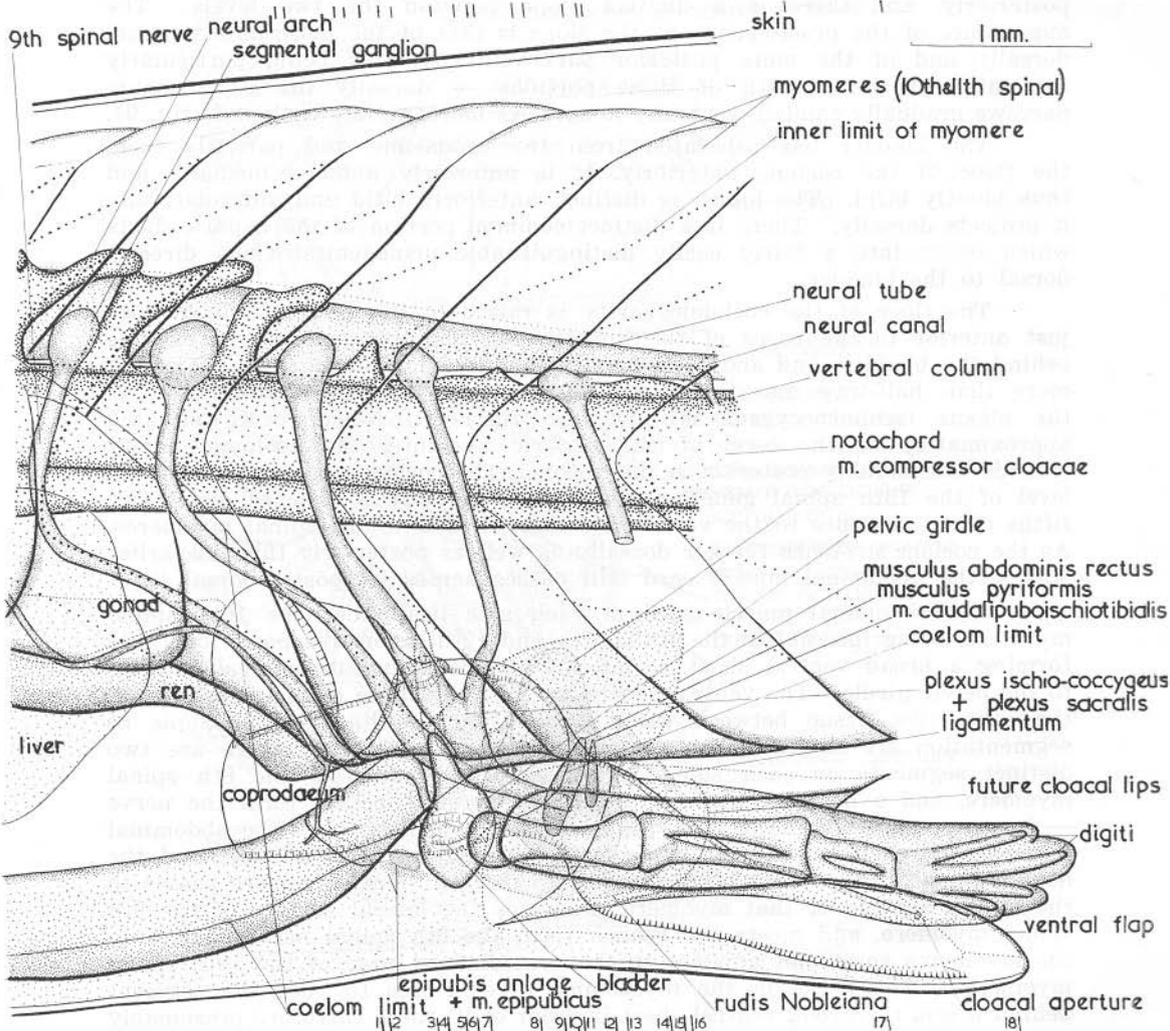


Fig. 8.

Norma lateralis sinistra. Graphic reconstruction from transverse sections.

and pes, each with its cartilaginous skeleton well differentiated. The position of the halves of the pelvic girdle and of the limbs indicates a forward and dorsal growth of the tissue at the bases of the hind-limb buds. This movement has resulted in the lifting of the coelom, proctodaeum and urodaeum in the pelvic region.

Anterior to the pelvic halves, at the medial edges of the myocommata between the ventral abdominal muscle sheets and the following segments, the anlagen of the epipubes (or halves of the epipubis) are visible as condensations of cells in the connective tissue stretching from these myocommata to the

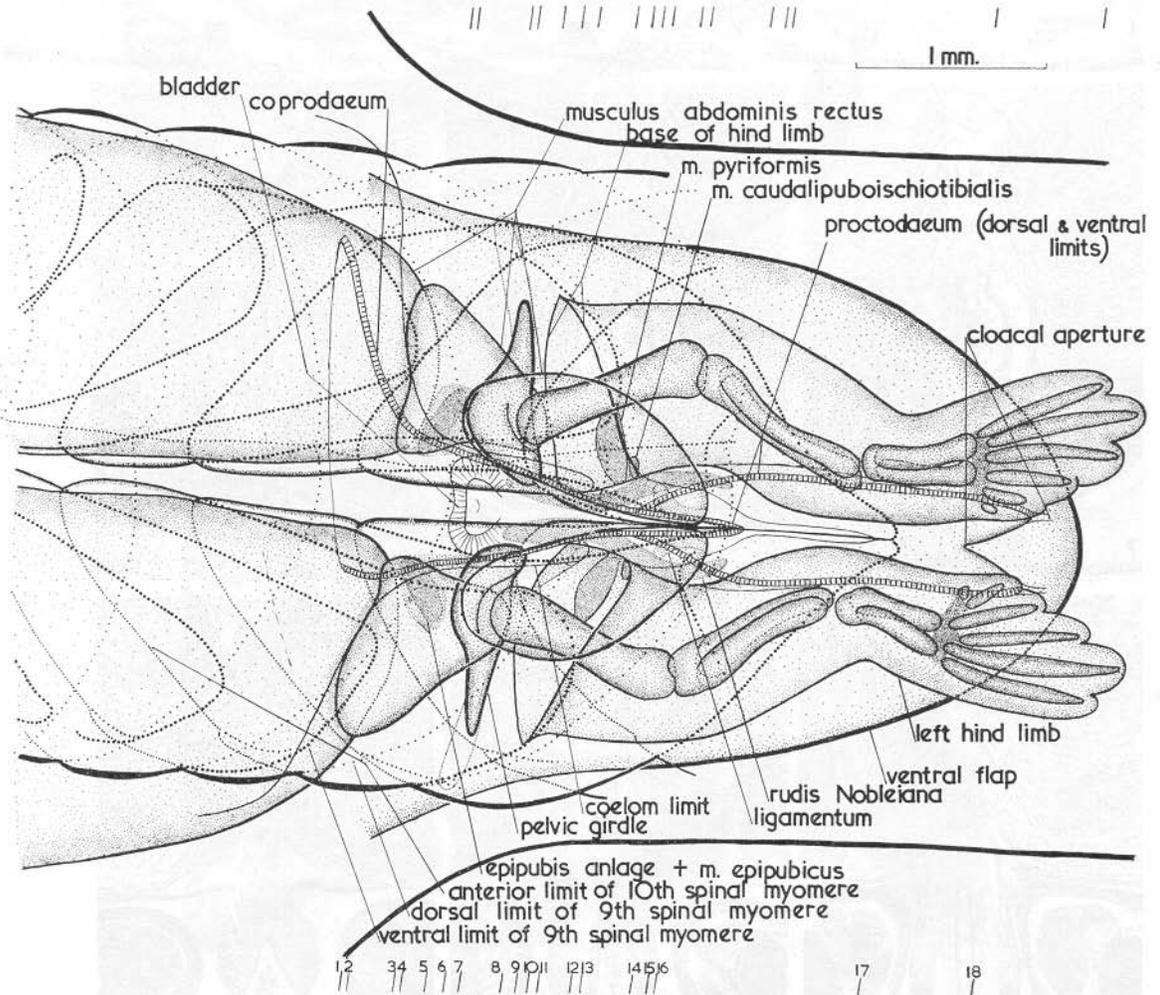


Fig. 9.

Norma dorsalis. Graphic reconstruction from transverse sections.

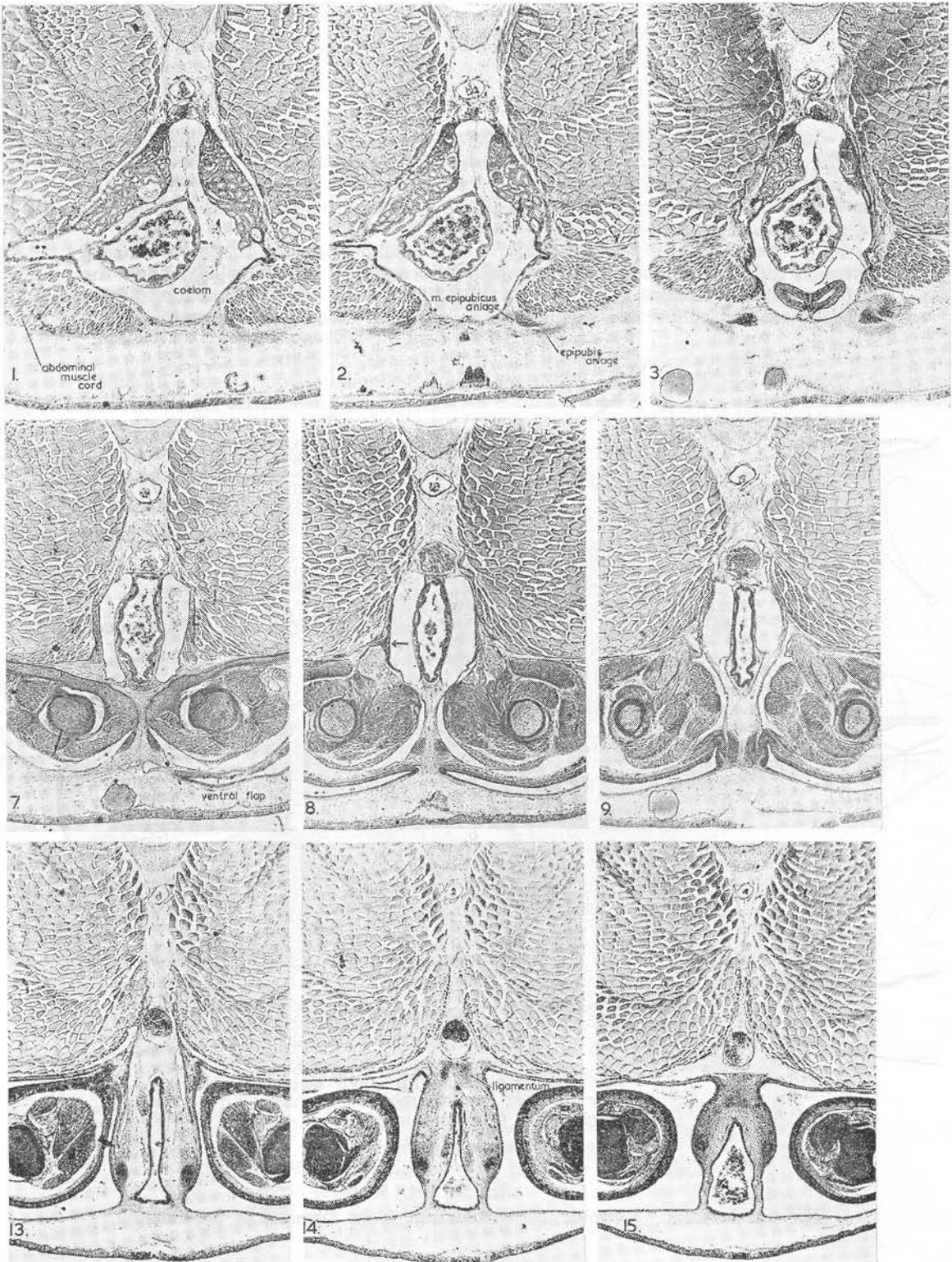
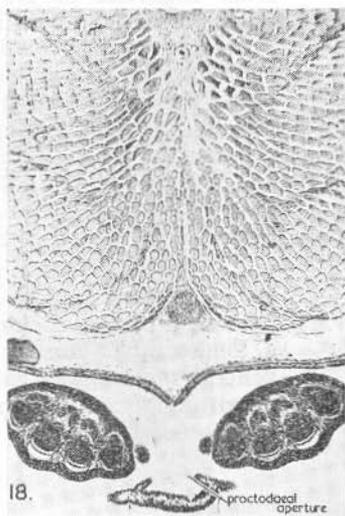
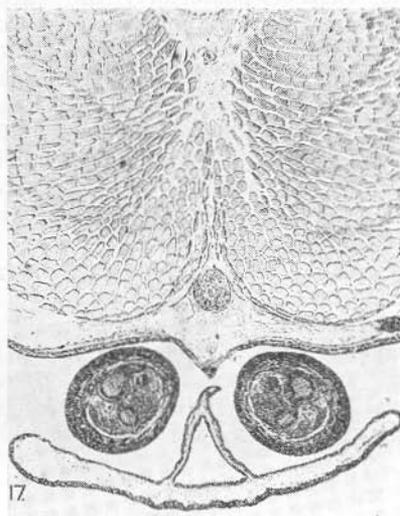
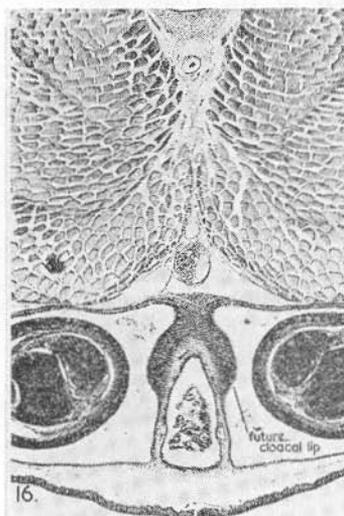
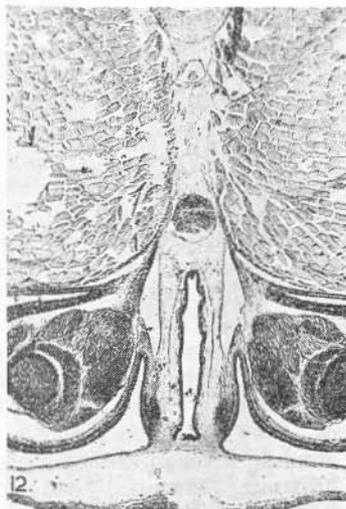
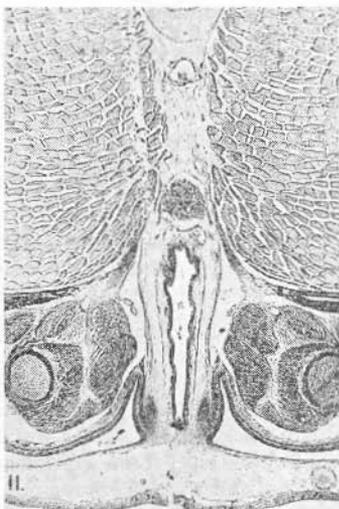
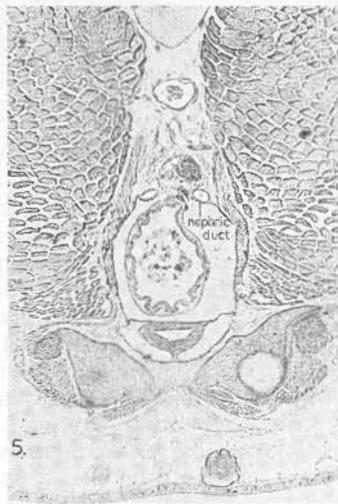
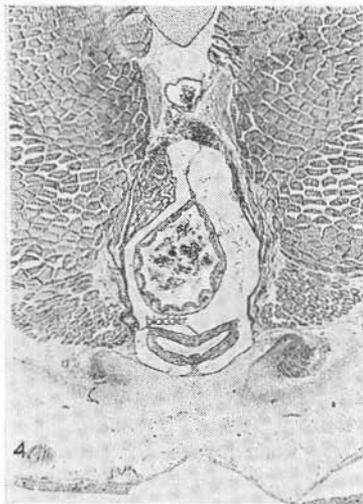


Fig. 10.

Norma posterioris. Photomicrographs of transverse sections (female specimen).

(The numbers refer to corresponding levels in figs. 8 and 9.)

An arrow in the figure at level 10 indicates the m. circumflexor arteriae.



pelvic halves. Dorsal to the epipubicanlagen, and considerably more distinct, are the *mm. epipubici*.

An aggregation of cells continues posteriorly from the epipubic anlage of each side medioventral to the pelvic girdle half, becoming the (post-pubic) rod of Noble posteriorly. These rods thus begin anteriorly medioventral to the posterior third of the pelvic girdle. Behind the pelvic girdle the rods of the two sides approach each other beneath the proctodaeum, parting again as the proctodaeum slopes posteroventrally between them and turning medio-dorsally at their posterior tips. The rods extend about two thirds of the way across the sides of the sloping portion of the proctodaeum. At their posterior tips the rods approach not only each other in the horizontal plane, but also a similar aggregation of cells forming a ligament which lies dorsal to the cloaca. From the anterior tips of the rods dense connective tissue stretches medial to the pelvic girdle halves dorsally.

In the region of the tips of the rods of Noble the skin laterodorsal to the proctodaeum is thicker than further posteriorly, these thicker walls representing the future cloacal lips. The median dorsal ridge of the urodaeum and proctodaeum is absent posterior to this point, but it is distinct somewhat anterior to it.

Near the posterior end of the bases of the hind limbs the *mm. caudalipuboischiotibiales* are located, lying dorsomedial-lateroventrally on either side of the dorsal half of the proctodaeum. Just anterior to the *mm. caudalipuboischiotibiales* the *mm. pyriformes* have their origins at the same horizontal and lateral levels. Their direction is more lateral than that of the former muscles, and they are longer, since they reach to the femurs. The *mm. pyriformes* just reach the level of the coelomic cavity anteriorly.

The *mm. compressores cloacae* have developed, stretching across the medial faces of the posterior segments of the abdominal muscles dorsally and passing on either side of the gut ventrally to approach the dorsal surfaces of the rods of Noble. Posteriorly the *mm. pyriformes* and the *mm. caudalipuboischiotibiales* lie between the *mm. compressores cloacae* and the abdominal muscles. Anteriorly the *mm. compressores cloacae* end some distance from the rods of Noble, further posteriorly they attach to the rods. Just posterior to the hind-limb bases the *mm. compressores cloacae* become indistinct. Posterior to the bladder the *mm. compressores cloacae* become continuous ventrally in the pelvic girdle region. Anteriorly slips of muscle continuous with the *mm. compressores cloacae* extend anterolaterally, a dorsal slip stretching along the lower half of the medial faces of the posterior abdominal segment of each side and a ventral slip curving around the *arteria ischiadica* and the *nervus ischiadicus* at the point at which the artery twists caudolaterad over the nerve. The ventral slip of muscle is here named *m. circumflexor arteriae* to facilitate reference.

The circular muscle layer of the gut can be detected approximately as far posteriorly as the *mm. compressores cloacae*, but it is incomplete ventrally posterior to the coelom. Posterior to the coelom there are few indications of a longitudinal muscle layer. Just posterior to the pelvic girdle, and to the ventrally complete *mm. compressores cloacae*, there are two cords of longitudinal muscle ventral to the gut. Indications of longitudinal muscle are also visible dorsolateral to the gut at this level.

The lymph sacs of the limbs have developed and extend from the hind-limb bases ventrally, and medially both cephalad and caudad, dorsal to the rods of Noble. The rods of Noble are firmly adherent to the ventral flap of skin posteriorly; anterior to the bases of the hind limbs there are small lymph spaces ventral to the rods separating them partially from the ventral skin.

Veins on either side of the proctodaeum just anterior to the future cloacal lips drain considerable sinuses around the proctodaeum in this region.

The gonads have developed, and the sex of some specimens may be determined at this stage.

### Stage 58, Male (figs. 11 and 12)

The proctodaeum has become considerably shorter by loss of the greater proportion of that part which was associated with the ventral flap of skin, the latter being greatly reduced in length and breadth while the skin which suspended it from the tail region is entirely absent. The proctodaeal aperture is now at the end of the downward-sloping portion behind the pelvic girdle. The median dorsal ridge of the gut epithelium now extends to the proctodaeal aperture, and the (post-pubic) rods of Noble, dorsal ligament, mm. compressores cloacae, and circular muscle layer, reach to a point dorsal to the posterior limit of the proctodaeal aperture; the rods of Noble, in extending dorsal to the proctodaeum, completely surround it laterally. A rudimentary "tail" is formed by these structures, little change in relative positions having taken place in its formation. The dorsal ligament is now much more distinct and projects dorsally at the level of the posterior limit of the fold between the thigh and the cloacal extension ("tail"), splitting to pass on either side of the caudal vein.

The approximation of the halves of the pelvis lifts the proctodaeum and urodaeum to the level of the ilia, so that the slope of the proctodaeum behind the pelvic girdle is much steeper than in earlier stages. As the process of approximation begins posteriorly and extends forward, the urodaeum and bladder are forced somewhat further forward, as they are lifted. Enlargement of the halves of the pelvic girdle posteriorly results in the ischial regions reaching the anterior edges of the ventral ends of the mm. pyriformes and thus almost to the posterior end of the coelom, the relations between coelom and mm. pyriformes having undergone little change. The halves of the pelvic girdle are separated only by a thin layer of dense connective tissue in the ischial region, anteriorly the halves are still well separated.

The bladder extends from the posterior edge of the ilium to a level in front of the pelvic girdle. It is bifid and well separated from the coprodaeum, on either side of which it projects anteriorly.

The floor of the coelom lifts steeply from in front of the pelvic girdle to a position between the ilia, but the posterior and dorsal limits are little changed.

The relation of the abdominal muscle cord to the coelom and the pelvic girdle is little altered; the increasing height of the body in this region, however, causes the muscles to assume an almost vertical position. At the same time degeneration of the posterior segments has taken place so that the dorsal portion of the cord is scarcely distinguishable. Close to the degenerating

segments on each side the *m. transversus* and *m. obliquus* have differentiated. On the ventral surfaces of the ventral muscle sheets the presence of smaller, more basophilic, fibres reveals the differentiation of secondary muscles — the superficialis layer of the rectus abdominis muscles. The dorsal degenerating portion of each abdominal muscle cord is associated with the 10th and 11th spinal myomeres of the corresponding side, but somewhat more anterior regions of these spinal myomeres than before. This is clearly shown by the relations to the nephric ducts, which open at a level between these degenerating muscles.

The *mm. compressores cloacae* extend to the tips of the rods of Noble posteriorly and attach to them ventrally as far forward as just behind the pelvic girdle. Further anteriorly the two muscles approach each other

Figs. 11 and 12.

*Ascapus* larva, Stage 58, male (both figs. from one specimen).

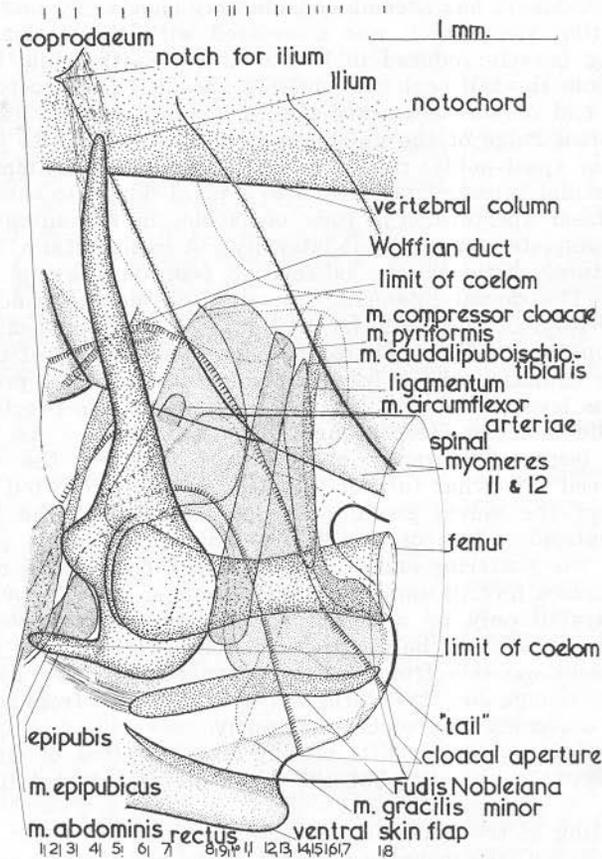


Fig. 11

Norma lateralis sinistra.

Graphic reconstruction from transverse sections.

ventrally, and, dorsal to the pelvic girdle, are again separate at their anterior limits, attaching to the pelvic girdle between the ilia. Dorsally the muscles extend to the ligament, which is the dorsal equivalent of the rods of Noble, posteriorly. Anterior to the ligament — which curves caudad dorsally — the *mm. compressores cloacae* extend further dorsal on the medial faces of spinal myomeres 10, 11 and 12, and are no longer separated from these by the abdominal muscle cords, as the latter have withdrawn just cephalad a little posterior to the ilia. The dorsal anterior slips of the *mm. compressores cloacae*, which were associated with the abdominal muscle cords, are not distinguishable, while the ventral anterior slips are quite distinct, here being designated (see previous stage) as *mm. circumflexores arteriarum* because they curl around the *arteriae ischiadicae*. The anteroventral tips of the *mm. compressores cloacae* lie considerably further forward, extending more than half-way across the pelvic girdle and well beyond the opening of the bladder. Relations to the coelom posteriorly indicate relatively little extension of the muscles posterodorsally.

The *mm. compressores cloacae* and the *mm. graciles minores* are closely associated, the former attaching to the dorsal surfaces of the rods of Noble at the same level as that at which the latter originate laterally from them.

The *mm. pyriformes* and *mm. caudalipuboischiotibiales* have extended both ventrally and dorsally without changing their antero-posterior relations to any extent. The *mm. pyriformes* reach to the ventral surfaces of the femurs and both pairs of muscles extend dorsally further than the *mm. compressores cloacae*, being associated with the medial faces of the 10th spinal myomeres — which are curved medially in this region.

The ilia have shifted slightly forward relative to the lateral surfaces of the 10th spinal myomeres, extending on each side from the anterior half of the myomere ventrally towards a notch on the posterior margin of the myomere. Dense connective tissue, in the form of strands, extends from the tips of the ilia into the myocommata between the 10th and 11th spinal myomeres at the notches. The tips of the ilia reach half-way up the hypochorda of the vertebral column.

The epipubic cartilages are well developed and are continuous with the pubic region of the pelvic girdle halves, but not with each other. The *mm. epipubici* extend from the anterior faces of the halves of the pelvic girdle and attach to the dorsal faces of the epipubic halves. From each half of the epipubis there is a dense connective tissue connexion with the rods of Noble which passes ventromedial to the halves of the pelvic girdle. Anteriorly the *m. rectus abdominis* of each side is attached to the epipubic cartilage.

The *m. pectineus* of each side extends forward dorsal to the *m. epipubicus* of that side, anteriorly curving somewhat medioventrally towards the epipubis, but not attaching to it.

The rods of Noble have been ventrally displaced anteriorly by the approximation of the halves of the pelvic girdle above them, and this has caused some inflected curvature anterior to the proctodaeum, the rods now having an elongated-S shape. The dorsal ligament lies in a narrow septum between the lymph sacs at the bases of the hind limbs, the septum therefore being the septum interfemorale between the sacci interfemorales.

The skin of the "tail" is beginning to separate from the proctodaeum

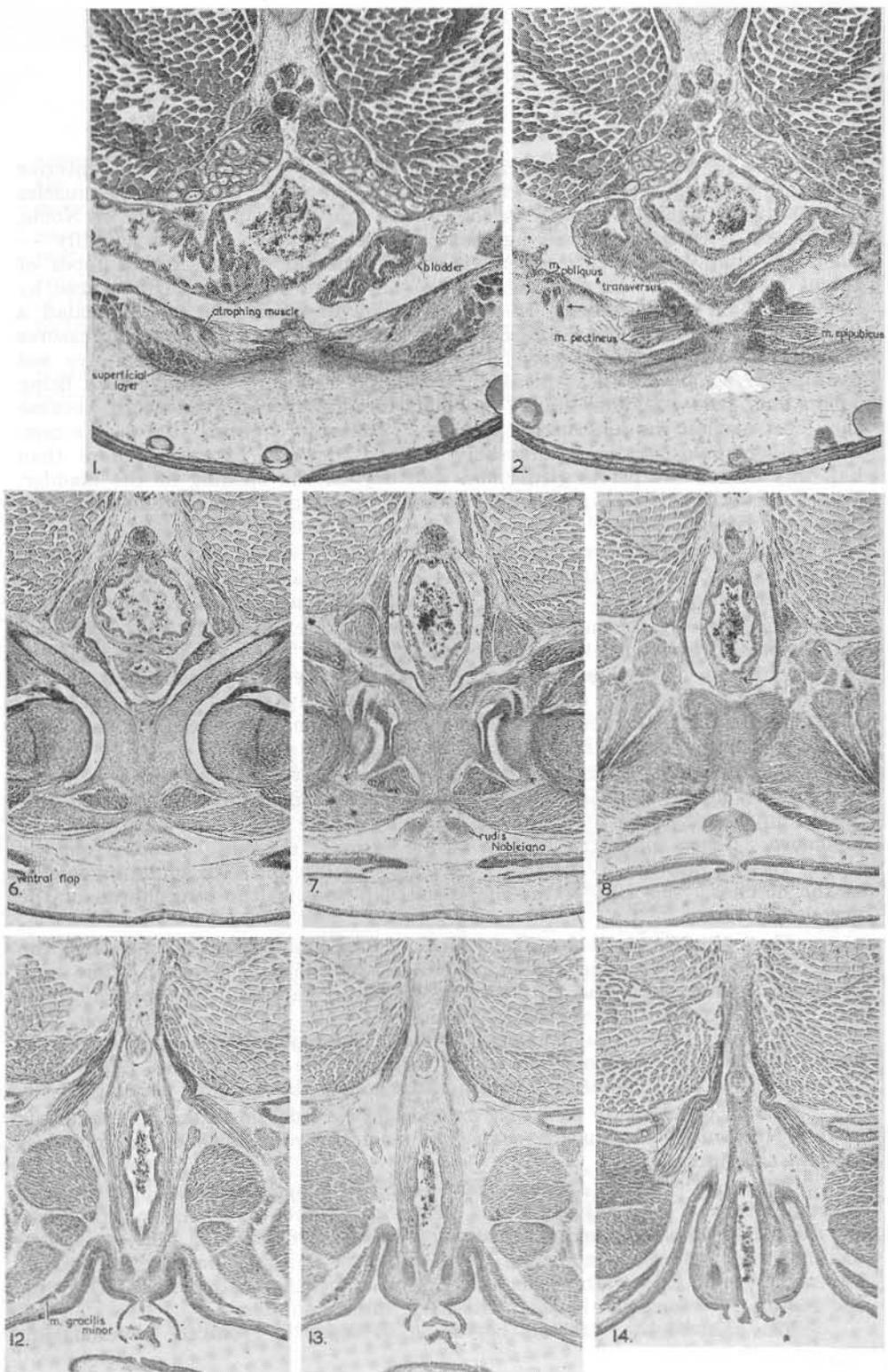
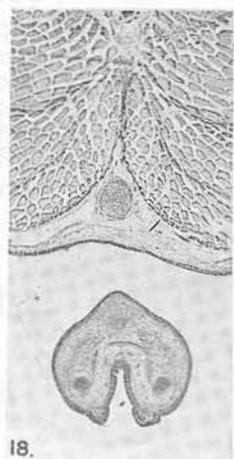
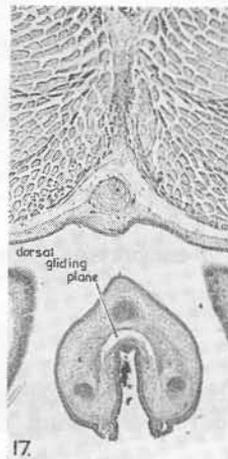
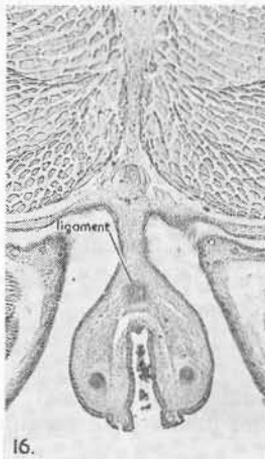
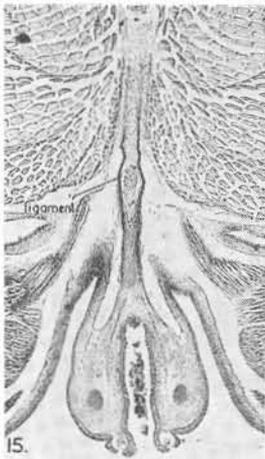
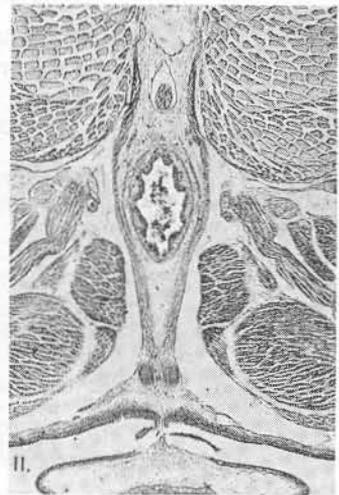
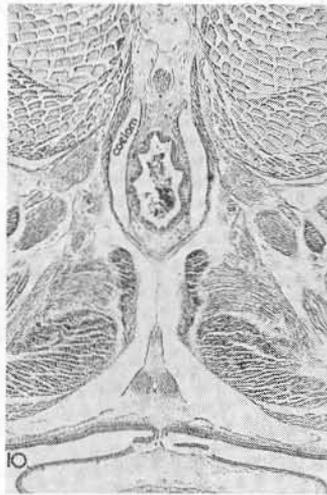
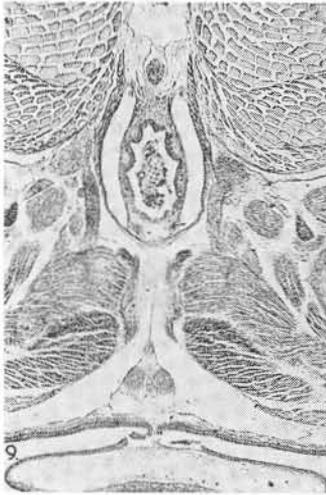
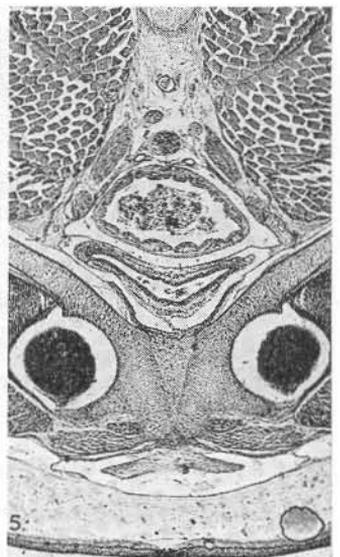
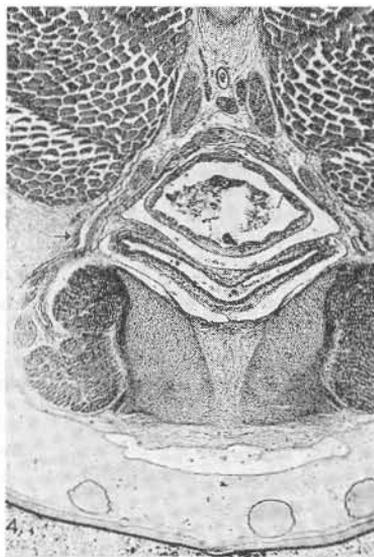
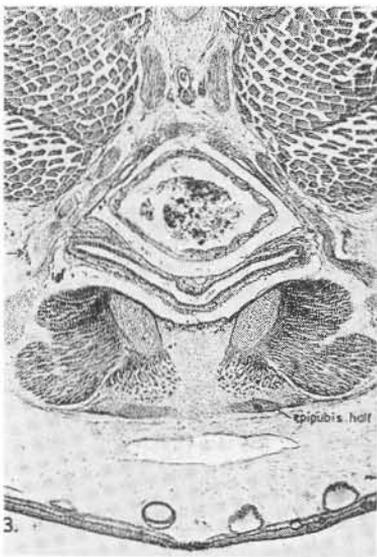


Fig. 12.

Norma posterioris. Photomicrographs of transverse sections. Arrows in the figure at levels 2—6 indicate the atrophying abdominal muscle cord, and at level 7 indicate the m. compressor cloacae and the m. circumflexor arteriae.



dorsally and dorsolaterally at the bases of the hind limbs, the lymph spaces produced being in communication with the sacci interfemorales, which extend a short way distally along the femurs. The dorsal gliding plane of the cloacal extension ("tail") has also developed.

The increase in the lymph spaces around the proctodaeum has left the

blood vessels and the nerves (12th) of this structure suspended between the posteromedial edges of the mm. pyriformes and the dorsolateral edges of the mm. compressores cloacae in the cloacal extension.

Stage 60, Male (figs. 13, 14 and 15)

Figs. 13—15.

*Ascapus* larva, Stage 60, male (all figs. from one specimen).

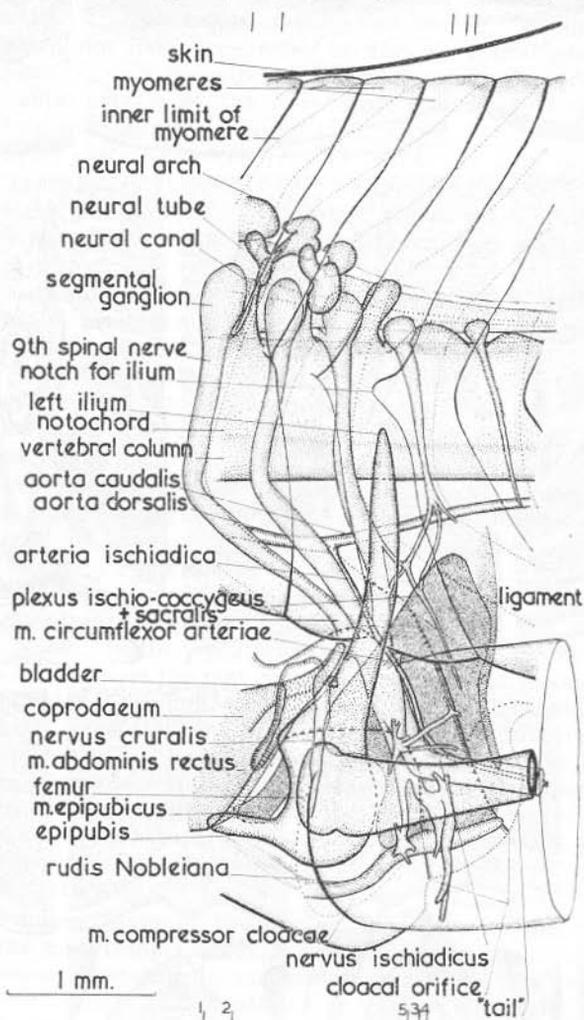


Fig. 13.

Pelvic region (norma lateralis sinistra).  
Graphic reconstruction from transverse sections.

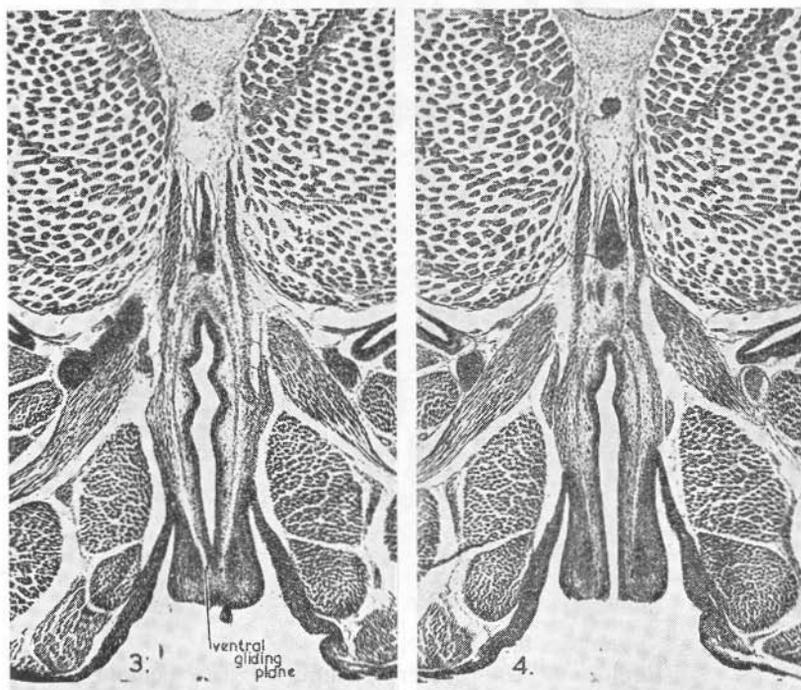
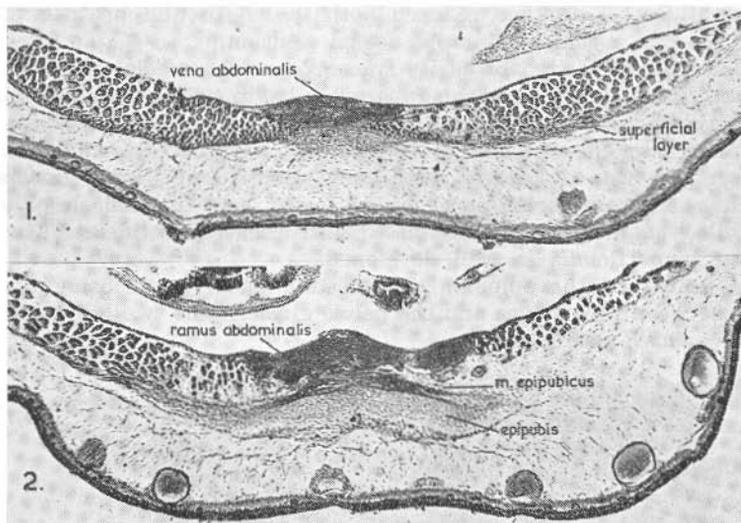


Fig. 14.

Epipubic and proctodaeal regions (norma posterioris) x 25.  
 Photomicrographs of transverse sections. (The numbers refer to levels in fig. 13.)

The ventral flap is further reduced both in length and breadth. The proctodaeal orifice has a somewhat more dorsal position.

The abdominal cords are not easily traceable on the medial faces of the spinal myomeres, the posterior segment apparently having disappeared. Ventral to the spinal myomeres two segments associated with the epipubis can be made out on each side, the anterior of these being part of the *m. abdominis rectus*. The more posterior and dorsal segment on each side is closely associated with the *m. iliacus internus*, being separated dorsally from this muscle only by *n. cruralis*, while ventrally it is associated with the *m. pectineus*. The *m. abdominis rectus superficialis* is well developed.

The two halves of the epipubis are fused anteriorly, enclosing a considerable space between themselves and the pelvic girdle. The two halves of the pelvic girdle have fused posteriorly.

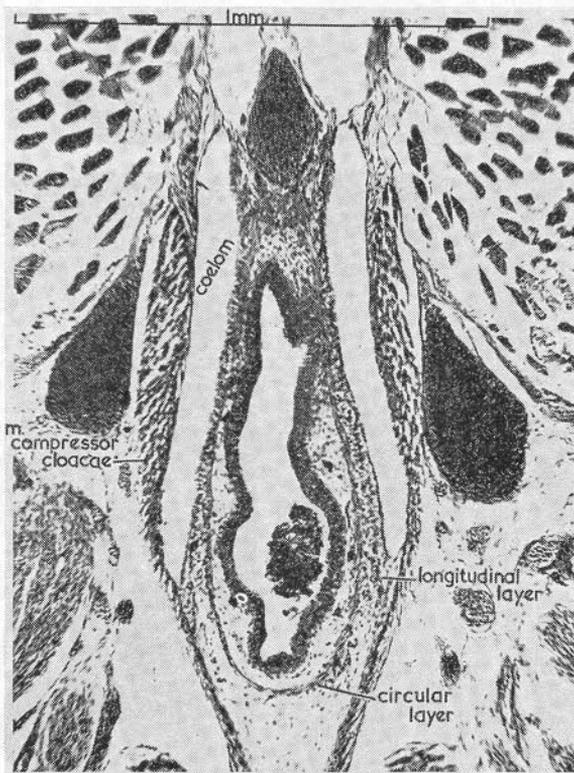


Fig. 15.

Region of the posterior limit of the coelom  
(norma posterioris) x 50.

Photomicrographs of transverse section.  
(The level of the section is indicated by 5 in  
fig. 13.)

With the formation of a vena abdominalis (showing signs of a dual origin) dorsal to the anterior tip of the epipubis and to the linea alba further anteriorly, the vascular system in the pelvic region has attained essentially the adult form.

Anterior to the ventral lip of the proctodaeal orifice a ventral gliding plane has developed; it is incomplete midventrally.

#### Stage 62—63, Male (figs. 16 and 17)

The proctodaeal aperture is somewhat more dorsal, particularly its upper rim, and there is a more marked fold between the cloacal extension ("tail") and the tail. The mm. compressores cloacae now reach further dorsally, to beyond the aorta caudalis, and are separated from each ventrolateral edge of the vertebral column by a short tendinous band only. Anteriorly the mm. compressores cloacae have shifted forward to a position lateral to the urogenital aperture. The mm. circumflexores arteriarum arising from the mm. compressores cloacae project beyond the anterior edges of the latter, the change in position being made possible by greater separation distally of the two muscles on each side. There is a forward shift of the plexus sacralis relative to the ilium.

The ilium now projects somewhat anterodorsally instead of posterodorsally or vertically upwards from the acetabulum. This change is associated with a shift of the ilium posteriorly relative to the ventrolateral face of the 10th spinal myomere, while the dorsal tip of the ilium enters the notch at the posterior edge of the myomere, which notch is now deeper and hence extends further anterior. The tip of the ilium approaches the transverse process of the 10th, the sacral, vertebra, which lies lateral to, but not in contact with, the arch of the vertebra. The secondary muscles associated with the transverse process are well-developed.

The abdominal muscle cord is represented by the m. abdominis rectus originating on the epipubis. The epipubis is connected to the rods of Noble by a tendinous sheet of connective tissue which has two thickened portions revealing its paired origin.

Posteriorly the rods of Noble are distinctly continuous with the dorsal ligament, which now reaches to the vertebral column. Associated with the rods of Noble cavernous tissue has begun to develop; this tissue surrounds the rods behind the pelvis and also lies lateroventral and medioventral to the rods in this region. The mm. compressores cloacae have the same transverse niveau with the rods as has the cavernous tissue. Between the rods just anterior to the ventral rim of the cloacal orifice there are fibres of the mm. compressores cloacae. These are well isolated from differentiated circular and longitudinal muscle layers.

There is a urodaeal diverticulum where the common nephric duct opens into the urodaeum. The wall of the urodaeum and proctodaeum is little folded in comparison with the other larvae, and there is no median dorsal ridge. Just within the proctodaeal aperture, and cephalad, cloacal glands have begun to develop anteriorly. They are visible as thickenings in the epithelium.

Ventrally the cloacal extension, rods of Noble and mm. graciles minores have separated from the skin except just anterior to the proctodaeal aperture.

Figs. 16 and 17.

*Ascaphus* larva, Stage 62—63, male (both figs. from one specimen).  
Pelvic and cloacal regions x 25.

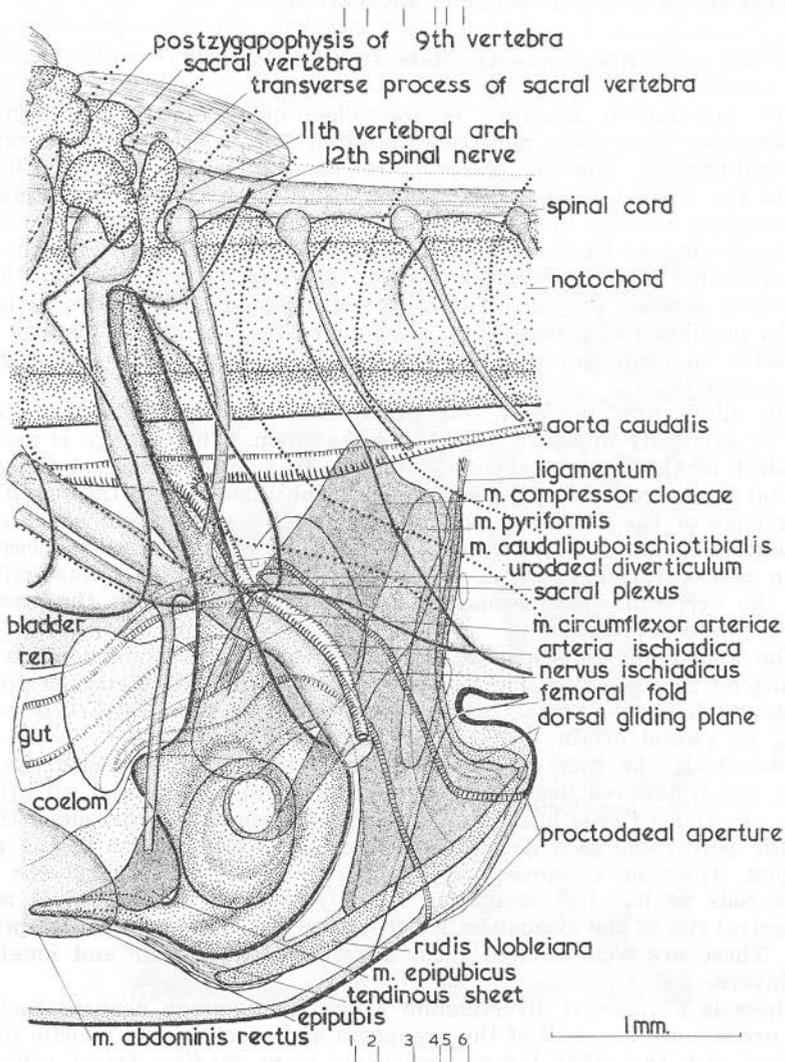


Fig. 16.

Norma lateralis sinistra.  
Graphic reconstruction from transverse sections.

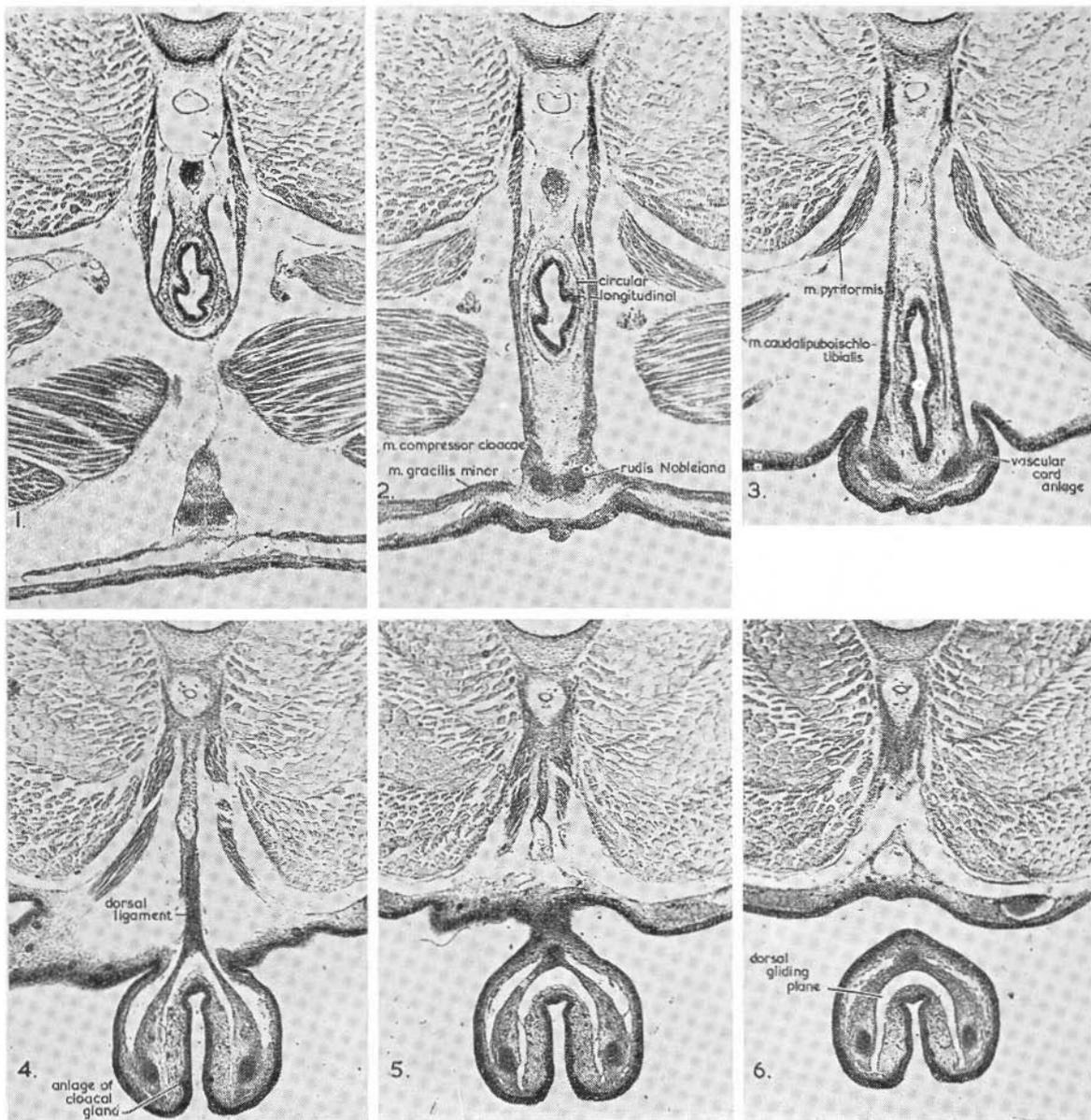


Fig. 17.

Norma posterioris (cloacal region). Photomicrographs of transverse sections. (The numbers refer to levels in fig. 16.)

The arrow in the figure at level 1 indicates the longitudinal muscle cord extending towards the vertebral column.

Stage 63, Female (figs. 18 and 19)

The fold between the tail and the cloacal extension has become still deeper. Cloacal glands are present in the ventral cloacal wall. The mm. compressores cloacae maintain practically the same relations,

Figs. 18 and 19.  
*Ascaphus* larva, Stage 63, female (both figs. from one specimen).  
 Pelvic region x 25.

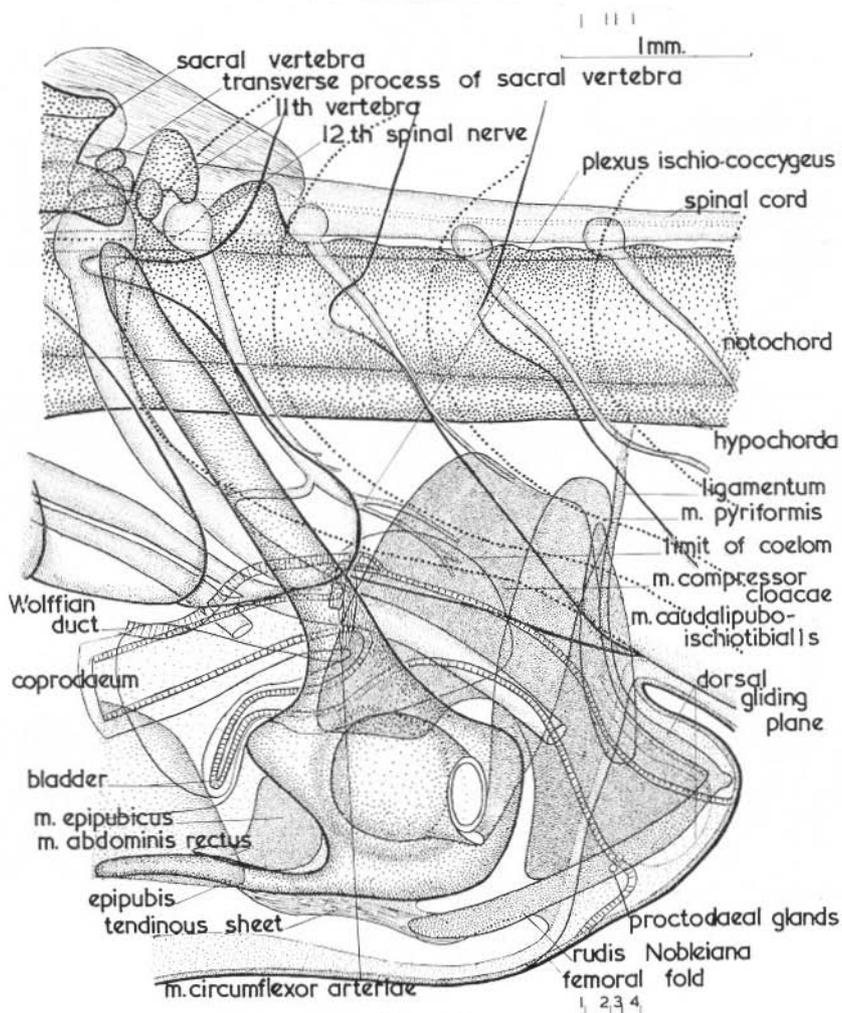


Fig. 18.  
 Norma lateralis sinistra. Graphic reconstruction from transverse sections.

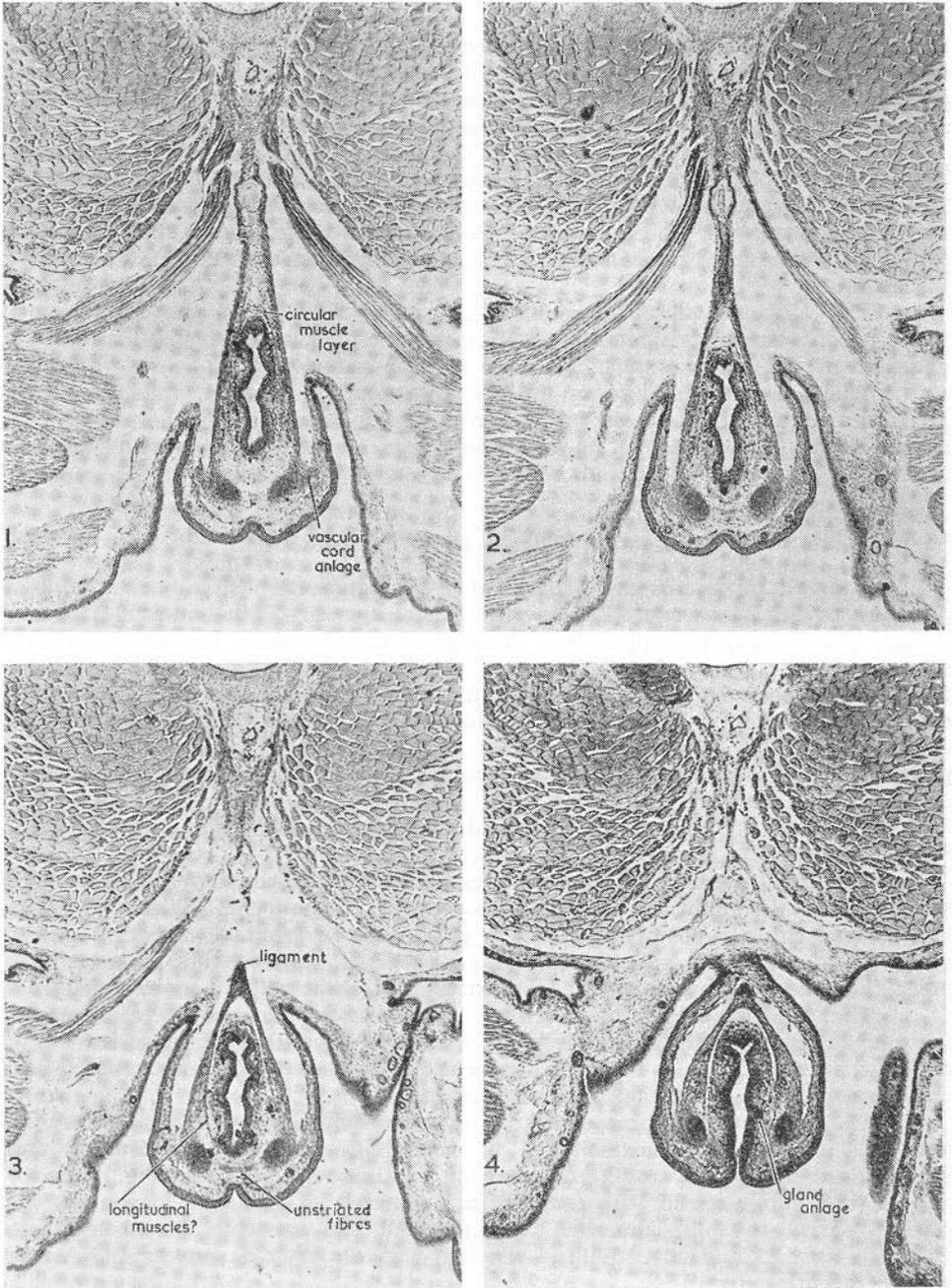


Fig. 19.  
 Norma posterioris. Photomicrographs of transverse sections.  
 (The numbers indicate levels in fig. 18.)

but in doing so have considerably lengthened antero-posteriorly, since the ischial region has become considerably larger. The origins of the mm. pyriformes are only slightly posterior to the ischia, and slightly posterior to a point at which the hypochorda becomes dorso-ventrally thinner.

On each side the dorsal portion of the ilium projects much more anteriorly, passing from a position lateral to the posterior extremity of the lateral face of the 10th spinal myomere to penetrate the very deep notch at the posterior margin of this myomere. It closely approaches the transverse process, now fused to the sacral vertebra. The plexus sacralis is displaced further anteriorly relative to the ilium.

The 12th neural arch reaches almost to the top of the spinal cord. The hypochorda is considerably smaller posterior to a point just behind the ganglion of the 14th spinal nerve than further anteriorly.

There is little change in the epipubis, tendinous sheet, rods of Noble, and dorsal ligament. Between the rods of Noble just anterior to the proctodaeal glands is a portion of the mm. compressores cloacae. The relation of this portion of muscle to the circular muscle layer is not clear.

#### Stage 64, Female (figs. 20 and 21)

The tail myomeres are considerably shorter dorso-ventrally, the ventral portions having been reduced. The proctodaeal aperture is considerably further dorsal, particularly its upper rim. The proctodaeal region has enlarged considerably so that the dorsal ligament is anterodorsally directed, instead of first being directed dorsally then curving posterodorsally nearer the vertebral column. This enlargement of the proctodaeum is partly due to the decrease of the tail, which is also more widely separated from the cloacal extension, providing more space for the proctodaeum. Cloacal glands are now distinct on the lateral walls of the cloaca.

The rods of Noble are longer and more sigmoid, the shape in the adult female being quite similar.

The mm. pyriformes and mm. caudalipuboischiotibiales are now close to the vertebral column and the former are dorsal to the ischia. The hypochorda has become thinner posteriorly from a point just in front of the level of the ganglion of the 14th spinal nerve (see previous stage), and it is at this point that the mm. pyriformes approach the vertebral column most closely.

The anterior edges of the mm. compressores cloacae have advanced further anteriorly. Since this is particularly so dorsally, the anterior edges are now nearly vertical. The plexus sacralis is relatively further cephalad and the ilium projects somewhat more anteriorly.

The mm. compressores cloacae are well developed in the region between the rods of Noble. The isolation of their fibres from the circular and longitudinal muscle layers is considerable in this region. Near the posterior end of the pelvic girdle the longitudinal muscles of the gut attach to the girdle ventral to these muscles and approach the urostyle dorsal to them (cf. also figs. 22 and 23). Behind the pelvic girdle they do not extend far as well-differentiated entities.

The 12th neural arch reaches the level of the top of the spinal cord at this stage.

Figs. 20 and 21.

*Ascapus* larva, Stage 64, female (both figs. from one specimen).  
Pelvic region x 25.

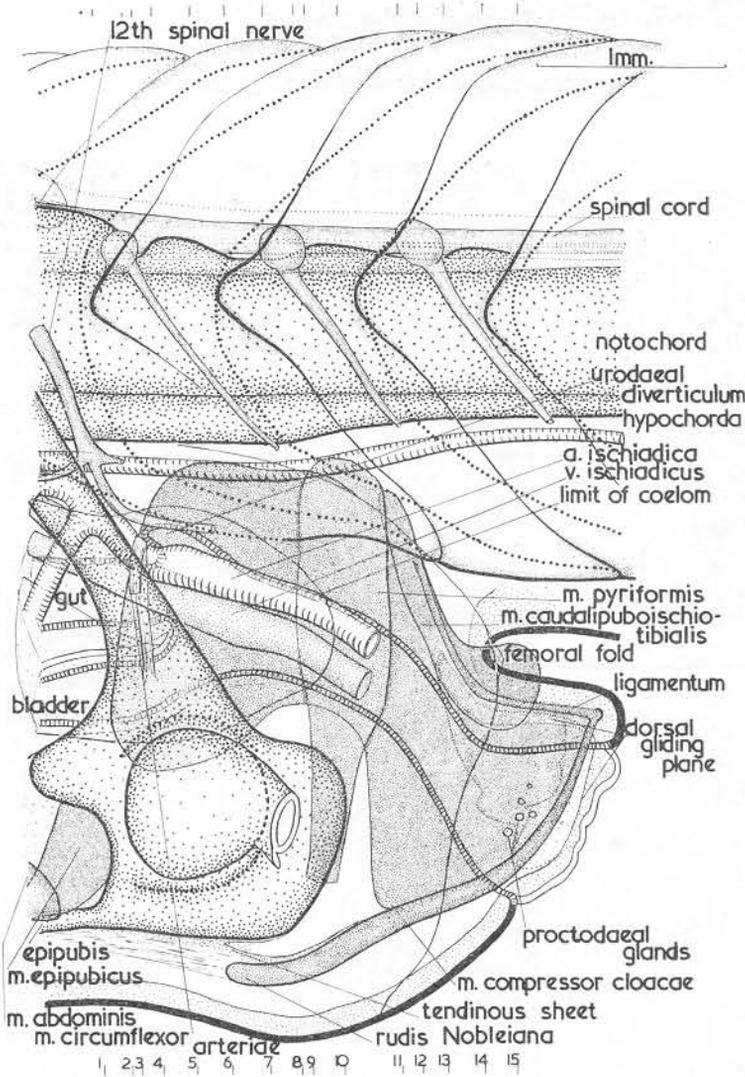


Fig. 20.

Norma lateralis sinistra.

Graphic reconstruction from transverse sections.

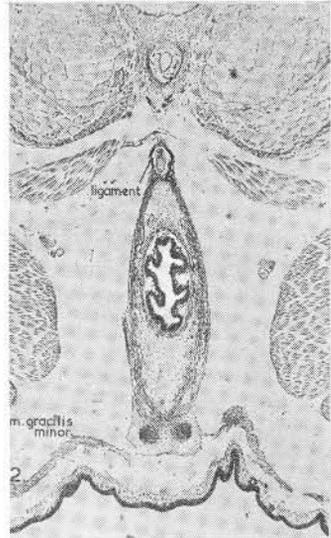
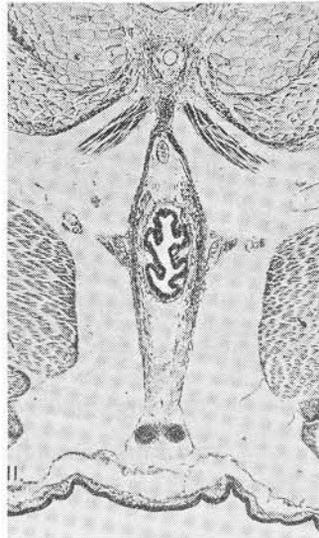
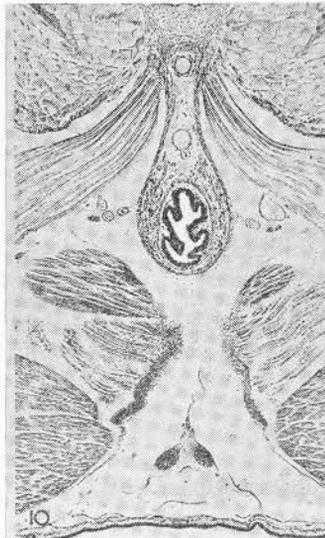
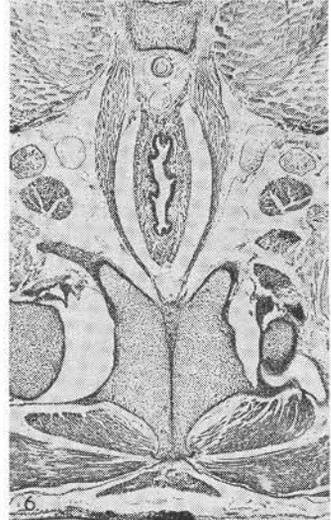
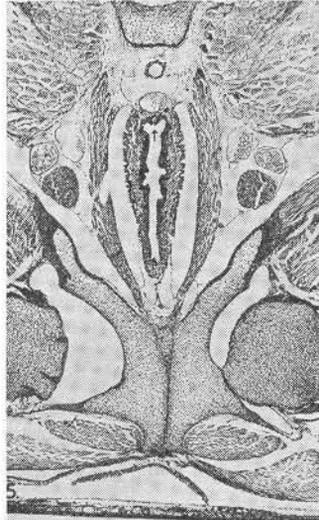
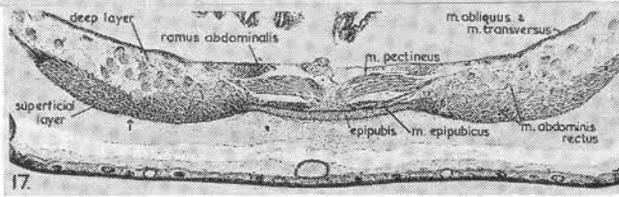
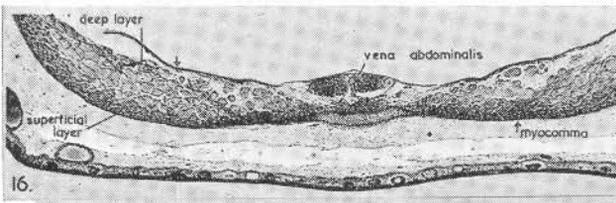
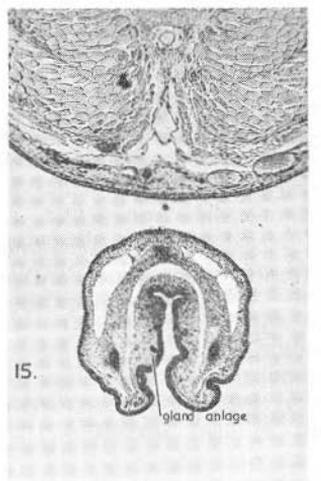
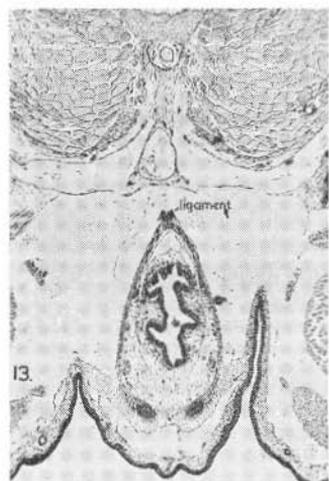
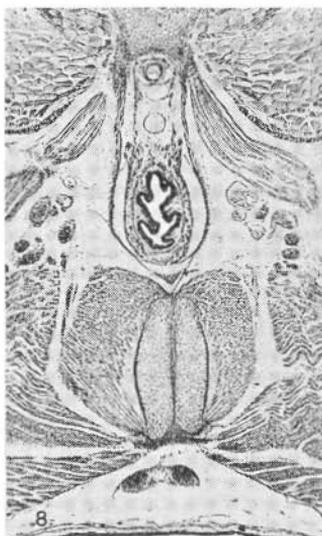
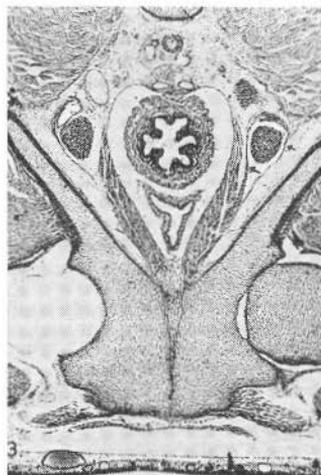
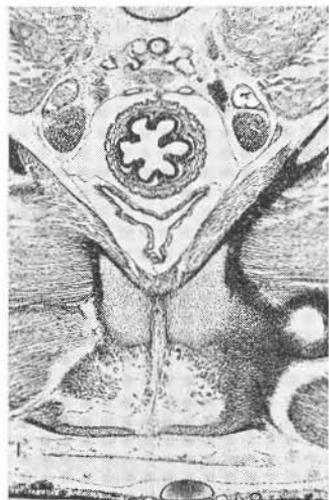


Fig. 21.

Norma posterioris. Photomicrographs of transverse sections.  
 (The number refer to levels in fig. 20.)

The arrows in the figure at levels 2 and 3 indicate the m. circumflexor arteriae,



at level 8 the arrow indicates the ventral longitudinal muscle cord, and the arrows at levels 16 and 17 indicate a myocomma between myomeres of the m. abdominis rectus.

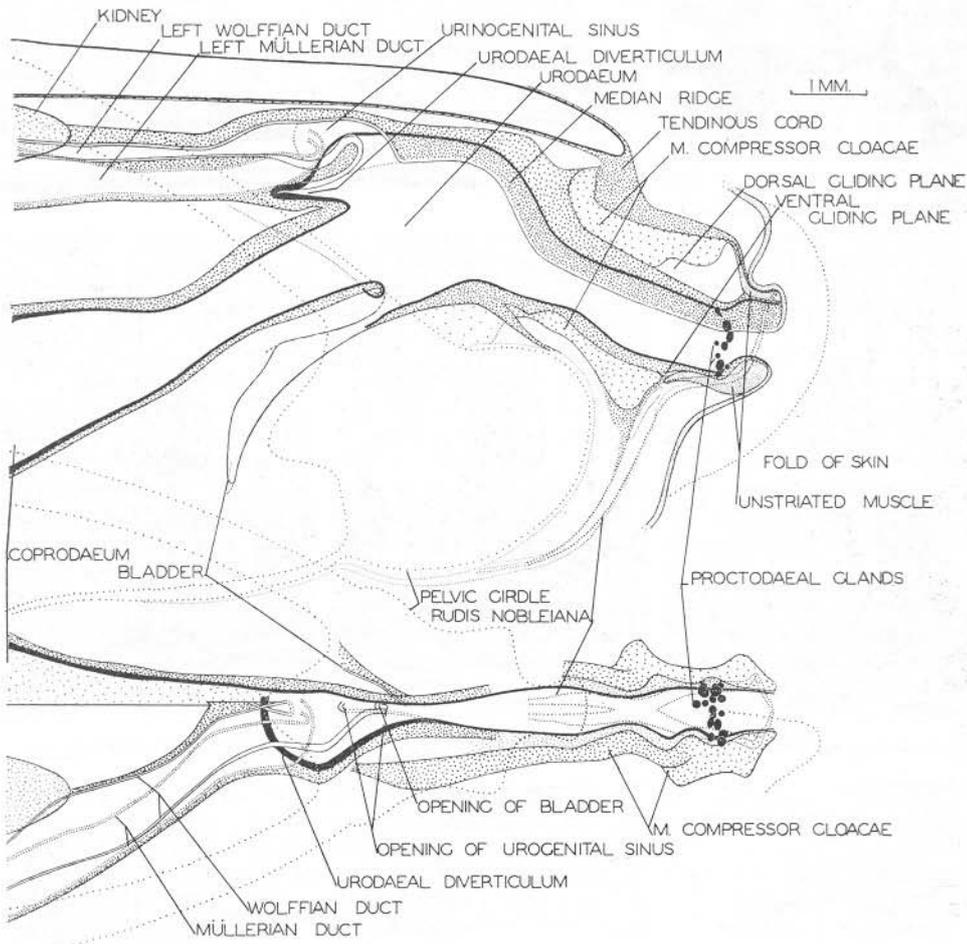


Fig. 22.

*Ascaphus* adult female. Pelvic region x 10.

Graphic reconstruction from transverse sections (simplified after van Dijk, 1955).  
 Norma lateralis sinistra (above) and Norma dorsalis (below).

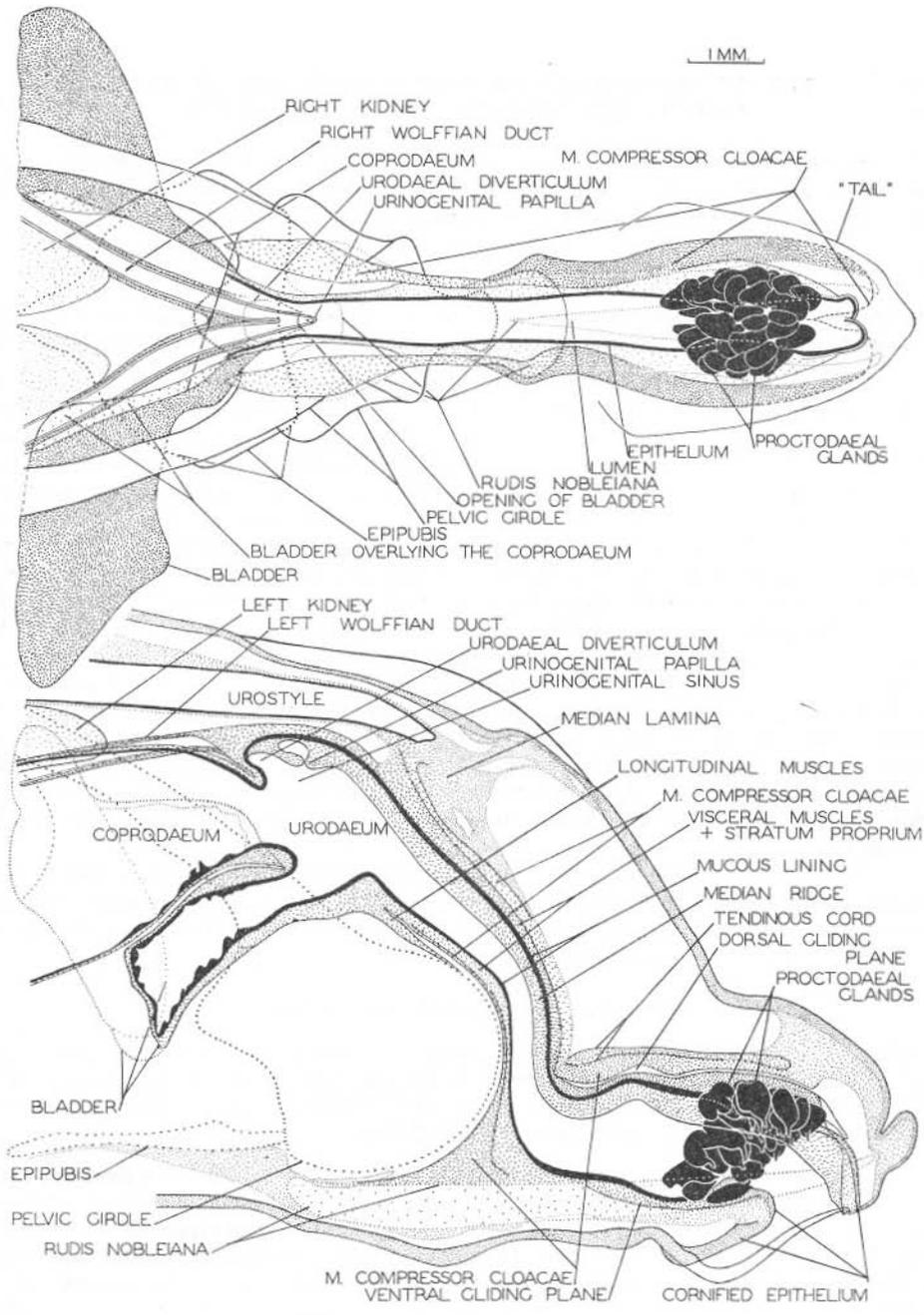


Fig. 23.

*Ascaphus* adult male. Pelvic region x 10.

Graphic reconstruction from transverse sections (simplified after van Dijk, 1955).  
 Norma lateralis sinistra (below) and Norma dorsalis (above).

## THE DEVELOPMENT OF THE PROCTODAEUM AND ASSOCIATED STRUCTURES IN *ASCAPHUS*

In the following descriptions reference will be made to illustrations of the stages described above; but not to the illustrations of the adult conditions (figs. 22, of the female, and 23, of the male).

### *Structures of Ectodermal Origin*

**Proctodaeal Invagination:** The relation of the proctodaeal invagination to the blastopore could not be determined from the limited *Ascaphus* material available.

**Proctodaeal Aperture:** The proctodaeal aperture in Stage 47 is situated on a prominence ventral and anterior to the tail fin, from which it is isolated by a notch (fig. 2). At Stage  $\pm$  50 the skin anterior to the proctodaeal aperture has expanded laterally and posterolaterally to form a ventral flap covering the hind-limb buds (figs. 3 and 4:2). The proctodaeal aperture is consequently present at the posterior tip of this ventral flap. The notch between the tail fin and the proctodaeal aperture is deeper at this stage, so that the aperture is posterior instead of ventral. At Stage 51 the proctodaeal aperture is directed posterodorsally from the tip of the ventral flap (fig. 5) and is triangular in shape with the base ventrally (cf. figs. 6:8 and 6:9, transverse sections through the ventral flap and the proctodaeum). There is little change up to Stage 56, figs. 8, 9, 10:17 and 10:18 illustrating the condition at Stage 56 well. At Stage 58 the ventral flap has become smaller and the proctodaeal aperture is situated on a prominence which is dorsal to, and which projects further posterior than, the ventral flap (figs. 11 and 12:13—12:18). As this prominence corresponds to the "tail" or "cloacal extension" of the adult the proctodaeal aperture undergoes little further change.

**Proctodaeal-Urodaeal Junction:** The proctodaeal-urodaeal junction is not distinguishable during development, consequently the anterior extent of the proctodaeum must be considered as being at some level posterior to that part of the hind-gut distinguishable as urodaeum.

### *Structures of Endodermal Origin*

**Urodaeum and Coprodaeum:** The coprodaeum passes over into the urodaeum posteriorly, the latter being distinguishable as that part of the cloaca into which the excretory ducts, and later the bladder, open. The urodaeum posteriorly abuts on the ectodermal proctodaeum.

**Aperture of the Nephric Ducts:** At Stage 47 the urodaeum extends into the somatopleura surrounding the proctodaeum as is shown by the nephric ducts opening into a part of the gut which laterally abuts on the body wall (fig. 1, right side). At Stage 51 there is a single common nephric aperture at the tip of a well-marked ridge in the dorsal wall of the urodaeum, in a region within the coelom. The aperture of the nephric duct moves slightly caudad during subsequent development, changing from a level just behind the posteroventral edge of the 9th spinal myomere at Stage 51 to half-way across the 10th spinal myomere at Stages 56 and 58, reaching to just posterior

to the ventrolateral edge of the 10th spinal myomere at Stages 62—63 and 63. The nephric ducts shift dorsally during development as a consequence of a similar movement of the gut in this region.

*Urodaeal Diverticulum:* At Stage 62—63 a slight anterodorsal diverticulum has developed anterior to the excretory aperture, and this develops slowly in subsequent stages (figs. 16, 18, 20; compare figs. 21 : 3 and 21 : 4).

*Bladder:* The anlage of the bladder is present in Stage 51 as a thickening of the ventral wall of the urodaeum, joining the latter to the floor of the coelomic cavity (figs. 5 and 6:1—6:3). It does not involve endoderm (gut epithelium) at this stage. At Stage 56 (figs. 8, 9, and 10:3—10:6) the bladder has developed a lumen, this presumably being a diverticulum of the urodaeum (and hence presumably lined with endoderm) projecting into the previously solid mesodermal anlage. Anteriorly the bladder is free from the gut dorsal to it, and from the floor of the coelom ventral to it, and is externally and internally bifid with anterodorsally directed horns (figs. 9 and 10:3—10:5). Further posteriorly the bladder is attached to the floor and side-walls of the coelom by membranes (figs. 10:3—10:6). The bladder retains its connexions with the coelomic walls while it increases in size in subsequent stages. At Stages 58 the lumen of the bladder begins to show convoluted outlines anteriorly (fig. 12 : 2), by stage 64 the anterior region of the bladder shows many epithelial folds.

#### *Structures of Mesodermal Origin*

**Coelomic Cavity:** At Stage 47 the coelom does not extend dorsally as far posterior as the level of the aperture of the nephric ducts (fig. 1), while laterally it extends posteriorly a short distance beyond the anterior margins of the hind-limb buds (fig. 2). At Stage  $\pm$  50 the relations of the coelom to the tissue of the hind-limb buds is similar. The coelom may be seen to extend posteriorly to a level not far from the posteroventral edge of the 9th spinal myomere (fig. 3). At Stage 51 the coelom reaches to the level of the bases of the hind-limb buds and extends considerably posterior to the level of the aperture of the nephric ducts, reaching to the posteroventral edge of the 10th spinal myomere (figs. 5 and 6 : 5). The coelom extends further posteriorly at Stage 56, reaching a level well within the region of the bases of the hind limbs and slightly beyond the posteroventral margin of the 10th spinal myomere (figs. 8, 9, 10 : 9 and 10 : 10). At Stage 58 the posterior edge of the coelom is still approximately level with the posteroventral margin of this myomere, at Stages 62—63 and 63 it has moved posteriorly half-way towards the posteroventral margin of the 11th spinal myomere and has come to correspond in extent with the posterior limit of the pelvic girdle. At Stage 51 the coelom does not extend far into the tissue between the series of spinal myomeres of the two sides; at Stage 56 it extends approximately  $\frac{2}{5}$  of the way from the ventral limits of these myomeres to the vertebral column (cf. figs. 5 and 8, and 6:1—6:5 and 10:1—10:10). In later stages the coelom extends somewhat further upwards. Lateral to the spinal myomeres the posterior limit of the coelom lies close to, and nearly parallel with, the lateral margins of the myocomma between the 8th and 9th spinal myomeres, anterior to them at Stage  $\pm$  50, posterior to them at Stage 56 (cf. figs. 3 and 8).

**Splanchnic and Somatic Mesoderm:** Splanchnic and somatic mesoderm are continuous with each other at the posterior limit of the coelom and a sharp distinction cannot be made between them. Nevertheless examination of sections close to the posterior limit of the coelom (fig. 15) does reveal that their individuality might be represented in the muscle layers arising in association with them. Somatic striated muscles laterally are distinguishable from splanchnic unstriated muscles medially to them; whether this distinction is valid in the post-coelomic tissue, which contains both these types of muscles, is uncertain.

#### **Visceral Muscle Layers:**

*Circular Muscle Layer:* Posterior to the bladder in Stage 51 the circular muscle layer of the gut is complete for a short distance; further posterior it is incomplete ventrally although present dorsally almost as far caudad as the point at which the limb buds project from the abdomen (figs. 5, 6:5 and 6:6). Ventrally, laterally and dorsally the circular muscles extend into the tissue behind the coelom. At Stage 56, and subsequent stages, the relations are little changed (figs. 10:6—10:13); the circular muscle layer is still complete behind the coelom although the latter extends further posterior than in earlier stages; the dorsal part of the circular muscle layer still extends to approximately the same level.

*Longitudinal Muscle Layer:* The longitudinal muscle layer of the gut is not as distinct as the circular layer at any level of any stage. This is partly due to the circular muscle layer being more easily distinguished by virtue of its arrangement being more easily detected in transverse sections; but the longitudinal layer does seem to be less developed, particularly posteriorly. The longitudinal muscle layer, in the early stages, is distinguished also from the coelomic epithelium only with difficulty, while posterior to the coelom it is often so close to the mm. compressores cloacae as to be difficult to detect. At Stage 56 the longitudinal muscle layer cannot be detected with certainty posterior to the coelom. At Stage 58 the longitudinal muscle layer is detectable dorsolateral to the gut (see figs. 12:11 and 12:12) almost to the level of the posterior tip of the ventral flap. At Stages 60 and 62—63 the fibres are detectable dorsolateral to the gut as far posterior as just anterior to the cloacal aperture (figs. 13 and 14:3, cf. 14:4; 16 and 17:2, cf. 17:3). At Stages 63 and 64, particularly the former, there are suggestions of the longitudinal layer extending further ventrally at the corresponding levels. The situation is complicated in these stages by increasing development of a gliding plane dorsal to the cloaca and apparently between the circular and longitudinal muscle layers. At Stage 58 there are paired concentrations of longitudinal muscle fibres between the gut and the pelvic girdle near the posterior edge of the latter (figs. 12:8 and 12:9). At the same level longitudinal muscle fibres turn posterodorsally dorsolateral to the gut and approach the vertebral column. These concentrations of longitudinal muscle fibres dorsal and ventral to the gut become better developed in subsequent stages until in Stage 64 it is seen that a substantial proportion of the longitudinal fibres attach to the urostyle above and the pelvic girdle below (cf. figs. 22 and 23). At Stage 64 the longitudinal muscle layer ventral

to the gut forms paired concentrations as in earlier stages, but these combine posteriorly to form a single dense cord triangular in cross-section (apex downwards) before attaching to the pelvic girdle (figs. 21 : 6 and 21 : 9). There are only slight indications of the paired origin of this cord except at the point of attachment to the girdle. The cords of longitudinal muscle fibres passing posterodorsally to the urostyle appear to give way further posteriorly to fibres passing anterodorsally from the post-pelvic gut (cf. figs. 17 : 1 and 17 : 2; 21:7—21:9).

#### **Anlagen of Pelvic Limbs and Girdle:**

*Pelvic Limbs:* The anlagen of the hind limbs develop ventral to the spinal myomeres on either side of the gut, and anteriorly overlap the coelom slightly (fig. 2, Stage 47). The anlagen bear similar relations to the gut and the coelom at Stage  $\pm$  50. At this stage the anlagen may be seen to be closely associated with the ventral limits of the 9th and 10th spinal myomeres, being just beneath the 10th and a caudad projection of the 9th myomere. Lateral to these spinal myomeres a dorsolateral projection of each anlage, representing the region of the ilial wing, may be seen on either side. Hind-limb buds are present as posterior projections of the hind-limb anlagen from the posteroventral aspect of the abdomen. The hind-limb buds are covered ventrally by the ventral skin flap (figs. 3 and 4 : 2). At Stage 51 the limb anlagen are no longer associated with the 9th spinal myomeres, as the caudad projections of the latter have disappeared. The dorsal limits of the bases of the hind-limb buds are at the level of the ventral margins of the myocommata between 10th and 11th spinal myomeres (fig. 5). At Stage 56 the base of the hind limb of each side reaches the ventral aspect of the 11th spinal myomere posterodorsally (fig. 8). The *m. pyriformis* and the *m. caudalipuboischiotibialis* project into the limb posterior to the ventral margin of the myocomma between the 10th and 11th spinal myomeres.

*Pelvic Girdle:* The pelvic girdle develops from paired chondrifications in a sheet of dense connective tissue which extends between and over the ventral surfaces of the spinal myomeres, just anterior to the point at which the gut passes posteroventrally through this sheet (figs. 3, 4 : 1 and 4 : 2). Extensions of this sheet laterally constitute the anlagen of the ilia and their muscles (Stage  $\pm$  50, figs. 3 and 4 : 2; Stage 51, figs. 5 and 6 : 4). Paired chondrifications develop in this connective tissue and approach each other at Stage 56, the gut being lifted above the region of their approximation (figs. 8, 9 and 10:3—10:7). By Stage 58 the halves of the pelvic girdle are separated posteriorly only by a narrow layer of dense connective tissue, while anteriorly they are still well separated (figs. 12 : 8 and 12 : 3). By Stage 60 the halves of the pelvis have fused posteriorly. The anlagen of the iliac regions of the pelvic girdle are directed towards the anterior tips of the lateral margins of the myocommata between the 10th and 11th spinal myomeres. This is seen at Stage  $\pm$  50 (fig. 3) and Stage 51 (fig. 5). When the pelvic anlagen have chondrified (Stage 56) the cartilaginous ilia lie nearly horizontally (in the transverse plane) with the ends turning dorsally (figs. 8, 9, 10 : 6 and 10 : 7). Dense connective tissue continues from the tip of each ilium towards the posterior bay in the lateral face of the

10th spinal myomere. At Stage 58 this bay is approached by the cartilaginous ilium, while the dense connective tissue at the tip of the ilium extends into the myocomma between the 10th and 11th spinal myomeres in a notch in this bay (fig. 11). The ilium is somewhat closer to the notch, which has deepened, at Stage 60 (fig. 13); while at Stage 62—63 the ilium enters the further enlarged notch (fig. 16). The ilia take in progressively more nearly ventro-dorsal positions (in the transverse plane) by addition laterally of dorsally directed portions, and by reduction in width and ventral extent of the spinal myomeres separating the ventral parts of the ilia (cf. figs. 10 : 7, 12 : 5, 21 : 2 and 21 : 3). From a condition where the ilia and the ventral parts of the pelvic girdle lie in the same transverse plane (Stage 56, figs. 8 and 9) there is progressive change in the relative anterior-posterior positions of the ilia and the ventral parts of the girdle. The ilia rotate anteriorly as they penetrate between the 10th and 11th spinal myomeres while the anterior limit of the ventral parts of the girdle remains more or less constant with reference to the spinal myomeres, the posterior limit, however, steadily extends backward from Stage 62—63 to Stage 64 (figs. 16, 18 and 20).

*Epipubis*: Paired chondrifications develop in the connective tissue anterior to the halves of the pelvic girdle, and are fused to the latter at Stage 58 (figs. 11 and 12:1—12:4). These cartilages are halves of the epipubis and have fused together anteriorly by Stage 60 (fig. 14 : 2). Incomplete fusion of the halves of the epipubis posteriorly results in the presence of a posterior notch in the epipubis of the adult (cf. Stage 64, figs. 21 : 16 and 21 : 17 with 21 : 1). The state of the epipubis anlagen at earlier stages (Stage 56) is best considered with the epipubic muscles.

*Rods of Noble and Interfemoral Ligament*: At Stage  $\pm$  50 strands of dense connective tissue extend from the ventromedial edges of the hind-limb buds along the sides of the gut close to the ventral flap (fig. 4 : 2). At Stage 51 the same situation is observed, the dense connective tissue disappearing posteriorly into less dense tissue lateral to the gut which extends dorsally to the posterior limits of the bases of the hind-limb buds (fig. 6 : 7). At Stage 56 the strands have developed into the rods of Noble, extending posteriorly from the ventromedial edges of the halves of the pelvic girdle to curve somewhat dorsally on either side of the gut behind the pelvic girdle (figs. 8, 9 and 10:6—10:16). A pair of posterodorsally directed cords of connective tissue above the gut ends close to the tips of the rods of Noble. These cords constitute the beginnings of a dorsal interfemoral ligament. The posterior tips of the rods of Noble and the dorsal interfemoral cords are enclosed in thickenings on either side of the gut. Posterior to this the gut is thin-walled (figs. 10:13—10:16). At Stage 58 this thin-walled part of the gut has disappeared and the rods of Noble and the dorsal interfemoral cords end in the rounded cloacal lips (figs. 11 and 12:15—12:18). The rods of Noble show little change in relation to neighbouring structures in subsequent stages. The anterior tips of the rods at Stage 60 end in a layer of connective tissue which stretches to the ventral surface of the epipubis. This tissue becomes progressively more distinct and at Stage 64 forms a well-marked tendinous sheet (cf. figs. 11, 13, 16, 18, 20 and 21:2—21:6). The dorsal interfemoral ligament at Stage 58 has attained a condition which remains up

to Stage 63. At Stage 64 the ligament slopes somewhat anterodorsally from the cloaca, whereas in Stages 58, 60, 62—63 and 63 the direction is more nearly vertical with the dorsal end turned posterodorsally (figs. 11 and 12:15—12:18; 13; 16 and 17:4—17:6; 18 and 19:2—19:4; 20 and 21:11—21:15). The ligament is a median structure ventrally, but is paired dorsally with the arms passing on either side of the caudal vein.

### Somatic Muscles:

*Abdominal Muscle Cords (Rectus Muscles):* At Stage 47 the abdominal musculature is represented by two cords which separate from the ventral surfaces of the myomeres of the tail (fig. 1) and pass anteroventrally and somewhat laterally. These muscle cords pass over the anterodorsal edges of the hind-limb anlagen (fig. 2), between these and the coelom. Reduction of yolk in the abdominal region results in a more cephalad and less ventrad direction of the abdominal muscle cords, as at Stage  $\pm$  50. The abdominal muscle cord of each side is clearly segmented at Stage  $\pm$  50 and the posterior-most segment is applied to the 9th spinal myomere (fig. 3). A small separate element is present on the inner face of the last abdominal myomere (9th, as it corresponds to the 9th spinal myomere) near its posterior end (figs. 3 and 4:1). There is a small gap between the posterior tip of the last (9th) abdominal myomere and the anterior tip of the hind-limb anlage (fig. 3). At Stage 51 the abdominal cords do not reach dorsally to the 9th spinal myomeres, but curve around the ventral surfaces of the 10th spinal myomeres and pass up the medial faces of these myomeres and the myomeres behind them. (The posterior limits of the cords could not be distinguished.) The 10th and 11th spinal myomeres are ventrally indented by the abdominal cords (figs. 5 and 6). Segmentation of the abdominal muscle cords is clearly shown at Stage 52, the relation of the abdominal myomeres to corresponding spinal myomeres also being possible (cf. description of Stage 52). The 1st abdominal myomere is absent, the 2nd rudimentary; the 9th meets the spinal myomeres. The n. iliohypogastricus, derived from the 8th spinal nerve and used for identifying myomeres, passes into the myocomma between the 8th and 9th abdominal myomeres. This situation is also found in Stage 56 (fig. 8), the 8th abdominal myomeres forming two broad ventral sheets separated by connective tissue (linea alba) while the 9th abdominal myomeres reach up to the ventral surfaces of the 10th spinal myomeres and curve medially round them. Slight ventral notching of the 10th spinal myomere is visible. (See figs. 9 and 10:1—10:6). Just anterior to the ventromedial edge of the myocomma between the 10th and 11th spinal myomeres of each side, the 10th abdominal myomere begins and stretches over the medial faces of the 10th, 11th and 12th spinal myomeres, just reaching to the 13th (figs. 8, 9 and 10, particularly 10:16). The 10th abdominal myomere is apparently the last, but the possibility must be borne in mind that this late-developing segment may derive material from more than one metamere without a myocomma developing between the fibres derived from each. At Stage 58 the 9th and 10th (?+) abdominal myomeres of each side have degenerated, the latter to such an extent that muscle fibres are no longer distinguishable. The remnants of these myomeres are associated with the medial aspect of the

ventrolateral edge of the 10th spinal myomere in much the same position as that occupied by the 9th abdominal myomere at Stage 56 (figs. 11 and 12:1—12:5). At Stage 60 the dorsal part of the degenerated myomeres is absent and the remnants still present do not reach as far as to the spinal myomeres (fig. 13). In subsequent stages these abdominal myomeres are absent. At Stage 58 each 8th abdominal myomere attaches to the epipubic cartilage of that side (figs. 11 and 12 : 1); at subsequent stages these myomeres approach each other more closely anterior to the epipubis, but show little other change in their relations to the latter (figs. 14 : 1 and 14 : 2; 21 : 16 and 21 : 17). At Stage 58 a superficial layer of secondary muscle develops on the ventral surface of the abdominal myomeres. The fibres are distinguishable by their more basophilic staining properties, characteristic of undifferentiated muscle, and by the oblique direction of the fibres in transverse sections (fig. 12 : 1). There is increasing development of this superficial layer in subsequent stages (figs. 14 : 1 and 14 : 2; 21 : 16 and 21 : 17).

*Obliquus and Transversus Muscles:* At Stage 58, while the 9th and 10th abdominal myomeres degenerate, differentiation of the obliquus and transversus muscles begins between the remaining abdominal myomeres ventrally and the spinal myomeres dorsally (fig. 12 : 1). There are no apparent indications of the origin of the obliquus and transversus muscles from either the remaining abdominal or the spinal myomeres.

*Epipubic Muscles and Anlagen of the Epipubis:* At Stage 56 the mm. epipubici may be identified as two widely-separated myogenic masses which anteriorly are in contact with the ventromedial edges of the 9th abdominal myomeres, extending along these as far as the myocommata between these myomeres and the 8th abdominal myomeres. Ventral to these developing muscles are the more diffuse anlagen of the epipubis, and beneath these, sheets of connective tissue (figs. 8, 9 and 10:1—10:4). The anlagen of the epipubis are posteriorly separate from the halves of the pelvic girdle, being connected to them by thin vertical strands of connective tissue (fig. 10 : 4); the mm. epipubici posteriorly reach to the pelvic girdle. The anlagen of the epipubis are derivatives of the connective tissue between and beneath the 9th abdominal myomeres, the two anlagen being separate from one another as well as from the pelvic girdle (as mentioned above). The connective tissue in which these anlagen arise might possibly be considered as linea alba although the fibres continue laterally around the abdominal myomeres and are not confined between them. The relations of mm. epipubici show that they should be regarded as secondary derivatives of the abdominal myomeres, either the 8th or 9th or both.

*Femorococcygeal and Crurococcygeal Muscles:* The tissue from which these muscles (mm. pyriformes and mm. caudalipuboischiotibiales respectively) arise is that at the posterior limit of the hind-limb bases (fig. 6 : 6). This tissue is associated with the medial faces of the abdominal muscle cords, where these lie on the anterior tips of the medioventral faces of the 11th spinal myomeres (Stage 51, fig. 5). At Stage 56, when the muscles are well differentiated, the same relations are preserved. It is seen at this stage that the part of the abdominal muscle cord with which the muscles of each side are associated is the 10th (?+) abdominal myomere, nearer its posterior end (figs. 8, 9 and 10:9—10:12). At Stage 58 the degeneration of the

abdominal muscle cords has resulted in the association of the mm. pyriformes and mm. caudalipuboischiotibiales with the medioventral faces of the 11th spinal myomeres. The lengthening of the mm. pyriformes and mm. caudalipuboischiotibiales results in their association with the medial faces of the 11th, 12th and 13th spinal myomeres at Stage 62—63, the latter of these muscles just reaching to the 14th spinal myomeres (figs. 16 and 17:3—17:6). At Stages 63 and 64 the mm. pyriformes extend to, and a short distance over, the medial faces of the 14th spinal myomeres (figs. 18 and 19:1—19:3; 20 and 21:8—21:13), while the mm. caudalipuboischiotibiales just reach to these myomeres.

*Femoropelvic and Cruropelvic Muscles:* These muscles develop from the myogenic material around the pelvic girdle and femur. At Stage 56 the mm. pectinei originate entirely on the halves of the pelvic girdle. At Stage 58 an additional origin is developing on each side, a slip of each m. pectineus passing mediad over the m. epipubicus in the direction of the medial edge of the epipubic cartilage of that side (fig. 12:1). At Stage 64 the epipubic origins are well developed (fig. 21:17).

*Coccygeopelvic Muscles:* The mm. coccygeoiliaci, which constitute in the adult the only muscles present between the pelvic girdle and vertebral column in the cloacal region (except the cloacal muscles), are not distinguishable at Stage 64. They are clearly derived from the spinal myomeres posterior to the ilia, the first of these being the 11th spinal myomeres. Comparison of their attachments to the urostyle in the adult with the origins of the mm. pyriformes suggests that the 11th, 12th and 13th spinal myomeres may contribute to the mm. coccygeoiliaci (cf. Stage 64, fig. 20).

*Cloacal Compressor Muscles:* At Stage 56 the mm. compressores cloacae may be distinguished in the lateral walls of the coelomic cavity, and extend from near the posterior tip of the halves of the pelvis to the end of the coelom and beyond to a level just posterior to the bases of the hind limbs. Ventrally the muscles are attached anteriorly to the posterodorsal tips of the halves of the pelvis, and posteriorly to the rods of Noble; dorsally the muscles extend over the medial faces of the 10th (?+) abdominal myomeres, separated by these myomeres from the medial faces of the 11th, 12th and 13th spinal myomeres. The mm. pyriformes and mm. caudalipuboischiotibiales are interposed between the mm. compressores cloacae and the abdominal myomeres just behind the coelom (figs. 8 and 10:8—10:12). At Stage 58 the mm. compressores cloacae have extended anteroventrally on either side of the opening of the bladder, and posteriorly to the tips of the rods of Noble and the dorsal interfemoral ligament (figs. 11 and 12:5—12:18). The mm. compressores cloacae extend further dorsally and the anterior edges come to acquire, in subsequent stages, (figs. 13, 16, 18, 20 and 21:2—21:15), a more vertical position by anterodorsal expansion.

*Arterial Circumflexor Muscles:* At Stage 56 slips of muscle continuous with the mm. compressores cloacae near the latter's anterior edges extend anterolaterally, a dorsal slip extending along the ventral half of the medial face of the 10th abdominal myomere of each side, and a ventral slip curling around the arteria ischiadica and the nervus ischiadicus at the point at which the artery twists caudolaterad over the nerve (figs 8 and 10:8). At Stage 58 the dorsal slips of muscle cannot be distinguished. The ventral slip of muscle, or m.

circumflexor arteriae, of each side is more distinct at Stage 58 and subsequent stages (figs. 11 and 12 : 7; 13; 16; 18; 20 and 21:1—21:3). At Stage 60 the point of attachment of each m. circumflexor arteriae to the corresponding m. compressor cloacae is close to the ventral attachment of the latter to the pelvic girdle (fig. 13), and this allows the m. circumflexor arteriae to shift antero-laterally relative to the anterior edge of the m. compressor cloacae during subsequent development. At Stage 64 there are indications of the continuation of the lateral-most edge of the m. circumflexor arteriae medioventrally, so that a complete loop is formed around the artery and nerve (figs. 21 : 2 and 21 : 3). The arteria ischiadica is clearly occluded by the m. circumflexor arteriae in the sections of all stages in which it appears. Whether this was true of the living specimens, and if so to what degree, is impossible to state (cf. figs. 21 : 1 and 21 : 4 with figs. 21 : 2 and 21 : 3).

*Unstriated fibres of the mm. compressores cloacae:* At Stage 62—63 a cord of tissue is present between the rods of Noble just anterior to the cloacal orifice (fig. 17 : 3). This cord is separated from fibres of the circular and longitudinal muscle layers. In Stage 63 (figs. 19:1—19:4) the cord is visible in the same position, clearly separate from the circular and longitudinal muscle layers anteriorly (fig. 19 : 1), less clearly further posteriorly. At Stage 64 the cord is well separated from these layers (fig. 21 : 14 and also 21 : 15). This cord occupies the position of unstriated muscle fibres occurring in the adult, particularly the female.

**Cavernous Tissue:** The cavernous tissue characteristic of the cloaca of the adults, female as well as male, has not developed by Stage 64, but there are indications of rudiments. At Stages 62—63, 63 and 64 there are distinct deeply-staining regions lateral to the rods of Noble, anterior to, and anterolateral to, the cloacal orifice (figs. 17 : 3 and 17 : 4; 19:1—19:4; 21 : 14 and 21 : 15).

**Lymph Spaces:** At Stage 51 there are no lymph spaces associated with the pelvic region. At Stage 56 lymph spaces have developed under the skin of the legs and extend ventrally beneath the pelvic girdle to a point anterior to the ilia (fig. 10 : 5). Medially the lymph spaces extend both caudad and cephalad from the bases of the hind limbs above the level of the rods of Noble. Lymph spaces have begun to separate the rods of Noble from the ventral skin, but are absent in the ventral flap of skin (figs. 10:7—10:10). At Stage 58 lymph spaces extend ventrally as far anterior as the epipubis (fig. 12 : 2), posteriorly small lymph spaces have begun to develop ventral to the rods of Noble posterior to the pelvic girdle (figs. 12 : 9 and 12 : 10). At this stage lymph spaces are also extending into the cloacal "extension" dorsolaterally (figs. 12:15—12:17). At Stage 62—63 there are lymph spaces ventral to the post-pelvic gut and these extend posteriorly as small ventrolateral spaces in the cloacal "extension". The lymph spaces dorsolaterally situated in the cloacal "extension" are well developed at this stage (figs. 17:1—17:6). The lymph spaces present at Stage 62—63 are enlarged at Stages 63 and 64 with little change in relations.

*Gliding Planes:* At Stage 58 a gliding plane develops dorsally between the gut epithelium and the mm. compressores cloacae near the cloacal aperture (figs. 12 : 16 and 12 : 17). At Stage 60 ventral gliding planes develop ventrolaterally between the gut epithelium and the mm. compressores cloacae (figs. 14 : 3 and 14 : 4). Both dorsal and ventral gliding planes develop closer to the

mm. compressores cloacae than to the gut epithelium. At Stage 62—63 the dorsal gliding plane has enlarged until it reaches the ventral skin posterolateral to the cloacal orifice (figs. 17:4—17:6). The ventral gliding planes remain much less distinct than the dorsal one, but the former do extend sufficiently far towards each other to be considered as a single ventral gliding plane. At Stage 63 (figs. 19:1—19:4) a strip of tissue lateral (distal) to the dorsal gliding plane has developed and extends around the gut ventrally (fig. 19:3), while the circular muscle layer may be detected dorsally between the gliding plane and the gut epithelium. This suggests that the lateral slip of tissue represents the longitudinal muscle layer. Stage 64 shows little further change in the gliding planes.

#### *Epithelial Structures*

**Cloacal Glands:** At Stage 62—63 thickenings have developed in the epithelium just within the cloacal orifice. These are the beginnings of cloacal glands (figs. 17:4 and 17:5). At Stage 63 the gland primordia are distinguishable from the general cloacal epithelium (figs. 18, 19:3 and 19:4). At Stage 64 they are considerably more developed and are raised from the cloacal epithelium, to which they are attached by narrower stalks (figs. 20 and 21:15). Lumina have not yet developed.

**Horny Spines:** There is no evidence of the horny spines, which occur on the proctodaeal epithelium of the adult male, at Stage 62—63 (the oldest male larva reconstructed).

### THE PROCTODAEUM AND ASSOCIATED STRUCTURES IN ADULT ANURA

In the following attempt at relating adult conditions in *Ascaphus* and other genera to larval conditions in *Ascaphus*, reference will also be made to larval conditions in *Xenopus laevis*, *Bufo angusticeps* and *Bombina pachypus*.

#### *Structures of Ectodermal Origin*

**Proctodaeal Invagination:** Whether the proctodaeum of the adult is, or is not, a persistent blastopore could not be established from the limited *Ascaphus* material available; in *Bufo angusticeps*, however, it was possible to show that the blastopore had closed before the proctodaeum was formed.

**Proctodaeal Aperture and Proctodaeal-Urodaeal Junction:** In *Bufo angusticeps* there is a reduction of the proctodaeum, during development, similar to that observed in *Ascaphus* between Stages 56 and 58, the portion disappearing in *Bufo* being that traversing the ventral fin. Since the dorsal interfemoral ligament and the rods of Noble of *Ascaphus* surround the cloaca close to its orifice, and a homologous ligament (see below) bears the same relations to the cloacal orifice in *Bufo* and also *Rana*, the proctodaeal apertures of *Ascaphus*, *Bufo* and *Rana* may be considered homologous (cf. figs. 20, 22 and 23). Equivalent losses of the proctodaeum would thus appear to have occurred in these genera during development. The proximity of the interfemoral ligament to the cloacal orifice makes it probable that the proctodaeum extends anteriorly beyond the ligament in *Ascaphus*, *Bufo* and *Rana*, and that the extra length of

Figs. 24—27.  
*Ascapus* adult males (each fig. from a different specimen).

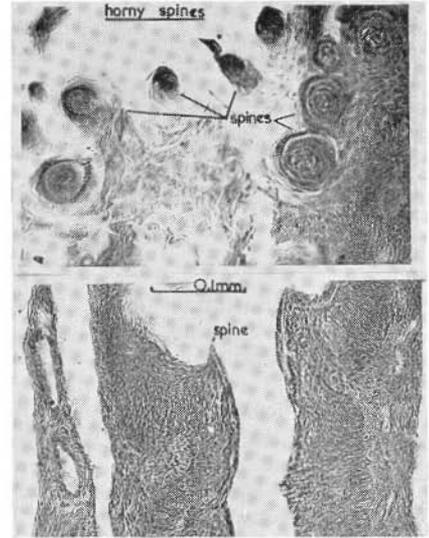
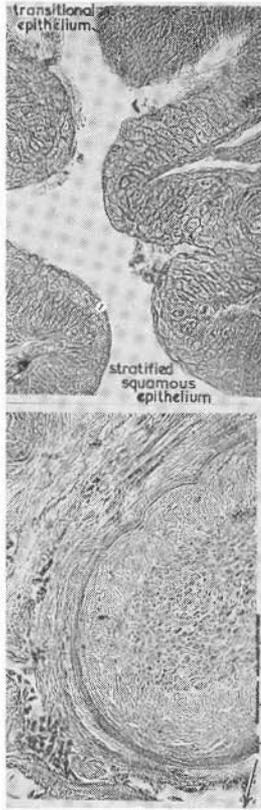
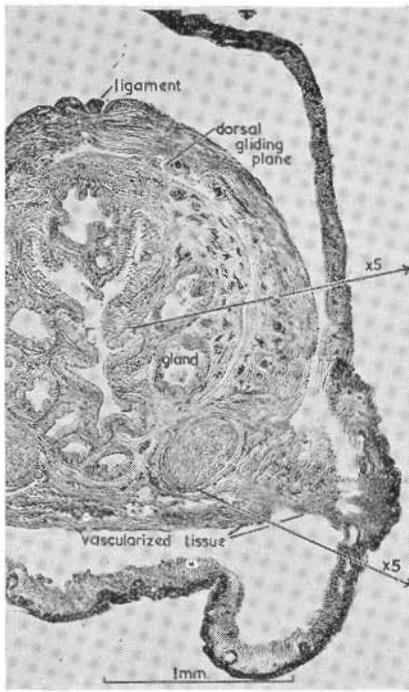


Fig. 25.  
 Photomicrographs of transverse and longitudinal sections of horny cloacal spines x 125, taken from longitudinal (tangential) and transverse sections of the cloacal wall.

Fig. 24.  
 Photomicrograph of a transverse section through the cloaca x 25 with enlargements (x 125) of the junction of transitional and stratified squamous epithelia and of a rudis *Nobleiana*.

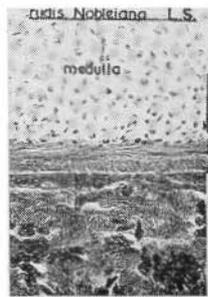


Fig. 27.  
 Photomicrograph of a longitudinal section of a rudis *Nobleiana* x 125, taken from a longitudinal section of the cloaca.

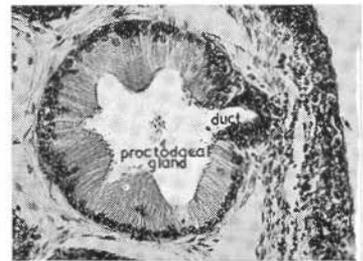


Fig. 26.  
 Photomicrograph of a cloacal gland x 125, taken from a transverse section of the cloaca.

Figs. 28—30.

*Ascaphus* adult female. Cloaca (norma posterioris).

Photomicrographs of transverse sections, (figs. 29 and 30 are enlargements from fig. 28).

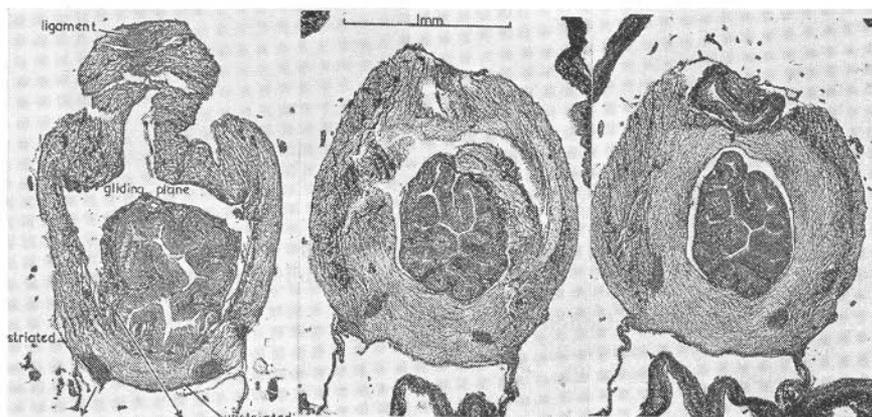


Fig. 28.  
Cloaca x 25.

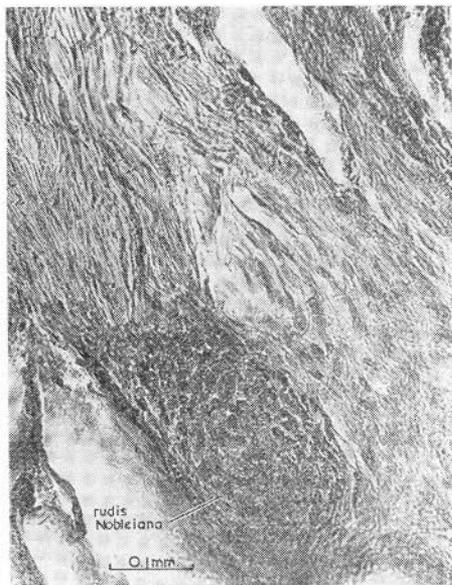


Fig. 19.  
Rudis Nobleiana and associated  
muscles x 250.

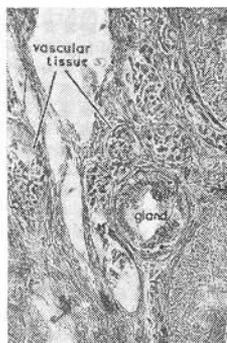


Fig. 30.  
Cloacal gland and  
vascular tissue.

Figs. 31 and 32.

*Rana* and *Ascaphus* adult females. Oviducts in the pelvic region.



Fig. 31.  
*Rana* oviduct sectioned tangentially showing oviducal glands x 25.

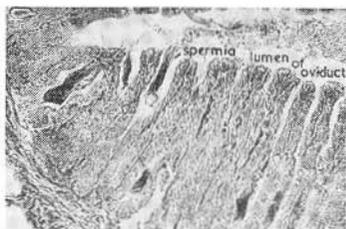


Fig. 32.  
*Ascaphus* oviduct sectioned transversely showing spermia x 125.

gut in *Ascaphus* may therefore be either the posterior part of the urodaeum, or the anterior part of the proctodaeum, or both.

**Proctodaeal Glands:** Dermal glands opening into the cloaca are present in both male and female adult *Ascaphus*, somewhat proximal to the rods of Noble and the dorsal interfemoral ligament (figs. 22, 23, 24, 28 and 30). The ducts of these glands open into a part of the cloaca lined with stratified squamous epithelium. Just anterior to the openings of the ducts the epithelium changes to a transitional epithelium with mucus cells, the line of contact between the two types of epithelium having, like the series of glands, a dorsocaudad slope. (Fig. 24, a transverse section through this region, shows the transitional epithelium dorsally, the stratified squamous ventrally.) this epithelial change may represent a functional change only, or may also represent the proctodaeal-urodaeal junction. If the epithelial change does represent the proctodaeal-urodaeal junction then *Ascaphus* has a relatively longer urodaeum, rather than proctodaeum, than other genera. In any event, it is probable that the cloacal glands are derived from the proctodaeum, rather than the urodaeum, and may hence be referred to as proctodaeal glands. Similar glands, distinct from the neighbouring skin glands, are present in *Leiopelma* near the cloacal orifice.

**Horny Spines:** Posterior to the proctodaeal glands in the adult male *Ascaphus* the stratified squamous epithelium is raised into horny spines (fig. 25). Such horny structures are characteristic of epithelia of ectodermal origin.

#### *Structures of Endodermal Origin*

The junction of the urodaeum with the proctodaeum has been dealt with above.

#### *Structures of Mesodermal Origin*

**Coelomic Cavity:** The posterior extent of the coelomic cavity is much the same in *Ascaphus* as in other genera, the post-pelvic gut ("cloacal extension") of the former being extra-coelomic. The relations of the coelom to the gut are essen-

tially similar in *Ascaphus*, *Leiopelma*, *Xenopus* and *Rana* as may be seen in fig. 40 (except the section of *Xenopus*, which is too far posterior) and fig. 35 : 3 (*Xenopus*).

**Visceral Muscle Layers:** The circular and longitudinal muscle layers extend along the post-pelvic gut of *Ascaphus*, fibres of the circular muscle layer being distinguishable as far posterior as just anterior to the proctodaeal glands. The extent of the visceral muscle layers thus tends to confirm the localisation of the proctodaeal-urodaeal junction at the stratified squamous and transitional epithelial boundary. The longitudinal muscle layer posteriorly concentrates into dorsal and ventral pairs of cords (taeniae) which attach to the urostyle and pelvic girdle, respectively, in all the genera examined except *Xenopus*, in which dorsal cords are absent. (The relative shortness of the urostyle in *Xenopus* may account for the absence of the dorsal cords.) Some longitudinal fibres continue posteriorly, independent of the cords, except in *Xenopus* and *Leiopelma*. In all the genera examined (except *Xenopus*) the dorsal pairs of longitudinal muscle cords are essentially similar, whereas the ventral pairs show some differences. *Leiopelma* has cords rounded in cross-section; *Bufo* and *Bombina* have well-separated dorso-ventrally elongated cords. (Metamorphosing larvae of the latter two were examined.) The cords in *Rana* are similar to those of *Bufo* and *Bombina*, although somewhat less separated, while in *Xenopus* the cords are more elongated and scarcely distinguishable at two separate entities. In *Ascaphus* the ventral cords are fused into a single cord triangular in cross-section, although the attachments are clearly paired at Stage 64. Tendinous material may be associated with the longitudinal cords near their attachments, in *Xenopus* a sheet of such material lying on either side of the muscle cords while in *Rana* the muscle cords are separated by a fused pair of tendons (cf. ligamentum interfemorale below). (The tendons were taken to be part of the muscle cords in *Rana* in the earlier study, with the result that the erroneous conclusion, that the muscle cords were united, was reached. op. cit., p. 29.) Fig. 40 shows the longitudinal muscle cords in *Ascaphus*, *Leiopelma*, *Xenopus* and *Rana*.

**Pelvic Skeletal Structures:** The linea alba and interfemoral ligament are conveniently discussed with the pelvic girdle and the epipubis, and are therefore included in this section.

*Linea alba:* The 8th abdominal myomeres in *Ascaphus* are the last segments of the abdominal cord to occupy positions ventrolateral to the coelomic cavity, the 9th and 10th myomeres (?+), developing lateral to the coelom (fig. 8). The 8th abdominal myomeres approximate medially during later development, those behind them atrophy. It follows that the term linea alba should be applied only to the median ventral fascia as far as the posterior tips of the 8th abdominal myomeres. Further posteriorly the median ventral fascia between the subsequent pairs of myomeres is represented by the ventral, ventrolateral, and even the dorsolateral, walls of the coelom (cf. figs. 8 and 10). It is in, and just ventrolateral to, this part of the median ventral fascia that the epipubis, the halves of the pelvic girdle, and the interfemoral ligament (including the rods of Noble), develop (figs. 8 and 10).

*Epipubis:* The epipubis and the ilia extend into lateral continuations of the median ventral fascia. In *Leiopelma* the epipubis in the adult underlies the m. rectus abdominis entirely. In *Ascaphus* the m. rectus abdominis attaches more

to the lateral aspects of the epipubis than the dorsal, and is not markedly underlain by the cartilage. This is apparently even more so in *Xenopus*.

*Pelvic Girdle:* The depth of the symphysis between the two halves of the pelvic girdle results in a dorsal displacement of the cloaca above it. As the tail atrophies during metamorphosis, the cloaca, which had opened at the base of the fully-formed tail, becomes shorter (very little so in *Ascaphus*, particularly the male). The posterodorsal position of the cloacal aperture in adult *Anura* is thus brought about by the shape of the pelvic girdle, the atrophy of the tail, and by the reduction in relative length of the proctodaeum (and possibly of the posterior part of the urodaeum).

*Interfemoral Ligament:* The septum interfemorale in *Rana* extends medially along the ventral, posteroventral, and posterior edge of the pelvis, from the anteroventral region of the pelvis to the cloaca. Along the length of this septum a strong band of connective tissue, the thickened lateral edges of which contain collagen fibres, extends in *Rana*, *Bufo*, *Bombina*, *Xenopus* and *Leiopelma*. This band of connective tissue has been termed ligamentum interfemorale in view of its relation to the septum termed septum interfemorale in *Rana*. The rods of Noble (and the dorsal ligament — see below), together with the tendinous sheet stretching between these rods and the epipubis are the homologues in *Ascaphus* of the ligamentum interfemorale of the other genera. In *Bufo* the (ventral) interfemoral ligament dorsally extends along the posteroventral edge of the cloacal sphincter muscle, then divides into cords on either side of the cloaca and its muscles. In *Bombina* the ligament forks dorsally as in *Bufo* (and *Ascaphus*), and this condition is clearly the primitive one. In *Xenopus* the ligament in the larva (fig. 36) has similar relations to the gut and is similar in form to the ligament in *Bufo* and *Bombina*, except that the dorsal ends are thickened. In the adult *Xenopus* the dorsal ends of the ligament are very much thickened and serve for the attachment of the mm. compressores cloacae to the pelvic girdle (figs. 35 : 3, 40 and 33). The staining techniques employed (cf. Techniques) showed that the dorsal thickenings in the adult *Xenopus* are of a ligamentous nature and do not consist of unstriated muscle as had previously appeared possible from their histology (van Dijk, 1955, p. 52). In *Rana* the ligament turns forward dorsally and forms a fused pair of tendons which attach to the pelvic girdle between the longitudinal muscle cords (fig. 40). At the point at which the ligament becomes a pair of tendons, the mm. graciles minores originate on the sides of the latter. In *Bufo*, *Bombina* and *Xenopus* the mm. graciles minores similarly originate on either side of the ligament, while in *Ascaphus* these muscles take origin from the homologous rods of Noble. The ligament is present in *Leiopelma* (as mentioned above), but the dorsal part and the mm. graciles minores were apparently removed when the specimen was skinned before sectioning. A short dorsal ligament occurs in *Bufo*, *Bombina* and *Xenopus* on the posterodorsal midline of the cloacal sphincter, much as in *Ascaphus*. It is absent in the adults of *Rana* and *Leiopelma*.

#### Somatic Muscles:

*Epipubic Muscles:* That the single m. epipubicus of *Xenopus* is formed from a pair of secondary muscles derived from the m. rectus abdominis, as in the case of the paired muscles of *Ascaphus*, is indicated by the conditions shown in fig. 37. A paired abdominal origin was suggested earlier (van Dijk, 1955, p. 11)

in contradiction to Ryke (1953, p. 54-55) who described a pelvic origin (presumably unpaired). That the m. epipubicus develops asymmetrically in *Xenopus* (as previously suggested, loc. cit.) is shown in figs. 34 and 35 : 1, and the relation of the muscle to the v. abdominalis is also shown in these figures and fig. 33. The presence of the vein on one side (right) may be responsible for the asymmetry. It would appear that the epipubis and epipubic muscles (or muscle) of *Ascaphus*, *Leiopelma* and *Xenopus* are perfectly homologous; but whether the m. epipubicus of *Xenopus* represents one or both of the mm. epipubici of *Ascaphus* and *Leiopelma* is not clear. The condition in *Xenopus* is

Figs. 33 and 34.

*Xenopus* young adult (both figs. from one specimen). Pelvic region x 25.  
Graphic reconstructions from transverse sections.

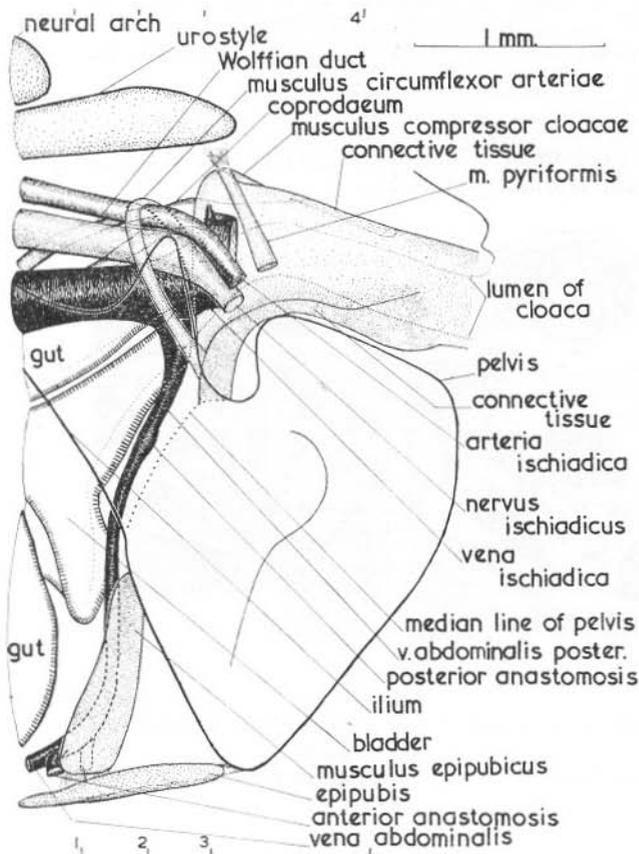


Fig. 33.  
Norma lateralis sinistra.

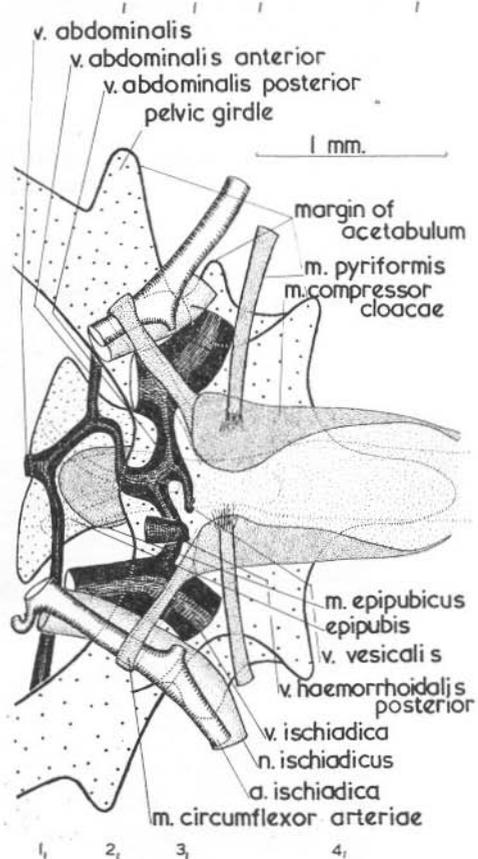


Fig. 34.  
Norma dorsalis.

clearly derived by specialization from a condition like that in the *Ascaphidae*. The presence of small cell-aggregations in *Bufo* tadpoles is similar sites to those at which the epipubis anlagen develop in *Ascaphus*, is noteworthy. In *Bombina* tadpoles a substantial cord of connective tissue passes from the linea alba to the pelvic girdle, from which it is isolated ventrally, in a position reminiscent of the m. epipubicus of *Xenopus*. These features of *Bufo* and *Bombina* might indicate that the epipubis was of wide distribution among primitive *Anura*.

*Cloacal Compressor Muscles*: Distinction may be made between three regions in these muscles, the anterior-most of which is dealt with separately in the next section under the name of Arterial Circumflexors. The other two divisions were termed mm. compressores cloacae and m. sphincter ani cloacalis by Gaupp (1904) (cf. van Dijk, 1955, p. 30). The m. sphincter ani cloacalis comprises that posterior part of the cloacal compressor muscles which is attached neither to urostyle nor pelvic girdle, but forms a complete ring around the cloaca. It is absent in *Leiopelma*, moderately developed in *Rana*, somewhat better in *Bufo* and very well developed by anterior extension in *Xenopus* and by posterior extension in *Ascaphus* (see fig. 33 for *Xenopus*). The whole post-pelvic part of the cloacal compressor muscles in *Ascaphus* actually represents the m.

Figs. 35—37.

*Xenopus* (each fig. from a different specimen). Photomicrographs of transverse sections x 25. Norma posterioris.

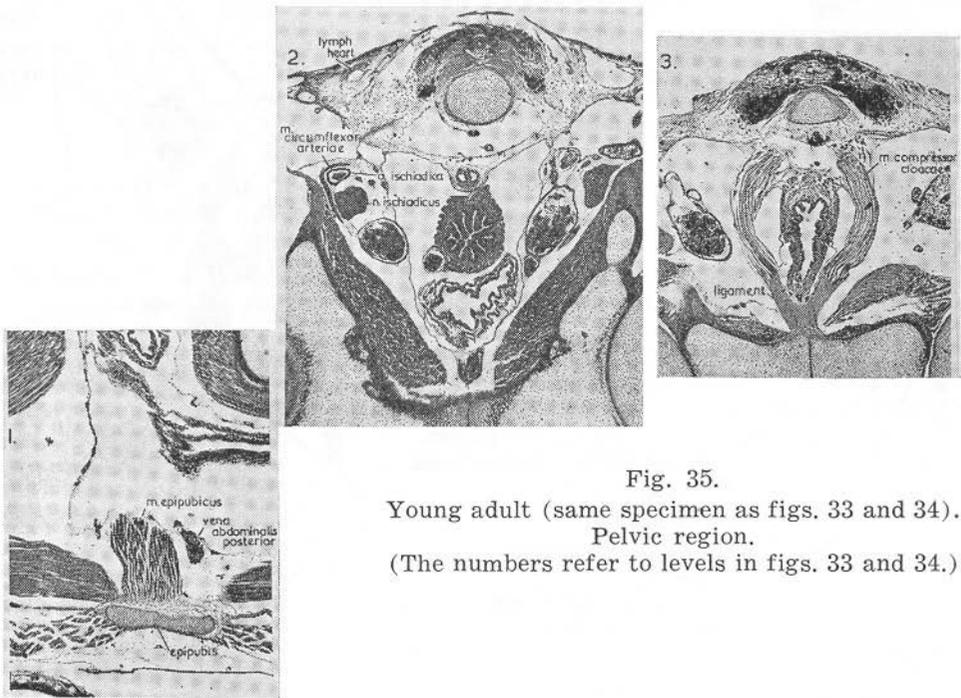


Fig. 35.

Young adult (same specimen as figs. 33 and 34).  
Pelvic region.

(The numbers refer to levels in figs. 33 and 34.)

sphincter ani cloacalis (cf. van Dijk, 1955, p. 31). The form of, and the degree of development of, the mm. compressores cloacae, the more anterior division of the cloacal muscles, is similar in *Ascaphus*, *Leiopelma*, *Rana*, *Bufo* and *Bombina*. In *Xenopus* these muscles have the typical form only as far as the tip of the urostyle, this extent constituting a very short length compared to other *Anura*. Behind this region in *Xenopus* the cloacal muscles are attached ventrally to the ligamentum interfemorale, but must be considered as m. sphincter ani cloacalis in view of the complete arch above the cloaca (figs. 33—35 and 40). In *Bufo* and *Bombina* the m. sphincter ani cloacalis attaches to the ligamentum interfemorale at the same level as that at which the mm. graciles minores originate from the posterolateral aspects of the ligament. This is similar to the situation in *Ascaphus* and in contrast to that in *Rana*.

Fig. 36.  
Larva. Cloacal region.

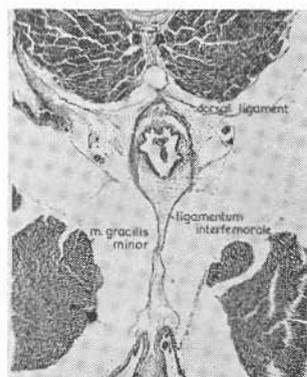
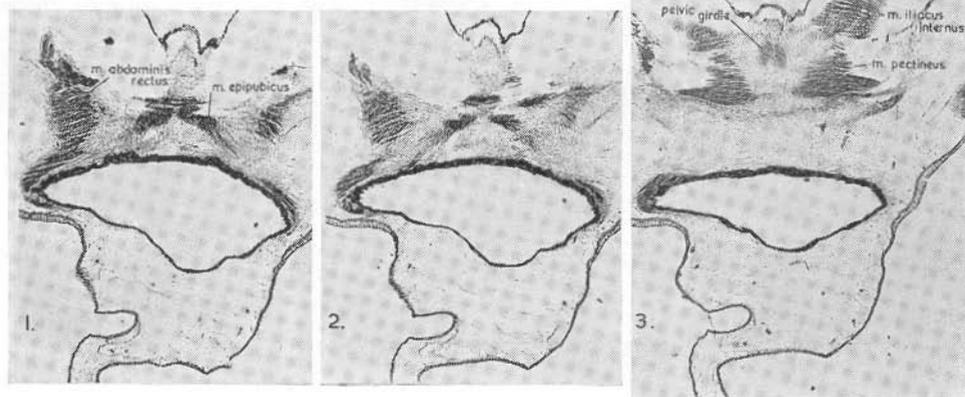


Fig. 37.  
Larva. Epipubic region.



*Leiopelma* adult male (both figs. from one specimen). Iliac region x 25.

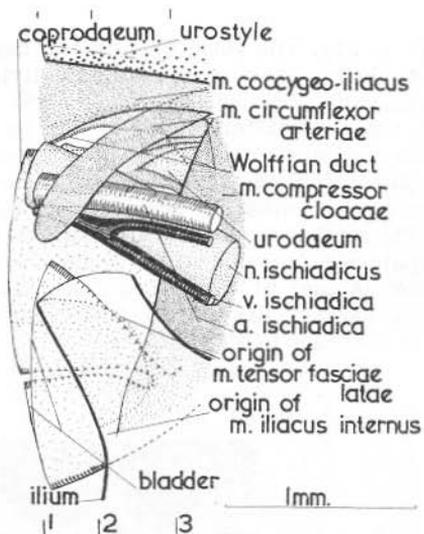


Fig. 38.

Norma lateralis sinistra.  
Graphic reconstruction from  
transverse sections.

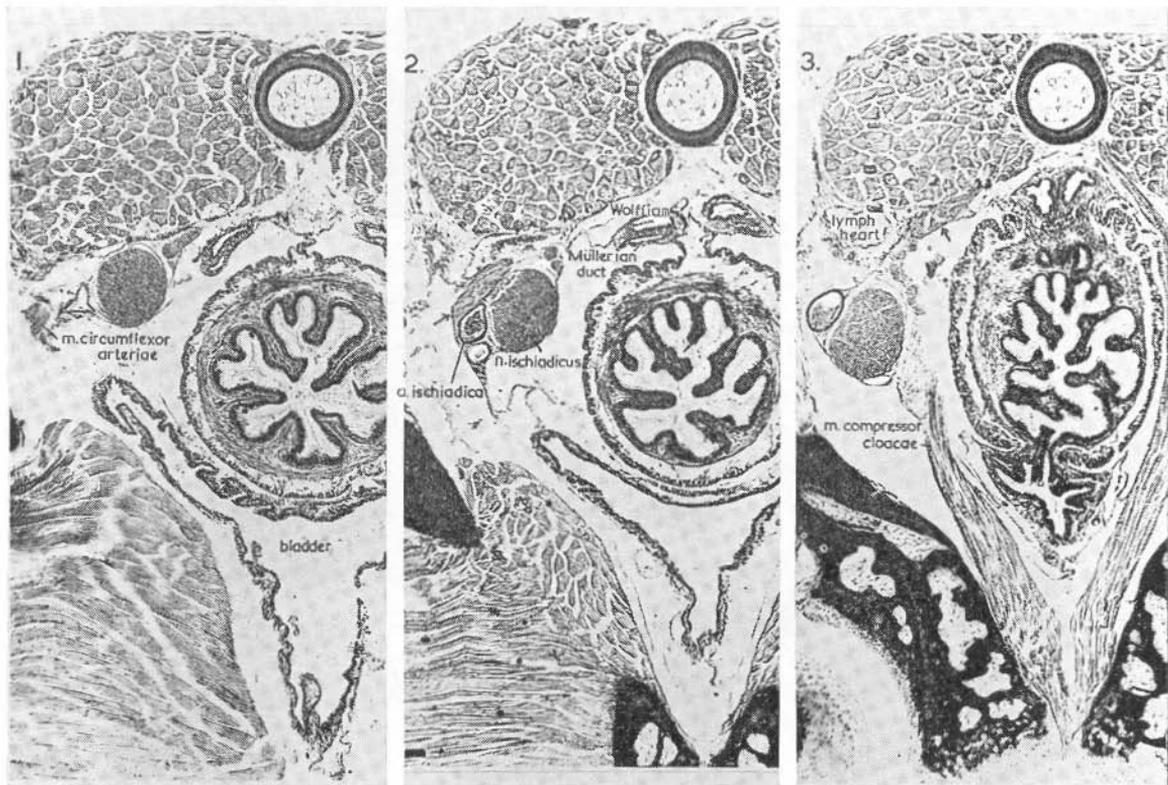


Fig. 39.

Norma posterioris. Photomicrographs of transverse sections.  
(The numbers refer to levels in fig. 38.)

*Arterial Circumflexor Muscles:* At Stage 56 in *Ascaphus* the arterial circumflexor muscles are represented on either side by a slip of muscle detaching from the m. compressor cloacae and dividing into ventral and dorsal portions. By Stage 58 the dorsal portion is no longer distinguishable and the m. circumflexor arteriae is represented only by the ventral portion. At Stage 64 each m. circumflexor arteriae shows a tendency to form a complete loop (cf. figs. 21 : 2 and 21 : 3 with 21 : 1 and 21 : 4). In the adult *Ascaphus*, and some species of *Rana*, complete loops are not formed by these muscles. In *Xenopus*, *Bombina* and *Bufo* complete loops are formed, as was observed in serial sections (figs. 33, 34 and 35 : 3). Dissections of representatives of all the South African anuran families (cf. Materials) revealed that the muscles were present in all cases, and in every case, including *Rana angolensis*, a complete loop appeared to be formed. The muscle is small and the dorsal dissection necessary does not expose it sufficiently to allow the completeness, or otherwise, of the loop to be established with absolute certainty. Sections of *Rana grayi* showed that the very small m. circumflexor arteriae of each side forms an almost complete loop. *Leiopelma* differs from all the other *Anura* examined in that the m. circumflexor arteriae of each side has no connexion with the m. compressor cloacae (figs. 38 and 39) and is clearly derived from the dorsal portion of a slip of muscle extending from the m. compressor cloacae at an earlier stage similar to Stage 56 in *Ascaphus*. In *Leiopelma*, as in all the other *Anura* examined, the m. circumflexor arteriae curves around the arteria ischiadica at the point at which this artery twists laterally over the nervus ischiadicus. It is for this reason, and because the muscle is not associated with the m. compressor cloacae in *Leiopelma*, that the muscle has been termed m. circumflexor arteriae. The occlusion, or partial occlusion, of the a. ischiadica between the n. ischiadicus medioventral to it and the m. circumflexor arteriae dorsolateral to it, seems the only function which contraction of the muscle could perform in *Leiopelma* and the other *Anura*. That the neighbouring coccygeal lymph hearts and their ostia may be influenced, as suggested by Gaupp (1904, p. 364), by contraction of the muscle, is unlikely because of variation in relations of these structures to the muscle. In sections the artery is seen to be partially or completely occluded, but this may be a post-mortem effect. Dissections of preserved and of lightly narcotized (ethyl urethane) specimens showed some constriction of the artery in every case. The relative volumes of blood flowing in the arteriae iliacae of numerous *Xenopus* adults under light narcosis (ethyl urethane), was studied by injection of fluorescein under ultraviolet light, such injections taking place into the dorsal aorta while it was in a position representing as nearly as possible its natural one (cf. Techniques). One m. circumflexor arteriae was cut after an initial injection, and a second injection then given, in every specimen. Changes in the blood-flow were frequently traced to slight movements of the legs occurring during the second injection, and attempts at staking the animal more firmly tended to stop the circulation to the legs. Consistent results could therefore not be obtained. The technique, however, holds promise.

*Unstriated fibres of the mm. compressores cloacae:* Unstriated fibres are present between the rods of Noble in the female (and, to a much lesser extent, the male) *Ascaphus*, and these fibres have the staining reactions of unstriated muscle. Since these fibres are closely associated with the mm. compressores cloacae (figs. 28, 29 and 21 : 14) it would seem that they are derived from these muscles. The mm. compressores cloacae, widening, have given rise to

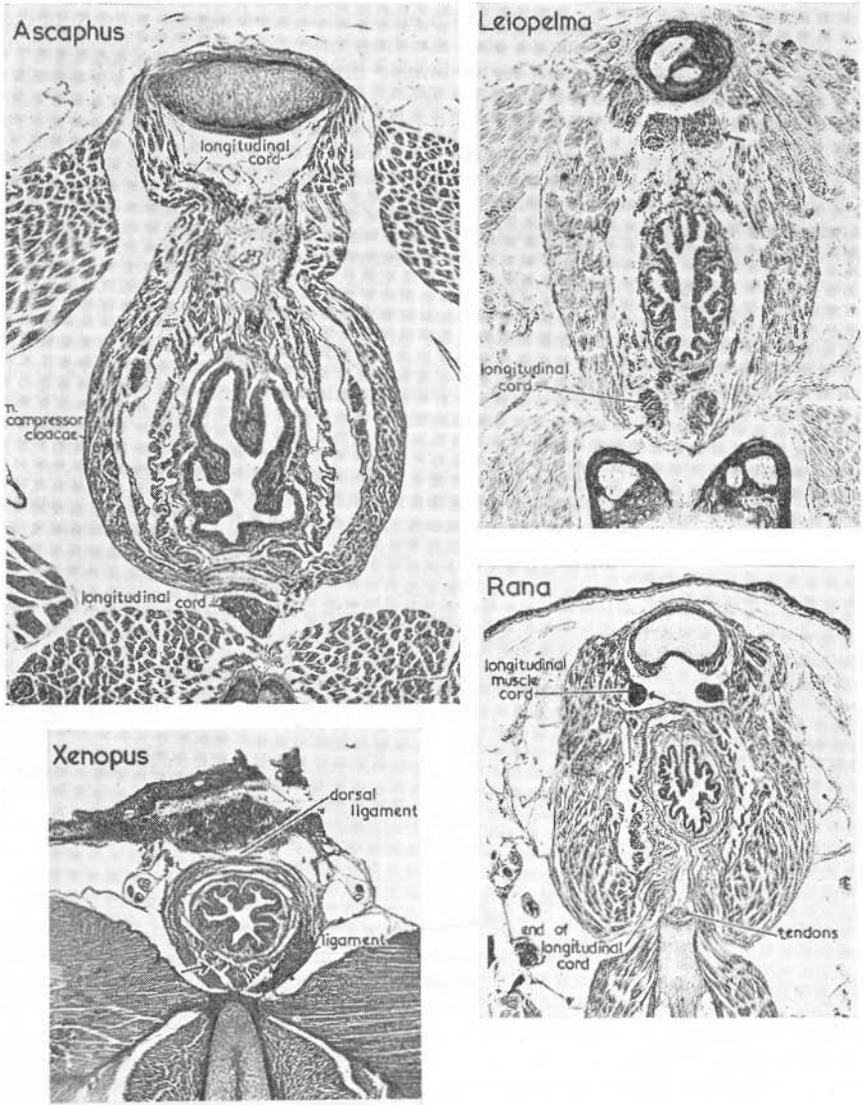


Fig. 40.

*Ascaphus*, *Leiopelma*, *Xenopus* and *Rana* adults. Cloacas showing longitudinal muscle cords x 25. Photomicrographs of transverse sections. Norma posterioris. The arrows indicate the longitudinal muscle cords. The *Xenopus* section is taken at level 4 of figs. 33 and 34.

Figs. 41—44.

*Bufo angusticeps* embryos and larvae (each fig. from a different specimen).

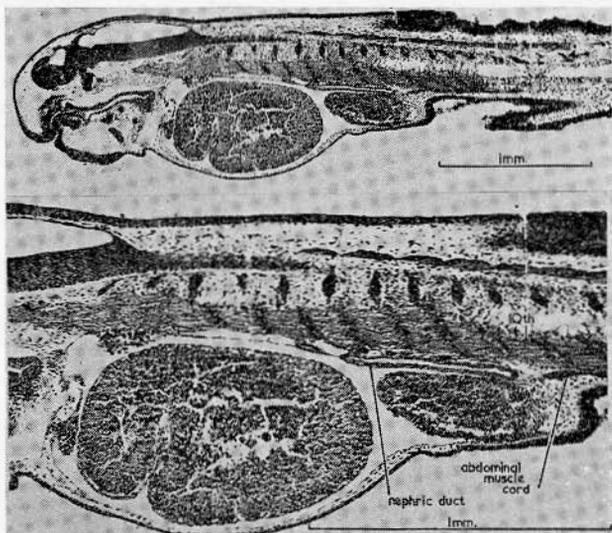


Fig. 41.

Embryo, Stage 42. Trunk region x 25 and x 50.  
Photomicrographs of a sagittal section  
Norma lateralis sinistra.

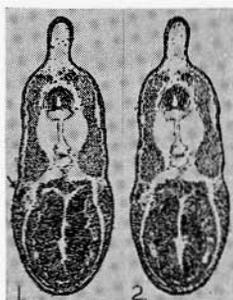


Fig. 42.

Embryo, Stage 42.  
Cloacal region x 50.  
Photomicrographs of  
transverse sections  
Norma posterioris.  
The arrow indicates  
the abdominal muscle  
cord.

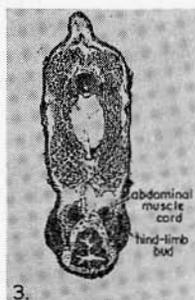


Fig. 43.

Embryo, Stage 46.  
Cloacal region x 50.  
Photomicrograph of a  
transverse section  
Norma posterioris.

striated fibres lateral to the rods as well, thus surrounding the rods with striated and unstriated muscle fibres (fig. 28). That the rods are derivatives of the same tissue as the muscles (as previously suggested — van Dijk, 1955, p. 30) is very doubtful.

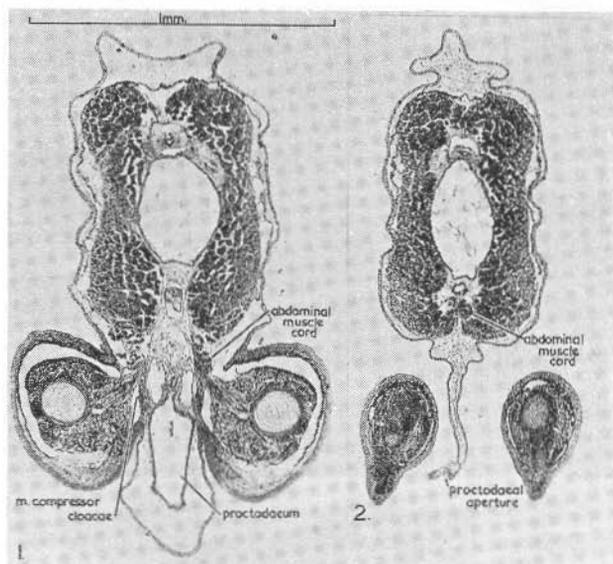


Fig. 44.  
Larva, Stage 54.  
Proctodaeal region x 50.  
Photomicrographs of transverse sections  
Norma posterioris.

**Cavernous Tissue:** The cavernous tissue of the cloaca of *Ascaphus* arises in association with the mm. compressores cloacae and in tissue containing fibres of the circular and longitudinal muscle layers. There does not appear to be de-differentiation of muscle tissue and subsequent vascularization, but rather the vascularization appears to take place in potentially myogenic tissue or in tissue which merely derives a rich vascularization from its proximity to muscle. The median vascularized cord appears to project further posterior in the adult than the point reached by the circular muscle layer at Stage 64. The ventrolateral vascularized cords develop close to the mm. compressores cloacae (figs. 19 : 3 and 19 : 4; 21 : 14 and 21 : 15).

#### DISCUSSION

A proctodaeal invagination is not the only possible source of an ectodermal, or apparently ectodermal, constituent of the cloaca. "Thus while the micromere layer gives rise to the ectoderm, it apparently also gives rise

to a part of the lining of the archenteron, as LWOFF (Bull. de la Soc. imper. des Naturalistes de Moscou, T. 8, 1894) maintained some years ago" (Wilson, 1901, p. 128). Rugh (1943) gives a description of the neurenteric canal of *Rana pipiens* and observes that "The cells of the floor of the neurocoel, of the floor of the neurenteric canal, and of the roof of the archenteron are of the same type and are, in all probability, continuous in early development" (p. 307). Earlier Erlanger (1891) had come to the conclusion "dass der After aus dem ventralsten Theile des Urmundes hervorgeht, während der dorsalste den Neuroporus und den Canalis neurentericus bildet" (p. 251). Whether the proctodaeum originates as an invagination of ectoderm, or whether it originates as a persistent blastopore (or part thereof) in the *Anura* has been the subject of several studies. Schanz (1887), Morgan (1890) and Erlanger (1891) have given reviews of earlier papers. The formation of the anus from a persistent blastopore (or part thereof) has been reported for numerous animals: by Shipley (1885) for *Petromyzon*; Caldwell (1885) for *Ceratodus*; Sedgwick (1884) and Johnson (1884) for *Triturus cristatus*, Johnson and Sheldon (1886) for *Triturus sp.*, Morgan (1890) for *Ambystoma* and Brachet (1902) for *Ambystoma tigrinum* (*Siredon pisciformis*); among the *Anura* by Spencer (1885) for *Rana temporaria*, Kupffer (1887) for *Rana temporaria* (?) and *Alytes obstetricans*, Gasser (1882) for *Alytes obstetricans*, and Pasteels (1942) for *Discoglossus pictus* and *Xenopus laevis*. The formation of the proctodaeum from a proctodaeal pit independent of the blastopore has been reported by Hatschek (1881) for *Amphioxus*, Scott (1880) for the lamprey; among *Anura* by Stricker (1860) for *Bufo cinereus*, Bambeke (1868) for *Pelobates fuscus*, Goette (1875) for *Bombina igneus*, Schultze (1884) for *Rana fusca*, Durham (1886) for *Rana sp.*, Sidebotham (1888) for *Rana temporaria*, Morgan (1889 and 1890) for *Rana halecina* and *Bufo lentiginosus*, Erlanger (1891) for *Rana fusca* and *R. esculenta*, *Bufo variabilis* and *B. vulgaris* and *Bombina igneus*, by Ziegler (1892) for *Rana sp.*, Brachet (1902) for *Rana temporaria*, Marchetti (1918) for *Bufo vulgaris*, Rondinini (1928) for *Bufo vulgaris*, Vogt (1929) for *Bombina*, Kagawa (1932) for *Bufo vulgaris japonicus*, Schechtman (1939) for *Hyla regilla* and Pasteels (1942) for *Rana fusca* and *Rana esculenta*, *Bufo vulgaris* and *Hyla arborea*.

Studies of Bytinski-Salz (1936), Risley (1939) and Schechtman (1939) were undertaken to elucidate the mechanism of proctodaeum formation and cloacal plate perforation. Schechtman (op. cit.) working on *Hyla regilla* suggested the following mechanism: "The ventral lip mesoderm induces the proctodaeal pit in that portion of the ectoderm which happens to come into contact with it. The invagination of the pit brings it into contact with the entoderm of the hind-gut. The latter completes the process of anus formation by inducing the perforation of the proctodaeal pit" (p. 49—50).

Risley (1939) remarked of anus formation in urodeles: "It is of particular interest that, under experimental conditions, a secondary anus may appear in conjunction with secondary tails even though no blastopore formation or gastrulation has occurred" (p. 114). This suggests that the formation of the "anus" from a part of the blastopore is not necessarily the primitive condition.

Pasteels summarizes the development of the proctodaeum as follows: "Le blastopore présente chez les Anoures deux modes d'évolution: chez les uns (*Discoglossus*, *Xenopus*), il se transforme en anus sans subir d'occlusion transitoire; chez les autres (*Rana*, *Bufo*, *Hyla*, probablement *Bombinator*), il s'obture de façon précoce, l'anus apparaissant à la suite d'une perforation

secondaire. . . Ces différences ne correspondent qu'à une variante chronologique de la même cinématique" (1942, p. 25).

In the descriptions of the formation of the proctodaeal pit, the position, in relation to the mesoderm, at which the cloacal membrane is formed, is rarely mentioned. Schanz (1887) does note of the proctodaeal pit that it had been supposed that "ein Stück des Urdarms wüchse ihm entgegen, . . ." (p. 10), and Marchetti (1918) observes that "Le cellule sticotropiche del fondo delle due fossette si vanno incontro, e per potersi raggiungere e fondere scacciano il mesoderma interposto" (p. 237).

The proctodaeum develops as an ectodermal fossa in *Bufo angusticeps*. The region of the cloaca into which the bladder and the nephric ducts open is apparently endodermal in *Ascaphus* (fig. 1) and *Bufo* (figs. 41, 42 and 43), as is inferred from the presence of yolk in the cloacal walls. According to Gadow's (1887) terminology this region may be described, by definition, as urodaeum. The urodaeum of *Ascaphus* and *Bufo*, then, is apparently endodermal. Shumway (1942) uses Gadow's terminology (Americanised) in describing the cloaca of the chick: "No urinary bladder is formed but the cloaca is ultimately divided into three regions: an anterior portion, the coprodeum, into which the rectum enters; an intermediate part, the urodeum, into which the urogenital ducts enter, and the terminal proctodeum" (p. 147). From this description it is clear what should be understood by cloaca and rectum. Unfortunately, other authors have been less precise. Huettner (1949) states of the young larval *Rana*: "The cloaca being continuous with the proctodaeum, is therefore partially formed by ectoderm. Its inner portion, which is continuous with the rectum, is formed from entoderm. Later on the *urinary bladder* is developed from the entodermal region of the cloacal portion of the hind-gut" (p. 134). This description does make it clear that the urodaeum is endodermal, at least ventrally, in Huettner's opinion. The region of the cloaca into which the nephric ducts open, is variously referred to as "cloaca", "rectum" and "proctodeum" in "NORMAL TABLE OF XENOPUS LAEVIS (DAUDIN)" edited by Nieuwkoop and Faber (1956). Thus the Wolffian ducts are said to open into "rectal diverticula" which originate as follows: "The *rectal diverticula* are indicated for the first time at STAGE 32, at which they appear as tiny slits in the dorsal wall of the proctodeum" (p. 132). It is further noted (p. 135, op. cit.) that "The *urinary bladder* develops as a ventral diverticulum of the rectum at STAGE 55 . . .". On p. 157 it is stated: "At its posterior end the rectum is continued beyond the last coil of the ileum, and then becomes the *cloaca*. The cloaca receives the ureters which enter by two separate openings lying close to one another in its dorsal wall. On the ventral side the cloaca communicates with the *urinary bladder*."

A study of O'Connor (1940) on *Pleurodeles waltii*, *Triturus taeniatus* and *Ambystoma* indicated that the posterior part of the gut might attract the nephric ducts during development and that the terminal parts of the ducts in adults might have a cloacal origin. Bijtel (1948), working on in *Triturus alpestris*, found that grafts of presumptive cloacal material attracted the nephric ducts to it during development. She refers to this material as "matériel cloacal", but refers to that which develops from it as a secondary proctodaeum. "Il est patent que le proctodaeum secondaire a exercé dans ce cas, donné en exemple, une action attractive sur l'uretère primaire". Bijtel noted one limitation: "Si regrettable que ce soit, la transplantation d'un peu de matériel sométique est inévitable". It is also important to emphasize that the exact limits of the

proctodaeum, in an animal in which it does not develop as a proctodaeal pit (as appears to be the case in urodeles), are difficult to determine. In *Xenopus* the cloaca is described as developing diverticula which grow anteriorly in the direction of the nephric ducts, later fusing to these (Nieuwkoop and Faber, editors, see above).

There is little literature on the development of the cloacal muscles in chordates. Lineback (1920) investigated the origin and development of the longitudinal muscle layer of the colon of *Homo* and refers to earlier remarks on the development of the visceral muscles in mammals; but in neither his own work, nor, apparently, in the works cited by him, is the relation of these muscles to the coelom described. The later development of the somatic muscles of the gut of *Homo* has been studied by Popowsky (1899), but the origins of these muscles would seem to be obscure. In *Tachyglossus* the sphincter cloacae appears to be a dermal muscle as it is continuous with the dermal muscles of the abdomen (Ruge, 1895). (Ruge's illustration appears in Wiedersheim's *Vergleichende Anatomie der Wirbeltiere* and other textbooks.)

Goette (1875) observed the relation of the abdominal muscle cords to the pelvic region. "Das hinter dem Becken gelegene Stück lässt er teils atrophieren, teils soll aus ihm das M. ischiococcygeus werden," to quote Lignitz (1897), p. 32. The m. ischiococcygeus is, in Gaupp's nomenclature (1896), the m. compressor cloacae. The abdominal muscle cords ("die ältesten Bauchmuskeln" — Kaestner) were subsequently studied by Kaestner (1892, 1893a and 1893b), Wiedersheim (1892), Field (1894) and Wild (1897), while Lignitz (1897) referred to their findings (except those of Wild) critically. Field and Lignitz were interested in the development of the fore-limbs. Maurer (1892), investigating the development of the trunk and abdominal muscles of urodeles, does not appear to have observed similar relations to the pelvic region in the case of the urodelan abdominal muscles as occur in the *Anura*. Green (1931) mentioned that "The 'primary abdominal muscle' preceding M. rectus abdominis at first passes through the pelvic basin to a point posterior to this. During the closure of the pelvis it moves forward and becomes attached to the pubic symphysis (p. 1287, of *Rana*). Recently Ryke (1953) described the development of the somatic muscles of the trunk of *Xenopus*, stating in his introduction (p. 2): "I do not intend reviewing the literature prior to MAURER, as it has been fully dealt with by this author in his above-mentioned works. Suffice it to say, that these include the publications of MECKEL (1828), DUGÈS (1835), STANNIUS (1854), HUXLEY (1873), GOETTE (1875), SCHNEIDER (1879) and KÄSTNER (1892)." The works of Maurer referred to are those of 1892 and 1895. Goette is later mentioned by Ryke in connexion with the m. sternohyoideus and appears in his bibliography, the other works mentioned (other than Maurer's) are not cited in the bibliography. Wiedersheim (1892), Kaestner (1893a and 1893b), Wild (1897), Lignitz (1897) and Green (1931) are not referred to at all. Ryke observed the abdominal muscle cords in the pelvic region: "An interesting point in the development of the m. rectus abd. is the occurrence (sic) of a backwardly directed strip reaching the root of the tail (fig. II) (sic). It runs along the outer side of the cloaca and possibly represents the m. rectus lateralis of some fishes. During metamorphosis this muscle strip atrophies" (p. 19). The m. rectus lateralis is situated much further dorsally than the abdominal muscle cords, being found at the level of the horizontal myoseptum.

The significance of the abdominal muscle cords for the interpretation of

the development of the pelvic muscles is summarized by Wild (1897, p. 33) as follows: "Kästner selbst sagt, dass es ganz verkehrt sei, den Versuch zu machen, den Wiedersheim und er selbst unternommen hätten aus einem wohl differenzierten Abschnitte der Bauchmuskulatur die Muskulatur der hinteren Extremität abzuleiten. Auf denselben Umstand macht auch Field . . . aufmerksam: 'Der bereits differenzierte Bauchmuskel habe mit der Bildung der Extremitätenmuskulatur nichts zu schaffen.'" Field (1894) and Lignitz (1897) advanced evidence that the fore-limbs are derived from the spinal myomeres and not from the abdominal muscle cords. Kaestner (1893a and 1893b) and Wild (1897) could not advance much evidence for the derivation of the hind-limb Anlagen from the spinal myomeres, but were convinced that the hind-limb Anlagen were not derived from the abdominal muscle cords. The present study confirms that the hind-limb Anlagen are not derived from the abdominal muscle cords and removes one obstacle to the derivation of these Anlagen (and hence also the cloacal muscles) from the spinal myomeres: The abdominal muscle cords are not interposed between the hind-limb Anlagen and the spinal myomeres at Stage  $\pm 50$  of *Ascaphus*. This is quite clear although the specimen is by no means a perfect one. It is difficult to understand the relationship of the abdominal cords at Stage 47 to the same muscles at Stage  $\pm 50$ , but subsequent development is easily followed. The hind-limb Anlagen develop independently of the abdominal muscles in *Ascaphus*, but differentiation of the cloacal compressor muscles occurs after the abdominal muscle cords have extended backwards to beyond the pelvic region (Stage 51).

When the cloacal compressor muscles first appear in the *Ascaphus* material examined (Stage 56), it is clear that they are not associated with the skin, and are therefore not dermal muscles; but they are quite closely associated with the abdominal muscles and the mm. pyriformes and mm. caudalipuboischiotibiales dorsally, and with the pelvic girdle anteroventrally. It is possible that the cloacal compressor muscles may be late derivatives of the same myogenic tissue which gave rise to the late-developing pelvic parts of the abdominal cords. In *Bufo* the abdominal muscle cords are complete when the hind-limb Anlagen are barely indicated (figs. 41 and 42), and the Anlagen subsequently develop ventral to the abdominal cords (fig. 43). When the abdominal muscle cords atrophy posteriorly the anterior regions of the mm. compressores cloacae, and, particularly, the mm. circumflexores arteriarum, are closely associated with the atrophying muscles. There is a marked increase in development of the mm. compressores cloacae while the abdominal muscle cords are atrophying in *Ascaphus* (Stages 58—60) and, particularly, in *Bufo* (cf. fig. 44, showing little development of the mm. compressores cloacae and maximally developed abdominal cords). That there may be a causal association between the atrophying of the abdominal cords and the differentiation of the mm. compressores cloacae, is a possibility. Goette (1875) may have been correct in his derivation of the "M. ischiococygeus" from the abdominal muscle cords. Kaestner (1893a) does not consider the derivation of Goette likely: "Nach meinen Untersuchungen geht jenes hinterste Stück bei Froschlarven ganz zu Grunde. Bombinator igneus scheint sich demnach etwas anders zu verhalten" (p. 280, discussing the abdominal cords). It is worth mentioning that the obliquus and transversus muscles also differentiate, between the abdominal cords and the spinal myomeres, while the abdominal cords are atrophying (Stage 58 of *Ascaphus*), their differentiation not beginning in *Bufo* until atrophy of the abdominal cords is con-

siderably advanced. In the comparison which can thus be made between the differentiation of the cloacal compressors and the obliquus and transversus muscles it is of importance to note that Ryke's (1953) interpretation of the abdominal muscle cords of *Xenopus* as constituting all the abdominal muscle layers anterior to the pelvis (loc. cit., fig. 6, p. 14; fig. 27, p. 39) seems to gain no support whatever from conditions in *Ascaphus* and *Bufo*, and is at variance with Kaestner's interpretation of the obliquus and transversus muscles: "Die erwähnte Muskelschicht liegt überall lateral — bezw. dorsalwärts vom Rectus abdominis, stösst, wenigstens anfangs, nirgends mit ihm zusammen, sondern es findet sich ein muskelfreier Zwischenraum zwischen der lateralen Grenze des Rectus und der medialen der neuen Muskelschicht" (1893a, p. 283).

If the cloacal compressor muscles are neither derived by division of the abdominal muscle cords when these have already formed (which derivation seems most unlikely), nor while these cords are developing or atrophying, another mode of origin from the abdominal muscle cords is still possible. It is possible that, like the mm. epipubici, they are derived in association with, if not from, the myocommata between segments of the abdominal cords. (The possibility that myocommata can contain myogenic elements must be admitted, since the proximity of the epipubic muscles to myocommata in *Ascaphus* suggests a possible histogenetic relationship.) It is probable that the mm. compressores cloacae are pelvic muscles unconnected with the abdominal muscles.

Experimental evidence, such as that obtained by Byrnes (1898), Lewis (1910) and Detwiler (1918 and 1929), has been that "There is no demonstrable contribution of the somites to the musculature of the limb in amphibians". (Nicholas, J. S., in "Analysis of Development" edited by Willier, Weiss and Hamburger, 1955, p. 431.) From the works of Lewis and Detwiler cited, there is evidence that extirpation of somites in the anterior region of urodeles results in the absence of ventrolateral abdominal muscles, but that the fore-limb still develops. Byrnes (op. cit.) found in both *Ambystoma* and *Rana* that suppression of the abdominal muscles is effected by treatment not affecting the hind-limb musculature. It would be interesting to learn to which category the cloacal compressor muscles belong on this criterion. While negative experimental evidence has questionable morphological significance, positive findings would be, at least, suggestive. There is evidence (based on carbon marking technique) that not only the limb buds, but also part of the ventrolateral muscles may form independently of the somites, at least in Aves (Rawles and Straus, 1948; Straus and Rawles, 1953). Detwiler (1955), however, finds the ventrolateral muscles of urodeles to be derived from the somites in studies based on extirpation and xenoplastic grafts. Since the abdominal muscles have already appeared in the earliest *Ascaphus* stage available (Stage 47), the study of their origin was unavoidably beyond the scope of the present investigation.

An investigation of *Salmo* and *Periophthalmus* showed the absence of striated cloacal muscles. Paired longitudinal muscles stretch from the pelvic girdle to the anal-fin skeleton in both of these teleosts, but these are widely separated in the cloacal region in both genera.

The thickening of the septum interfemorale which has here been named ligamentum interfemorale, and which in *Ascaphus* is modified to form the rods of Noble ventrally, was observed by Gaupp (1896) in *Rana*. Describing the m. gracilis minor, he wrote: "Dieser schmale, ganz am medial-hinteren Rande des Oberschenkels gelegene Muskel entspringt mit dem der anderen Seite von einem

sehnigen Streifen, der den hinteren Rand der Beckenscheibe deckt, vorn an der Insertion des *Rectus abdominis*, hinten an der *Spina pelvis posterior* befestigt ist. An dem hinteren Abschnitte dieses Streifens, bis zum Ursprunge des *Gracilis minor*, entspringen die hinteren Bündel des *Sphincter ani*" (p. 182). Grobbelaar (1922) did not mention this "sehniger Streife", but described the *m. gracilis minor* as originating on the ischium, close to the symphysis, in *Xenopus*. Bigalke (1926) divided the *m. gracilis minor* (*M. cutaneous femoris*) into a *Caput superius* and *Caput inferius*, referred to Gaupp's description and stated: "Bei *B. vulgaris* aber entspringt das *Caput superius*, zusammen mit dem der anderen Seite, mit dünnem Ursprung gerade unterhalb der Afteröffnung von der hinteren Fläche des *M. sphincter ani cloacalis* und nimmt gleich an Breite beträchtlich zu" (p. 335). In both *Xenopus* and *Bufo* the ligament is nevertheless present. In *Xenopus* the ventral and dorsal parts were reconstructed and labelled "connective tissue" before their significance was realized (fig. 33). In *Ascaphus* the rods of Noble bear essentially similar relations to the cloaca and the *mm. graciles minores* as do the dense edges of the *ligamentum interfemorale* in other *Anura*. Since the possession of these rods has been regarded as evidence of great specialization in *Ascaphus*, this genus must now be considered as being less specialized than has been maintained. The glands of the oviduct in which spermia are to be found in *Ascaphus* are essentially similar to the oviducal glands of *Rana*, as may be seen from figs. 31 and 32. Here specialization for internal fertilization has not produced any marked morphological changes.

Szarski (1957), discussing the larval state in *Amphibia*, observed: "All amphibian and fish larvae have a similarly constructed tail fin, consisting of a skin fold." The shortening of the proctodaeum during metamorphosis, which occurs in the *Anura*, would thus also take place in fish larvae when the tail fin becomes reduced. This may be seen, for example, in Stages 27 and 28 of the development of *Gasterosteus aculeatus* (Swarup, 1958).

*Bufo* exhibits no marked specializations in the cloacal region which distinguish it from the other *Anura* studied. Green (1931) remarked that "the oldest definitely accepted Anuran is of Upper Jurassic age (*Eobatrachus agilis* March; Moodie 1912)" and "*Eobatrachus* is structurally almost indistinguishable from modern Bufonids" (p. 1259). In respect of the cloacal region there is no reason to believe that this is not because the bufonids are rather primitive *Anura*. *Rana* and *Xenopus* reveal several specializations in the cloacal region, while *Bombina* resembles *Bufo* quite closely. Szarski's evidence that *Rana* shows greater specialization than *Bufo* and *Bombina* in the vascular system (1948) is significant in the light of conditions in the cloacal region. *Ascaphus* and *Leiopelma* show specializations from the bufonid condition in the cloacal region, and these are typically in divergent directions. An example is afforded by the enlargement and modification of the *m. sphincter ani cloacalis* in the male *Ascaphus* and the absence of this muscle in *Leiopelma*.

In *Ascaphus* and *Leiopelma* there is one more presacral vertebra than in typical *Anura* such as *Bufo*, and this is regarded as a primitive feature. Green (1931) offers evidence of this and remarks: "The phylogenetic forward extension of the ilia is represented during larval development" (p. 1288). Smit (1953) states: "If we consider that during the evolution of the *Anura* a forward shifting of the ilio-sacral articulation has taken place (Watson, 1939—1940, and Piveteau, 1937), . . . then the urostyle must represent original trunk vertebrae, as suggested by Piveteau (1937), and not caudal vertebrae" (p. 127—128). Despite the dif-

ference in sacral vertebrae in the *Ascaphidae* (in which it is the 10th vertebra) and in *Bufo* and *Rana* (in which it is the 9th) the plexus ischiadicus and the innervations of the pelvic muscles, including the mm. compressores cloacae, is essentially the same. Information in this connexion has been given for *Ascaphus* by Ritland (1955a and b), and van Dijk (1955), for *Leiopelma* by Stephenson (1952) and Ritland (1955b), for *Rana* by Gaupp (1899) and others; and for *Bufo* by Bigalke (1926). Goodrich (1930) remarked of movements of the hind-limb: "For instance, in the frog the straining backwards of the pelvic plexus shows that the base of the hind-limb has moved backwards with the elongation of the pelvic girdle" (p. 138). The innervation of the cloaca by the 11th spinal nerves in *Rana*, by the 11th, 12th, and sometimes the 13th (according to Ritland) spinal nerves in *Ascaphus*, and by 11th to 13th nerves in *Leiopelma* (as appears to be the case), suggests a process of transposition (Rosenberg, 1875 and 1907) by which the innervations have been shifting to more anterior nerves during phylogeny. On the other hand it is clear that the hindlimbs shift backwards relative to the spinal nerves during ontogeny. Comparison of Stages 56 and 64 of *Ascaphus* (figs. 8 and 21) shows the pelvis moving from a position ventral to the 12th spinal nerve to one behind the 13th. This movement is even more strikingly illustrated by comparing Stage 56 with Ritland's (1955a) fig. 11 and Stephenson's (1952) fig. 2, when it may be seen that the origin of the 12th nerve, which marks the original level of the pelvic girdle, is far forward on the urostyle in both *Ascaphus* and *Leiopelma*. The same movement of the pelvis clearly takes place in all *Anura* during development. The cloacal nerves of tetrapods are apparently rarely presacral, and it seems likely that the 11th vertebra is the posteriormost sacral vertebra to have occurred in an anuran with the ascaphid arrangement of the plexus ischiadicus. The relation of the sacral plexus to the sacrum in *Urodela* is well illustrated by Davidoff (1884). The 13th spinal nerve is apparently rarely retained in the adult *Ascaphus*, although Ritland has detected it (pp. 166—167 and fig. 11, 1955a) and reports that it supplies the cloaca. In none of the larvae reconstructed in the present study, nor in the adults reconstructed previously (1955) does the 13th nerve participate in the plexus ischiadicus.

Of the tail muscles of the larval *Anura*, only those in the region of the urostyle are retained in the adult, becoming the mm. cocygeo-iliaci. Since these muscles only become differentiated at metamorphosis, when the cloaca has almost achieved its adult form, their development is of little consequence to cloacal differentiation. The differentiation of the spinal myomeres early in metamorphosis has here been studied in *Ascaphus* and the comparative material (*Bufo*). As the youngest *Ascaphus* embryo studied (Stage 47) has well-differentiated spinal myomeres, the earlier development of the spinal myomeres is not relevant to the present study, which is centred on *Ascaphus*. It may be mentioned, however, that there have been a number of studies on the origin of the tail and the early stages of its subsequent development. Notable among the workers in this field is Bijtel, whose thesis (1929) and some of whose subsequent works (Bijtel 1930, 1931, 1936 and 1958) have been consulted in the present study. Other works consulted are those of Chuang Hsiao-Hui (1947), Nakamura (1942, 1947 and 1952) and Bijtel and Woerdeman (1928), as well as others also mentioned in other connexions (e.g. Marchetti, 1919; Rondinini, 1928).

Of the *Amphibia*, the anurans *Ascaphus* and *Nectophrynoides*, the salamandroid *Urodela*, and all the *Gymnophiona* practice internal fertilization.

The copulatory apparatus of *Gymnophiona* has been investigated by Tonutti (1931, 1933) and others (cf. van Dijk, 1955, pp. 51—52), and comparisons with the copulatory apparatus of *Amniota* have been made by Tonutti (1932 and 1934). The cloacal and caudal regions of *Gymnophiona* are specialized, the absence of the pelvic girdle being a notable specialization. For this reason, and because the development of the copulatory apparatus in *Gymnophiona* has not been worked out, the nature of the muscle layers of the cloaca in these animals is obscure and comparisons with amniote conditions must be considered tentative. Since Tonutti further compares the more specialized *Gymnophiona* with specialized *Amniota*, and compares the cloacas of the males while the female condition is probably more primitive, his conclusions must be considered doubtful. In *Nectophrynoidea* and those *Urodela* which copulate, the cloaca is little specialized for copulation. The cloaca of *Ascaphus* similarly appears to have undergone relatively slight modifications for copulation when compared with the conditions in most *Amniota* and in *Gymnophiona*. Conditions in the *Anura* and the *Urodela* therefore suggest that internal fertilization developed in early tetrapods without the evolution of a complicated intromittent organ. The absence of an intromittent organ in *Sphenodon* (Gadow, 1887) suggests that such organs evolved late in tetrapod evolution and independently in divergent directions in the *Squamata*, the *Synapsida* (and hence the mammals), and the *Chelonia* and *Archosauria-Aves* lines.

#### SUMMARY AND CONCLUSIONS

1. In *Bufo angusticeps*, as in other *Bufo* species, the proctodaeum forms from a proctodaeal pit after closure of the blastopore.
2. In *Bufo* the proctodaeal pit meets the archenteron in the tissue posterior to the coelom, i.e. in the somatopleura (somatic mesoderm).
3. In *Ascaphus* the junction between the proctodaeum and the enteron lies posterior to the coelom within the somatopleura (somatic mesoderm) at an early stage.
4. In early stages of *Bufo* and *Ascaphus* the nephric ducts open into a region of the gut (which is hence, by definition, urodaeum) with yolk-laden walls.
5. In *Bufo* and *Ascaphus* the urodaeum, as shown by the presence of abundant yolk in its walls, is part of the endodermal enteron and is (at least initially) endodermally lined. (The urodaeum is widely accepted as being endodermal in *Amniota*.)
6. In *Bufo* and *Ascaphus* the longitudinal and circular layers of visceral muscle develop in the (splanchnic) mesoderm around the endodermal enteron in the coelomic region, and posterior to the coelom in the somatopleural region.
7. Splanchnic mesoderm may have accompanied the enteron into the post-coelomic somatopleural region when enteron and proctodaeum approached each other, thus giving a layer of splanchnic mesoderm between the enteron and somatopleura (somatic mesoderm).
8. The post-coelomic visceral muscle layers in *Bufo* and *Ascaphus* may, like those of the coelomic region, be derived from splanchnic mesoderm.
9. The visceral muscle layers persist behind the coelom in adult *Ascaphus*, *Leiopelma*, *Bufo*, *Xenopus*, *Bombina* and *Rana*.
10. The posterior limit of the visceral muscles probably corresponds closely to the urodaeal - proctodaeal junction in *Anura*.

11. The posterior limit of the visceral muscles in *Ascaphus* corresponds approximately to the level at which there occurs during metamorphosis differentiation of the cloacal epithelium into transitional, proximally, and stratified squamous, distally.
12. The urodaeal-proctodaeal junction in adult *Ascaphus* may be represented by a transitional — stratified-squamous epithelial junction.
13. A cluster of dermal cloacal glands surrounds the transitional — stratified junction in adult *Ascaphus* of both sexes.
14. The ducts of the dermal cloacal glands of *Ascaphus* are directed somewhat posteriorly and thus open into the stratified squamous epithelium region.
15. The dermal cloacal glands of *Ascaphus* develop during metamorphosis as solid thickenings of the stratified squamous epithelium, separating (except for the future ducts) as solid structures from the epithelium and becoming hollowed out later in metamorphosis.
16. There is no evidence that the cloacal glands are not proctodaeal glands. In the light of the evidence that they are of proctodaeal origin they should be considered as proctodaeal glands.
17. In *Leiopelma* there are, at least in the male, glands similar to the proctodaeal glands of *Ascaphus* near the cloacal orifice and distinct from the glands of the skin near them.
18. In the adult male *Ascaphus* horny spines develop in the stratified squamous epithelium of the cloaca, anlagen of these spines not being visible late in metamorphosis.
19. The development of horny spines, which are characteristically ectodermal structures, is further evidence that the proctodaeal part of the cloaca extends at least as far as such spines occur.
20. The cloaca lengthens posteriorly, during development, in traversing the ventral skin flap in *Ascaphus* (and probably *Leiopelma*, which also possesses this structure) and the ventral fin in *Bufo*, *Xenopus*, *Bombina* and probably most other *Anura*.
21. The cloaca in the region of the ventral fin or ventral skin flap is without a tunica muscularis in all the *Anura* examined, and is presumably proctodaeum only.
22. Early in metamorphosis the loss of the ventral flap or the anterior part of the ventral fin results in a shortening of the proctodaeal part of the cloaca.
23. The cloacal lips are differentiated in the adult position before the shortening of the proctodaeum occurs in *Ascaphus*, *Bufo*, *Xenopus* and *Bombina*. This is very well seen in *Ascaphus*.
24. The cloacal lips bear similar relations to the pelvic structures, particularly the septum interfemorale, in *Ascaphus*, *Bufo*, *Xenopus* and *Bombina* and appear to be in homologous positions in these *Anura*.
25. In *Ascaphus*, when the hind-limb buds become distinct (Stage  $\pm$  50), the pelvic anlagen are situated immediately ventral to the spinal myomeres at the level of the 10th and caudad processes of the 9th of these myomeres.
26. The topography of the 9th, 10th and 11th spinal myomeres (especially the 10th), is such as to make their contribution to the anlagen of the pelvic muscles, or their derivation from the same source as these anlagen, possible and even probable.

27. Anterior to the pelvic anlagen in *Ascaphus* (Stage  $\pm$  50) the 9th myomeres are differentiated into spinal myomeres dorsally and abdominal myomeres ventrally; further cephalad (8th myomeres etc.) there is a separation of spinal and abdominal myomeres.
28. In *Bufo* the 10th trunk myomere is divided into a spinal myomere dorsally and an abdominal ventrally and anterior to this the abdominal myomeres, from their first appearance, are separate from the corresponding spinal myomeres.
29. In *Bufo* and *Ascaphus* the abdominal myomeres thus form abdominal muscle cords metamERICALLY segmented in a manner corresponding to the segmentation of the spinal myomeres with which they are (at least initially) continuous posteriorly.
30. In *Ascaphus* the 9th abdominal myomeres separate from the spinal myomeres early in development (Stage 51) and the 10th, and possibly more caudad, abdominal myomeres develop and project over the faces of the 10th and subsequent spinal myomeres without showing metameric correspondence to them.
31. In *Bufo* the abdominal myomeres extend posteriorly along the ventromedial edges of the 10th and subsequent spinal myomeres, with which they serially correspond.
32. In *Bufo* the hind-limb anlagen are scarcely visible and the hind-limb buds are absent when the abdominal muscle cords are completely formed.
33. In *Ascaphus* the abdominal muscle cords have extended caudad beyond the pelvic anlagen, by development of the 10th myomeres, before the mm. compressores cloacae develop.
34. In *Bufo* and *Ascaphus* the mm. compressores cloacae may be derivatives of the pelvic muscle anlagen or of the abdominal muscle cords.
35. The mm. compressores cloacae differentiate in the region of the somatic mesoderm (somatopleura) lateral and posterolateral to the coelom between the posterior region of the pelvis and a level somewhat caudad to the posterior edge of the coelom. (*Bufo* and *Ascaphus*.)
36. The mm. compressores cloacae are more closely associated (anteroventrally) with the pelvis than with the vertebral column until late in development. (*Bufo* and *Ascaphus*.)
37. The mm. pyriformes in *Bufo*, and these muscles and the mm. caudalipuboischiotibiales in *Ascaphus*, develop near the posterodorsal limit of the mm. compressores cloacae. The posterodorsal limits of the three pairs of muscles in *Ascaphus*, and the two pairs in *Bufo*, lie close together throughout development.
38. There is no evidence of differentiation of the abdominal muscle cords to give rise to the mm. compressores cloacae in *Bufo* or in *Ascaphus*.
39. The mm. compressores cloacae might be late derivatives of the same stratum as had given rise to the posterior parts of the abdominal muscle cords in *Ascaphus*.
40. The abdominal muscle cords in *Bufo* develop so much earlier than the mm. compressores cloacae that late derivation of the latter from the same stratum as gave rise to the former is extremely unlikely.
41. The 9th and subsequent abdominal myomeres atrophy during metamorphosis in *Ascaphus* (Stages 58—60) and *Bufo*.

42. The atrophy of the abdominal myomeres in the pelvic region becomes evident, in *Ascaphus* and *Bufo*, shortly after differentiation of the mm. compressores cloacae takes place.
43. In *Ascaphus* and *Bufo* atrophy of the abdominal muscle cords posteriorly, is closely synchronous with the differentiation of obliquus and transversus muscles in the region between the remaining anterior parts of the abdominal cords (rectus muscles) ventrally and the spinal myomeres dorsally. The beginning of atrophy of the abdominal cords is clearly evident in *Bufo* before the obliquus and transversus muscles develop, and these muscles have differentiated before atrophy of the abdominal cords is complete in *Ascaphus*.
44. There might be causal relation between the atrophy of the abdominal muscle cords and the development of the mm. compressores cloacae and obliquus and transversus abdominal muscles.
45. A superficial secondary muscle layer develops on the ventral surface of the m. abdominis rectus (prepelvic portions of the abdominal cords, separated in the midline by the linea alba), at the time when the abdominal muscle cords are atrophying posteriorly in *Ascaphus*. Such a m. abdominis rectus superficialis has been reported from *Xenopus* (Ryke), but the stage at which it develops is unknown. Ryke could not find the muscle in *Ascaphus*.
46. There is no evidence in *Ascaphus* or *Bufo* that the mm. compressores cloacae develop as secondary derivatives of existing muscles, abdominal or spinal, in a manner comparable with the origin of the m. abdominis rectus superficialis in *Ascaphus*.
47. The mm. epipubici develop as secondary muscles associated with the myocommata between the 8th and 9th abdominal myomeres, in *Ascaphus*.
48. There is evidence that the m. epipubicus of *Xenopus* is a secondary derivative of the rectus muscles as in *Ascaphus*.
49. The mm. epipubici of *Ascaphus* are innervated by the crural (9th spinal) nerves.
50. There is no apparent evidence that the mm. compressores cloacae arise in association with the myocommata of pre-existing segmental muscles, abdominal or spinal.
51. The cloaca of *Ascaphus*, including the mm. compressores cloacae, is innervated by the 11th and 12th spinal nerves, sometimes also by the 13th spinal nerve (Ritland).
52. The mm. compressores cloacae show no indications of dual (or triple) origin. This makes a derivation similar to that of the mm. epipubici unlikely, as the mm. compressores cloacae would originate from cells from more than one myocomma (because of their compound innervation).
53. Early in the development of the mm. compressores cloacae in *Ascaphus* the anterodorsal edges give off slips of muscle curving ventrolaterad and dorsomedial over the faces of the abdominal muscle cords.
54. The dorsomedial slips of muscle from the anterior edges of the mm. compressores cloacae are transient in *Ascaphus*, while the ventrolaterad slips develop around the aa. iliacae and nn. ischiadici, showing a tendency later in development to form loops beginning medially from the mm. compressores cloacae and ending at the pelvis near the midline.
55. In *Leiopelma* there are muscles in the adult occupying the positions of the dorsomedial slips of the mm. compressores cloacae in larval *Ascaphus*, and curving around the aa. iliacae and nn. ischiadici. These muscles are isolated

- from the mm. compressores cloacae and hence have been given a separate name, mm. circumflexores arteriarum. This name is also applied to the homologous muscles in *Ascaphus* etc. (dorsales may be appended to distinguish the *Leiopelma*, ventrales the *Ascaphus*, condition).
56. In all the families of *Anura* found in South Africa mm. circumflexores arteriarum occur and appear to form complete or nearly complete loops extending from the mm. compressores cloacae around the aa. iliacae and nn. ischiadici to the pelvis.
  57. In *Ascaphus* and *Rana* (some species only, apparently, e.g. *R. temporaria* according to Gaupp) the mm. circumflexores arteriarum form half-loops only, the lateral parts being absent.
  58. The mm. circumflexores arteriarum have no known function, but they have the same relation to the aa. iliacae and nn. ischiadici in all the *Anura* examined, whether they are separate from the mm. compressores cloacae as in *Leiopelma* or joined as in other *Anura*.
  59. The aa. iliacae may be compressed against the nn. ischiadici by the mm. circumflexores arteriarum.
  60. The posterior parts of the mm. compressores cloacae meet each other mid-ventrally and mid-dorsally to form a sphincter, the m. sphincter ani cloacalis, in *Rana*, *Bufo*, *Bombina*, *Xenopus*, *Ascaphus* and probably most other *Anura*.
  61. A sphincter ani cloacalis is absent in *Leiopelma*, the cloacal sphincter in this genus consisting of a circular visceral muscle layer.
  62. The teleosts *Salmo* and *Periophthalmus*, have no striated cloacal (anal) sphincters.
  63. The absence of a striated cloacal sphincter in *Leiopelma* may be a primitive feature.
  64. The m. sphincter ani cloacalis is enlarged in the male *Ascaphus* to form the striated muscle layer of the "cloacal extension".
  65. The bilateral origin of the m. sphincter ani cloacalis may be reflected in the presence of fascial sheets in the dorsal and ventral midlines, such fasciae forming thickened cords on the surfaces of the muscles.
  66. The cords on the dorsal and ventral midlines of the m. sphincter ani cloacalis form part of a ligament lying in the septum interfemorale, and hence have together been named ligamentum interfemorale.
  67. Dorsal to the cloaca the ligamentum interfemorale consists of a single dorsal ligament attached to the urostyle, and penetrated by the v. caudalis at least in the larvae, in *Ascaphus*, *Bufo*, *Bombina* and *Xenopus*.
  68. A dorsal ligament is absent above the cloaca in *Rana* and *Leiopelma*.
  69. Ventral to the cloaca the lateral edges of the ligamentum interfemorale are thickened into tendinous cords in *Bufo*, *Bombina*, *Xenopus*, *Rana* and *Leiopelma*, while in *Ascaphus* these thickenings are represented by the rudes Nobleanae (rods of Noble, "post-pelvic" rods, "post-pubic" rods, "Nobelian bones", etc.).
  70. The ligamentum interfemorale stretches from the pubic region along the ventral, posteroventral and posterior edge of the pelvis to the cloaca in *Bufo*, *Bombina* and *Rana*.
  71. In *Xenopus*, *Leiopelma* and *Ascaphus* the ligamentum interfemorale reaches the epipubis anteriorly (= tendinous sheet in *Ascaphus*).

72. In the male *Ascaphus* the rods of Noble are widely separated from the posterior edge of the pelvis and reach to a level just anterior to the cloacal orifice.
73. The ligamentum interfemorale of *Bufo* and *Xenopus*, and the rods of Noble of *Ascaphus* fork posteriorly to extend on either side of the cloaca near its orifice.
74. In *Xenopus* the fork of the ligamentum interfemorale below the cloaca is greatly thickened and extended forwards, and serves to attach the mm. cloacae to the pelvis.
75. In *Rana* the end of the ligamentum interfemorale below the cloaca is turned forward, and it attaches to the pelvis.
76. In *Bufo*, *Bombina*, *Xenopus* and *Rana* the mm. graciles minores originate from the ligamentum interfemorale just below the cloaca. In *Ascaphus* these muscles originate from the rods of Noble in a comparable position.
77. The cloacal orifices of *Bufo*, *Bombina* and *Xenopus* bear the same relations to the ligamentum interfemorale as does that of *Ascaphus* to the rods of Noble: these orifices are hence homologous.
78. In *Leiopelma*, *Bufo*, *Bombina* and also *Rana* (cf. van Dijk, 1955) there are two cords of longitudinal visceral muscle attached to the urostyle, and two to the pelvis, in the cloacal region.
79. In *Xenopus* there are no longitudinal visceral muscle cords attached to the urostyle, and the cords to the pelvis are closely associated with each other.
80. In *Ascaphus* there are two longitudinal visceral muscle cords attached to the urostyle, but there is only one cord attached to the pelvis, although this has a dual origin.
81. The vascularized tissue in the cloacal region of *Ascaphus* develops in association with the visceral muscle layers and the mm. compressores cloacae, but does not arise from de-differentiated muscle tissue.

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