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XXXI.—The Life-History of *Xenopus laevis*, Daud.

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XXXI.—The Life-History of *Xenopus laevis*, Daud. By Edward J. Bles, B.A., B.Sc.,
Assistant in Zoology at the University of Glasgow. (With Four Plates.)*

(Read January 18, 1904. MS. received January 11, 1905. Issued separately November 8, 1905.)

INTRODUCTION.

The present communication is intended to be the first of a series dealing with observations on the life-history of the Anura Aglossa and their anatomy at different stages of development. *Xenopus laevis*, with its small ova and protracted larval free-swimming stages, must necessarily form a basis for the study of the development of that other remarkable Aglossan, *Pipa americana*. Although the adult Aglossan is an aberrant and specialised Anuran, there are Urodele features in the development of *Xenopus* which make its embryology of great general interest. These primitive features, combined with others peculiar to the genus, impress a character upon the early life-history of this frog which is widely divergent from that of the Phaneroglossa with small ova.

The fullest account of the development of *Xenopus* is contained in a short paper by BEDDARD, published in 1894. He has cited and reviewed the scanty earlier literature. Nothing has since been contributed to the subject but a note on the breeding habits by myself (1901). BEDDARD's observations were made on material obtained at the gardens of the Zoological Society of London. Specimens of *Xenopus laevis* from Zanzibar spawned there a few months after their arrival. The earliest stage observed was the larva shortly after hatching; some frogs were reared from the tadpoles. The most important new fact made known in the paper was the presence of a cement organ ("sucker"). Its structure was described. W. K. PARKER's observations on the presence of external gills and the absence of so-called internal gills were confirmed. Some details of the internal structure were described. Figures drawn from fresh specimens are given of three tadpole stages, early and late.

BEDDARD confirms the absence of horny teeth already noted by PARKER ('76) and LESLIE ('90). But he did not connect this deficiency with the absolutely different method of feeding which must necessarily follow. The food of all our European tadpoles is obtained by the scraping action of the lips with their rows of horny teeth, sometimes, but rarely, aided by the biting horny jaws. The teeth act exactly like the radula of a gasteropod and are used to rasp away animal or vegetable matter from any substratum. BEDDARD found numbers of Cyprids and nothing else in the alimentary canal of the *Xenopus* tadpoles, and concluded

* Grateful acknowledgment is due to the Carnegie Trust for generously defraying the cost of reproducing the plates illustrating this paper.

that they were purely carnivorous and adopted this diet from choice, and states that "there was plenty of water-weed on which they could have fed." These statements it is impossible to bring into harmony with the observations recorded in this paper, and it is difficult to conceive, taking the facts as known to BEDDARD, how a tadpole without any buccal hard parts could feed on water-weed. The other observations which BEDDARD records I can confirm, with the exception of only one or two minor points regarding the cement organ.

It may be convenient to give here a brief summary of the main observations described and conclusions reached in this paper.

(1) The conditions are enumerated and discussed under which *Xenopus* and other Amphibia can be induced to breed freely in captivity (pp. 795 and 796).

(2) A detailed account is given of the breeding habits of *Xenopus* (pp. 797-798).

(3) The remarkable method of oviposition is described in some detail (p. 798).

(4) It is concluded that fertilisation is external in *Xenopus* (p. 799).

(5) The egg-envelopes are described and the occurrence of a rudimentary egg-shell noted (p. 800).

(6) The segmentation of the ovum and the development of the embryo within the egg are described and figured for the first time (pp. 801-806).

(7) The late embryo is shown markedly to resemble corresponding stages in Urodele development.

(8) The posterior ends of the medullary folds are found not to enclose the blastopore nor meet behind the anus (p. 803).

(9) The early development of the face is described and frontal views figured (pp. 806 and 811).

(10) The "frontal gland" and its secretion are shown to be functionally concerned in the hatching process (pp. 807-809), and the development of the gland is described (pp. 804, 805, and 806).

(11) It is shown not to be connected with the formation of the neuropore and, as it is not a sense-placode, lends no such support as v. KUPFFER claimed it did to his theory of monorhiny and amphirhiny (p. 809).

(12) The process of hatching in an Anuran is described for the first time (p. 807).

(13) An account is given of the habits of the tadpole after hatching and before it begins feeding (p. 810).

(14) The development of the pectoral lymph-hearts at this early stage is noted for the first time in an Amphibian (p. 812).

(15) The *Xenopus* tadpole is shown to breathe by its lungs as soon as it begins to feed (p. 812).

(16) The development of the cement organ is traced from the first appearance to its disappearance (pp. 803, 804, 805, 806, 811, and 813).

(17) A brief account is given of the feeding habits. The manner of taking food is seen to be remarkably similar to that of *Ammocetes* (pp. 813-814).

(18) A few cases of branching tentacles in old tadpoles are figured, and it is argued from the frequent symmetry of the branching that there is a congenital tendency to branch and that the branching is not due to regeneration after injury. The branching, if the above view is correct, is evidence in support of the theory that the tentacles are external gills of the mandibular arch (pp. 814-816).

(19) A very curious difference between the behaviour of the dark chromatophores of the head and abdomen and those in the distal part of the fin-fold is described. The latter expand at night, while the former contract (p. 816).

(20) The arm is developed in a sac shut off from the gill-chamber. When it is protruded it is found that the action of the branchial current is not interfered with as in terrestrial Anura and feeding by the branchial current goes on as before (p. 817).

(21) The external features of the process of metamorphosis are described (p. 817).

(22) The young frog is found to feed on small Crustacea, chiefly *Daphnia*, like a young Urodele.

(23) One specimen, a male, was seen to become sexually mature when two years old.

In this paper very little is said about the internal anatomy of embryos and tadpoles. It is hoped that this omission will be made good later.

METHODS OF PRESERVATION, EXAMINATION, ETC.

It is not proposed to give full particulars here of all the methods used in this investigation. There are, however, one or two new devices which may be useful to others and are therefore worth recording.

The early stages of *Xenopus* (segmentation, gastrulation) are best preserved in a 4 per cent. formaldehyde solution, after stripping all the jelly from the vitelline membrane. The latter is so close-fitting that it cannot be removed from the living egg in these early stages. When the embryo elongates, the vitelline membrane swells up and can easily be removed. From this stage onwards the best preservative for general purposes is the one formulated below. Of course, for some special stains, special preserving fluids are indicated, such as corrosive sublimate for HEIDENHAIN'S iron-haematoxylin, and so on.

The following mixture was made as the result of experiments to discover a killing and fixing fluid with the advantages and without the faults of PERENYI'S fluid. As in PERENYI'S fluid, the basis is strong alcohol, but glacial acetic acid replaces the nitric acid, and formalin the chromic acid. The fluid has greater penetrating, fixing, and hardening power than PERENYI'S, and it has the same great advantage of not making the yolk hard and brittle. The nuclear structures are far better preserved than they are by PERENYI'S fluid, mitotic figures are often perfectly fixed, and the embryos and tadpoles of all ages are killed almost instantaneously if they are transferred to the preservative with a minimum quantity of water.

Mix 90 c.c. of 70 per cent. alcohol with 3 c.c. of glacial acetic acid. Any

quantity of this mixture can be made as a stock solution, as it keeps indefinitely. Just before use add 7 c.c. of formalin (40 per cent. formaldehyde solution) to each 93 c.c. of the above stock solution. This killing and preserving fluid contains :—

90 vols.	70 per cent. alcohol,
3 ,,	glacial acetic acid, and
7 ,,	formalin, in every
<hr/>	
100 vols.	

The fluid cannot be used with confidence when more than a fortnight old for killing, but embryos and larvæ of Anura, if killed in a large quantity of the fresh fluid, may be left in it indefinitely for preservation. The same reason can be given for both these statements, viz. that the fluid after a fortnight has begun to decompose, which impairs its killing and hardening powers but not its preserving property. The formalin and acetic acid both disappear from the fluid sooner or later, as can easily be proved by the disappearance of their characteristic odours; these are replaced by an aromatic odour mixed with that of the spirit, and this mixture is as good a preservative as pure 70 per cent. alcohol. This property of purifying itself, as it were, makes the fluid particularly useful for recommending to collectors at a distance. Specimens can be killed in it and then either sent off in the same fluid or forwarded in a change of the fluid after twenty-four hours, according to the bulk of the specimen and the relative size of the bottle or jar. It will then travel any distance without further preparation.

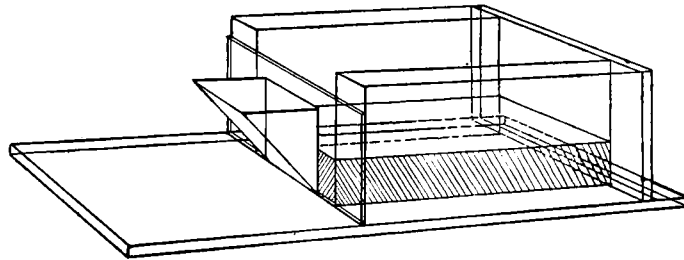
From the reports of friends and colleagues who have used this mixture and from my own experiments, I gather that it is useful for the most diversified objects, from the eggs and yolky larvæ of Echinoderms to the larvæ and adults of *Anopheles*, the newly hatched fry of *Salmo fario*, and a full-grown *Ammocete*. It is, judging from these examples, worth a trial on almost any object, especially yolky embryos. Of course it decalcifies and is useful for preserving and decalcifying small Craniates or their heads when required for sections. The specimens should be transferred from this fluid to 50 per cent. alcohol, washed and passed into 70 per cent., and can then be treated as required.

A Simple Prism Reflector.—All students of Anuran tadpoles have sooner or later felt the want of a convenient method of examining these objects from all points of view without the risk of damaging the specimen. A simple and cheap means of carrying this out is to build a trough as shown in Text fig. 1. The base is an ordinary 3 inches by 1 inch or 3 inches by 1½ inch glass slide. The sides of the trough are of plate glass; the right-hand end is a piece of ordinary thin glass; the end over the middle of the slide is a piece of No. 2 or No. 3 cover-glass.

The cementing can be done in a few minutes if marine glue is used; the objects can then only be examined in water or formalin, but if the trough is to contain spirit the cement used must be carefully applied and allowed to thoroughly harden before use. Bichromated gelatine, Lovett's cement, or some such spirit-proof cement can be used.

When an object is to be examined under the microscope, a thick piece of glass or several thin pieces are laid in the bottom of the trough, the appropriate fluid poured in, and the object then placed as close as possible to the cover-glass end of the trough, with the surface of the object so placed that by looking through the end of the trough horizontally the required view of the object can be obtained. But the same view can be seen by fixing a right-angled prism against the cover-glass and examining the reflection of the object thrown up vertically from the internal surface of the hypotenuse side, and the whole arrangement can be put on the stage of a vertical binocular microscope with the objective over the horizontal face of the prism and the reflected image enlarged by the use of low powers. With the arrangement shown in Text fig. 1 it is possible to use a Zeiss A objective. The prism is fixed to the cover-glass with a drop of cedarwood oil or castor oil or glycerine. The object is easily moved about into any required position while the eyes are at the microscope, as the lower end of the tube does not come in the way of the right hand.

The side views of eggs and the frontal views of larvæ figured on the plates were drawn with the help of this little appliance.



TEXT FIG. 1.—Glass Trough with prism for internal reflection. (Natural size.)

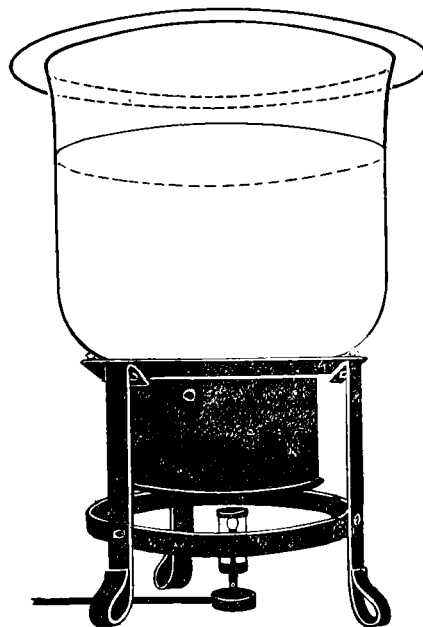
Before concluding these introductory remarks, I wish to acknowledge very gratefully the help of the artists who have so patiently and carefully carried out my wishes in making the illustrations for this paper. A member of the zoology class of 1903, Mr HORATIO MATTHEWS, kindly provided the drawings of figs. 12, 13, and 14 from the living embryos. The rest of the drawings (excepting four) are from the skilful brush of Mr A. K. MAXWELL, whose work I have controlled and confirmed throughout. Figs. 16, 17, 18 and 19 were sketched by myself from the larva as it hatched, and these sketches have been carefully elaborated, with the help of the identical specimen (killed five minutes after hatching), by Mr E. WILSON of Cambridge.

OBSERVATIONS ON BREEDING AND DEVELOPMENT.

Breeding Habits.—My embryological material has all been obtained from specimens of *Xenopus* which I have now kept in captivity for seven years, since December 1896. Spawn was first obtained in February 1899, when the frogs were in the Tropical Lily Tank of the Cambridge University Botanic Garden, and a note on the

observations then made has already been published (BLES, '01). Since then the same female has spawned during the spring and summer of 1901, 1902, and 1903, under conditions easily established anywhere. The methods adopted are possibly more or less applicable to the breeding of other Amphibia in confinement, and I will therefore enter into details.

In the first place, the most necessary condition of success in this and similar cases is that the frogs should be allowed to hibernate. But, in order to accomplish this successfully, a frog must be in the best health and condition when the winter sets in, and must have passed the summer in the best circumstances as regards heat, light, and food supply.



TEXT FIG. 2.—Tropical Aquarium described in text. The diameter of the bell-jar is 20 inches; other parts are in proportion.

Xenopus is practically a purely aquatic animal, probably more so than any caducei-branchiate Urodele, and should be kept in an aquarium at a tropical heat during the summer in a place which is reached by the early morning or the evening sunshine. The direct rays from the sun will thus not strike the aquarium for more than an hour or so. The form of tropical aquarium I have found perfectly successful is one devised by my friend the late Mr J. S. BUDGETT, who kindly gave me permission to describe it here.

Text fig. 2 shows a bell-jar 20 inches in diameter standing on an iron tripod. The circular ring at the top of the tripod is slightly dished inwards to adapt it to the bottom of the bell-jar. Upon the upper surface of this ring rests the flange of a galvanised iron tank containing water, and heated below by a Zeiss micro-burner (Bunsen). This tank acts as a water-bath, and is kept 10°-15° C. hotter than the aquarium, according to the quantity of water in the latter. I find that this particular

burner is very convenient, as the temperature of the aquarium remains constant sometimes for weeks together, and a variation of a degree can be put right by slightly lowering or increasing the flame. Between the bottom of the aquarium and the flange of the water-bath I have a coil of three turns of asbestos cord, which keeps the glass away from the hot metal, and prevents evaporation from the water-bath.

In such aquaria my *Xenopus* have now lived for years, and in them they have spawned, and the tadpoles been reared, in one case to maturity.

The bottom of the aquarium is covered with earth and stones, and *Vallisneria* thrives in it. During the summer the *Xenopus* are kept at about 25° C., and the temperature may rise occasionally to 28°–30° C. They are fed daily with small earth-worms or thin strips of raw calves' liver, and are fed until they refuse to eat more, which they do in a comical manner, by pushing aside the food with the palm of the hand when it is held near them. The water in the aquarium is never changed.

In December the temperature is allowed to sink to 15°–16° C. during the day, and it may sink to 5°–8° during the night. The frogs then become lethargic and torpid, take no food for days, eat very little at a time and move about rarely, spending very little time, at any rate during the day, at the surface of the water. I have on a few occasions approached the aquarium very quietly and found one or the other of the frogs lying flat on the stones with what appeared to be a translucent film dimming the brightness of its eye.

A sudden noise, however slight, makes the frog start up, and the film moves slowly outwards and forwards, uncovering the eye, and is recognised as the lower eyelid. The frog then moves away. Judging from analogy, one might conclude that the frog had been aroused out of its sleep. Whether this conclusion is justified or not, the facts seem worth recording, as the evidence of the occurrence of sleep in the lower vertebrates is somewhat slender.

When the temperature of the aquarium rises in the spring and the days become brighter, a change in the behaviour of the frogs becomes evident. The female and males spend a great part of the day at the surface of the water with eyes and nostrils above the surface. The males become exceedingly restless, swimming about with an air of wishing to escape from the aquarium. Both sexes are now very shy, and difficult to feed.

There may or may not already be attempts at pairing, but by taking the following measures pairing will take place immediately (with or without spawning), or at least the male, after being silent the whole winter, will commence to croak at once. First, the temperature of the aquarium is raised to 22° C. and, secondly, when it has become constant, a certain amount of the water, say 2 gallons, is drawn off morning and evening, allowed to cool for twelve hours, and then run in slowly in the following manner, in order to simulate the fall of rain. The cooling vessel is raised above the level of the aquarium, and a syphon is used to run off the water. The lower end of the syphon is drawn out to a fine point, and turned up in such a way that the water rises up like from a fountain, and falls as spray into the aquarium. The third condition

to be established is to bring the water into such a state that the larvæ will find their food when it is required. This condition will be explained later (pp. 813-14).

By carrying out such measures I obtained from one female between April and July 1903 more than fifteen thousand eggs. Of these, twelve thousand were taken out of the aquarium and counted, and the remainder were estimated at three to five thousand.

Some general significance may, I believe, be attached to the results in breeding this frog, the more so as they are in accordance with other similar results obtained by SEMPER (1878) with Axolotls and by myself with Axolotls, *Triton waltlii*, and *Discoglossus*.* SEMPER showed that by feeding them copiously, and by keeping Axolotls crowded together in small vessels, he could obtain spawn from the same individual three or even four times a year after a sudden transference to an aquarium stocked with growing plants, with stones on the bottom and supplied with running water. I have repeated his experiments and can fully confirm his statements. With *Discoglossus pictus* I have had a similar experience. Specimens kept in a small vivarium for four and six years have been given a superabundance of food during the summer, allowed to hibernate, and, when they showed signs of readiness to breed, a little tank in a corner of the vivarium has been filled with suitable pond water, and invariably within forty-eight hours the frogs have spawned. On two occasions the males have taken to the water in the spring and assumed their nuptial characters, but for several weeks the water which had stood in the tank during the winter has been allowed to stand. No pairing took place, but as soon as the water was changed spawn was deposited in twenty-four hours. Two female *Discoglossus* have each spawned twice every summer for the last three years, just as they do when free. Two pairs of *Triton waltlii*, which I have reared from larvæ, have spawned when two years old. In their case the same treatment was carried out. They were well fed in the summer, kept cold in the winter, and fresh water added to the aquarium when they showed readiness to pair.

These various experiences appear to indicate that the difficulty met with in breeding Amphibia kept in confinement is not due to any toxic influence on the gonads due to the results of close confinement. Darwin was inclined to believe that the functions of the generative organs were sometimes impaired by captivity, but unless and until concrete evidence is given to show what specific influence is at work, it would very often be simpler to assume that the external conditions are unfavourable for breeding, or deficient.

In the case of *Xenopus* all the other conditions may be present, but if there is no daily change of the water there is no oviposition, and although the male may embrace the female, the behaviour of the latter clearly shows that she is not ready to spawn.

If the view is correct that breeding is brought about in animals, especially in those with a fixed breeding season, as the response to a certain set of definite external stimuli on the sexually mature of the species, it may help to explain

* And also by P. KAMMERER ('04) with *Salamandra maculosa* and *S. atra*.

why some animals appear in great numbers in one year and are much less numerous in another. Entomologists are familiar with this phenomenon, and it may be worth considering whether such fluctuations in numbers are not due to causes of the nature indicated.

It is obvious that changes in the environment affecting the breeding habits might lead to rapid divergence through the action of Natural Selection, and the diversity in the breeding habits of allied tailless Batrachians has perhaps been established through the agency of such induced sterility.

The male *Xenopus* begins to assume nuptial characters a couple of days after the temperature is raised to 22° C. The dorsal surface of the hand darkens and the area covered with nuptial asperities extends along the arm towards the axilla; the whole patch blackens from the hand inwards in the course of about two days. The shape of the patch has already been figured (BLES, '01).

The abdomen of the female becomes very much distended during the winter by the enormously enlarged ovaries, so much that the lungs are displaced upwards and raise the dorsal body-wall on either side of the vertebral column into two great projecting longitudinal humps. The three flaps of skin surrounding the cloacal aperture are flaccid until the spring, when they become swollen and turgid and more highly vascularised. I was unable to detect any change in the epidermis of the breeding female until last year (1903), when the back of the hands became darker at the same time as the nuptial asperities appeared in the male. Special attention was paid to this point in the seasons before, and it is certain that nothing of the kind occurred then, so that it appears that a secondary sexual character of the male is making its appearance with age in the female (see BOULENGER, '97, p. 72, for similar cases).

During the first week of the newly established spring conditions the males become vocal. They have been silent throughout the winter, and their first attempts are intermittent and low in tone. Their voice strengthens from day to day, and at night-fall, especially if fresh water has been added, becomes a loud and continuous metallic rattle, kept up for hours with hardly a break. The noise made by a single frog is loud enough to be heard at a distance of 100 yards or more in the open.* It resembles the voice of *Hyla arborea* more than that of any European frog, but has two alternating notes extremely like those made in winding up an old grandfather's clock with a crank handle. By rubbing the corrugated handle of a pair of large forceps backwards and forwards against the rounded edge of an empty tin tobacco box, I have imitated the sound so exactly that the frogs have responded. The croak is produced under water, and although air is no doubt passed to and fro between the lungs and the buccal cavity, there is no movement of the pectoral or gular region visible externally.

Normally, pairing only occurs at night. The male croaks loudly and incessantly

* I had not heard its full strength when I made the statement in my former account (BLES, '01, p. 211).

during the twilight until he seizes the female. There is nothing resembling the courtship of Urodeles. The male makes a sudden dash and clasps his mate round the lumbar region; his arms are too short to meet on the ventral surface. In the amplexus being inguinal, *Xenopus* resembles the Discoglossidæ and Pelobatidæ (BOULENGER, '97, p. 69). The note of the male changes at the commencement of pairing. In a low tone he utters "cô, cô, cô, cô," and at each syllable strikes the under side of his head against the back of the female. Between each stroke the floor of the mouth of the male is seen to bulge considerably so as to carry his head away from the back of the female. When spawning begins he is silent, but every now and again while the amplexus lasts he croaks loudly during the short intervals when oviposition stops.

The account given by LESLIE ('90) of the breeding of *Xenopus* speaks only of spawning taking place in August, i.e. the South African spring.

According to my observations, I should conclude that, like Discoglossids, the same female may spawn in the wild state several times during the spring and summer, and that the males are ready to pair at any time in those seasons. My female *Xenopus* had spawned for the first time in the year in February (Cambridge), April (Cambridge), and May (Glasgow), in successive years, for a second time in June, and a third time at the end of August in the same summer (Glasgow, 1902); in the following year three batches of eggs were laid at corresponding times. Thus the animals became acclimated to a difference of six months in the seasonal changes.

Each batch of mature eggs was usually deposited in the course of five days, one night in which pairing did not take place intervening between the nights when eggs were laid. On one occasion spawning took place on five nights; between the third and fourth night of spawning there was an interval of three nights; the others were on alternate nights as usual.

It has so often happened that male and female have been seen to cast their skin the morning after pairing, that it is probable that ecdysis is usual at this time. The skin is loosened all over the body, the legs are then freed, and the skin which is attached to the snout is drawn forward, crammed into the mouth, and eaten in exactly the same way as it is by many terrestrial frogs.

Oviposition.—The amplexus is continued from the evening until the next morning, and may last until 9 A.M. Spawning does not commence immediately, but may begin an hour after pairing. From this time onwards eggs are laid at frequent intervals all through the night. As a rule the eggs are laid singly, and the pair swim about or come to the surface to breathe between each act of spawning. But occasionally three or four eggs are laid in quick succession in the same spot, and somewhat rarely eight to ten eggs will be emitted in a group.

The egg is held between the three protruding flap-like lips of the cloacal spout, while the pair swim about restlessly for half a minute to two minutes; the female then grasps a long leaf or a twig of water-plant between her outstretched feet, and the pair

come to rest in such a position that the cloacal spout of the female becomes applied to the anterior end of a shallow median groove on the ventral surface of the male, which runs back to the cloacal opening of the male for about three-quarters of an inch. This groove is formed by two skin folds over the ventral edge of the pelvic symphysis. The egg is passed out, travels rapidly along this groove, over the cloacal aperture of the male and directly backwards about 4 inches to the weed held by the outstretched legs of the female, where it adheres. The egg has to travel about 5 inches from the female to the weed, and is carried this distance in a straight course. This is partly due to the fillip it receives from the tumid lips of the cloaca as it passes out, and partly to a backwardly directed current in the water, created by gentle swimming movements of the feet of the male. The pair immediately swim away, another egg appears in the cloacal spout of the female, and the process is repeated. As each egg or group of eggs is laid, a spasmodic quiver can be seen momentarily passing over the body of the male, and at this time, I have reason to believe, a very small number of spermatozoa are emitted.

Fertilisation.—The curious method of oviposition resembles in the action of the female the spawning of Urodeles and is so unlike that observed in the Phaneroglossa, that the question of fertilisation is raised. Quite a number of considerations point to the conclusion that each egg is fertilised as it is laid and after it has passed into the water, but all attempts to secure spermatozoa in the water as the eggs were laid proved unsuccessful, as were also attempts made to observe fertilisation in the living egg.

One set of observations repeated at different times proves, I believe, that fertilisation does occur after or during deposition of the egg, as in other Anura. The eggs when attached always have the dark pole below, and within half an hour rotate within the egg-membranes, so that the dark pole is above and the light pole below. In *Xenopus* eggs which are unfertilised this rotation does not take place at all as a rule, or may be incomplete or take an hour or more to complete. This agrees exactly with the rotation described in eggs of other Anura with external fertilisation (R. HERTWIG, '03, p. 534).

Further, there are at each spawning a number of eggs (100–200) which do not become attached, presumably by accident, but fall to the bottom of the aquarium. It is exceedingly rare to find a fertilised egg among these. This seems to point to the conclusion that a very limited number of spermatozoa are emitted, otherwise it is difficult to understand why these eggs should not have spermatozoa carried to them in a small aquarium with water kept in constant motion by the active pair of frogs. Every now and then during spawning an egg is passed which does not pass along the ventral groove of the male in the normal manner, and these drop to the bottom. This would account for these eggs not being fertilised. It is hardly probable that they are all immature eggs; that would not account for them not having been attached, as they are in other respects quite normal. One of these ova has been figured in fig. 1. Plate I.

The Egg-envelopes.—The diameter of the whole egg when laid varies between 2.75 and 3.0 millimetres. It is surrounded by a layer of transparent jelly-like substance,

the outer coat being extremely adhesive. One result of this is that the eggs stick to the first foreign body they touch in the water. Another consequence is that the eggs become coated with a thin layer of mud when laid in turbid water; the suspended particles stick to the surface. The appearance of an egg laid in fairly clear water is shown in fig. 11, Plate II. Within a few hours the outer layer changes in consistency; it becomes hard and horny, and floating particles no longer stick to it. The horny coat is exceedingly tough, and might be regarded as a rudimentary egg-shell. In this capacity its function would only be transitory, as on the second day after spawning this outer envelope splits and its contents ooze out. The substance of the outer coat is so tough and unyielding that the contents are tightly pinched as they issue; the jelly and the soft embryo itself are both constricted between the lips of the chink. The egg thus freed is composed of a thick outer layer of jelly, the vitelline membrane and the elongated embryo lying in the fluid within the vitelline membrane (see fig. 12, Plate II). The whole remains adherent to the original place of attachment; the outer coat shrinks and forms a shrivelled ring round the place of attachment of the egg to the substratum. The escape of the egg-contents only occupies a few minutes.

A very similar shell-like structure, which seems to be undescribed, is found in the egg of *Hyla arborea* var. *meridionalis*. Here it is not superficial, but enclosed in a thin, soft, adhesive layer which holds together a number of eggs in a clump. Inside this layer is a tough, thin, whitish translucent membrane; then follows a layer of jelly and then the vitelline membrane. This "egg-shell" is also burst at an early stage. On the third or fourth day after the spawn is laid, the spherical shell is found in two hemispherical pieces lying embedded next to each egg in the jelly of the clump. In this case the split extends meridionally completely round the "shell." The splitting of this membrane in the case of both *Xenopus* and *Hyla* is most likely due to the absorption of water by the inside jelly and its consequently swelling until the internal pressure bursts the non-extensible membrane. This membrane is comparable with the true membranous egg-shell discovered by GUPPY in the large eggs of *Rana opisthodon*, which are laid in the crevices of rock and enclose the young frog until perfectly formed.

The Ovum.—The history of the ovum is here taken up at the period of oviposition. It then measures 1.5 millimetres in diameter, so that it ranks among the smaller Anuran eggs.

While rotating within the egg-membranes after fertilisation, it can easily be seen that the pigmented and unpigmented areas of the surface are approximately equal (see fig. 1, Plate I). The pigmented area usually covers rather less than a complete hemisphere. The dark half is of a rich brown colour, while the yolk is of a very pale greenish-blue colour.

Fig. 1 represents an unfertilised egg about twenty-four hours after oviposition. It is easily recognised by the patch of unpigmented protoplasm which has risen with the egg-nucleus inside it to the surface, and has displaced the superficial layer of dark pigment from the upper pole. This appearance is very characteristic of unfertilised

eggs on the second day after spawning. In other respects the egg shows the normal appearance before segmentation commences.

Segmentation.—The details of segmentation do not appear to differ much from those seen in small eggs of other Anura, and are, therefore, not described in detail. I have had figures of a few stages made as accurately as possible to show the general course of the processes.

The first furrow is, as usual in Anura, meridional and divides the eggs into two equal blastomeres. It is completed one hour to one and a half hours after fertilisation. The next vertical furrow appears within two and a half hours after fertilisation, and the third (horizontal) one within the next hour. The third furrow does not form exactly parallel to the equator, but is bent in the manner shown in fig. 2, Plate I. The egg is viewed here from the right side, according to the statement made by O. SCHULTZE and KOPSCH to the effect that the unpigmented portion of the egg reaches much nearer the upper pole on that side of the egg which is to become the posterior end of the embryo. In this egg there is at the upper pole a marked departure from the radial symmetry which, as shown in fig. 2A, is still present at the lower pole. The egg has become bilaterally symmetrical with an elongated and a more rounded cell on each side at the upper pole. The pigment is not altogether confined to the cells of the upper hemisphere; there is a patch posterior to them (fig. 2, Plate I). The egg represented in figs. 3 and 3A, Plate I, is at a stage reached about four hours after fertilisation. The segmentation has become irregular, especially of the yolk cells. There is still a marked difference in the bulk of the cells of the upper and lower hemispheres, but the latter are now rapidly dividing. Furrows start from the edge of the pigmented areas and extend downwards over the yolk cells until they meet an existing furrow near to, but not accurately at, the lower pole of the egg. At the stage shown in fig. 4, the cells of the upper and lower hemispheres are almost equal in size and are again arranged fairly regularly. This return to a regular arrangement must be a result of the mobility of the superficial cells, together with the need for a geometrical arrangement in order to accommodate a given number of cells of a certain size in a limited space. That the cells of both the upper and lower poles are movable to a certain extent can easily be observed in the living egg. Newly divided cells may push apart two cells which were in contact with each other until the edges which were touching are separated by the whole diameter of the intruding cells. If there are intercellular strands of protoplasm at this stage they must certainly become broken in the shifting about of the cells.

At the stage shown in fig. 5, the cells have become smaller and have lost both the regular arrangement and the roughly hexagonal outlines seen in the earlier stage. The yolk cells near the equator are dividing more rapidly than those at the yolk pole and are appreciably smaller. The living segmenting egg at this stage has a feature which is shown here in the figure (fig. 5) of a preserved specimen. A number of cells at the margin of the spreading epiblast are of much paler brown

than the neighbouring epiblast cells. This difference in tint is found, by watching the cells in a living egg, to be the sign of an approaching division of the pale cell. The pigment reappears apparently undiminished in quantity after division. The cause of such cells turning pale at this particular time I hope to discuss later, as there appears to be some parallel between this process and that of contraction in the chromatophores.

Gastrulation.—A stage of the development of the blastopore is shown in figs. 6 and 6A. In the side view (fig. 6), the egg is shown in the vitelline membrane and lying with its orientation as in life, the lowest point on the egg sphere being 30° behind the middle of the dorsal lip of the blastopore. This view shows very clearly features not easily shown in the ventral view, namely, the extent of the depression of the posterior lip of the blastopore and the sharp, slightly puckered edge of the dorsal lip, which is not depressed, but remains up to the edge coincident with the surface of the sphere of the egg. At this stage indications are already noticed of the arrangement of ectoderm cells in rows, forming alternating light and dark streaks lying in the position of great circles roughly coaxial with the great circle passing through the longitudinal axis of the embryonic area. These streaks are indicated in fig. 6, passing from the posterior edge of the upper pigmented area towards the blastopore. In fig. 6A, the lower ends of these streaks are seen near the right-hand angle of the blastopore. In this figure (6A) the blastopore has reached the greatest lateral extension it attains as a crescentic opening.

In fig. 7 the egg is seen from behind, and a stage is represented where the blastopore has just become circular. The yolk plug does not protude; at no time does it become prominent in *Xenopus*. The pigmented cell-area now extends back to the edge of the upper lip of the blastopore. A peculiar feature of this stage is the constant occurrence of alternating bands of darker and lighter cells arranged as described above in the previous stage figured (fig. 6). The observation that epiblast cells undergoing mitotic changes become pale at certain stages of the process leads to the interesting conclusion that the lighter patches are areas in which the epiblast cells are proliferating simultaneously.

The Embryo.—In figs. 8 and 8A, representing views of the same egg seen from behind and from before, an early stage of the development of the medullary folds is shown. The view from behind (fig. 8) shows that the medullary folds are externally much less obvious at the hinder end than they are in the head region seen in fig. 8A. On the dorsal contour of the latter figure, the gentle elevations are due to the swollen medullary folds, and the slight notch between them indicates the neural groove (*Rückenrinne*). The neural groove extends far forwards, as far as the anterior wall of the brain, and can be seen between the paired dark patches on the exposed floor of the thalamencephalon. These areas are occupied by the pigmented cells of the optic vesicles, and closely resemble those described by EYLESHEIMER in *Rana palustris* ('93). Returning to the medullary folds, it will be seen from fig. 8A that at about the middle of their length the medullary folds have converged from the anterior end to lie side by side. It is thus possible to identify

the elevations on the upper contour of fig. 8, Plate I, near the middle line as the medullary folds, otherwise it would be difficult to make out their position in the posterior view of this stage. They flatten out and disappear towards the blastopore. On the outer side of each medullary fold is a band of pale ectoderm cells which can be seen at the points *a* and *b* to be slight ridges. These bands of proliferating ectoderm cells meet at a point just anterior to the future anal perforation, marked at this stage by a small pigmented area at the hinder end of the closed and elongated blastopore. As these bands lie external to the medullary folds, it is certain that at this stage the medullary folds do not enclose the blastopore or meet behind the anal opening. The archenteron is now completely closed to the exterior, as for a short time the blastopore is quite closed: there is no anus. The anal opening is formed in a few hours after this stage has been passed through. The neurenteric canal and postanal gut persist for a long time; they are present at the stage shown in fig. 14. The dark area (fig. 8A) anterior to the medullary folds, and extending a short distance back along the sides of the fore-brain region, is a region covered with modified, deeply pigmented ectoderm cells, the greater number of which are destined to become secretory epithelium cells. The median ventral portion of this area is the precocious rudiment of the cement organ ("Sucker"); the lateral dorsal portions contribute to the formation of the frontal gland ("Stirnknospe," v. KUPFFER, "Stirnstreifen," HINSBERG). This area corresponds in its position and relations to other parts exactly to O. SCHULTZE's "Sinnes-platte," MORGAN's "Sense-plate." It is not to be regarded as a lateral extension of the medullary plate (MORGAN, '97, p. 57). It is a part of the general ectodermal covering without any obvious connection with the central nervous system and formed by differentiation in the cells of the superficial epidermic layer of the ectoderm only. The deeper nervous layer of the ectoderm is not modified and there would seem to be no justification for the term "Sinnes-platte."

The portions of the ectoderm from which the epithelium of the nasal pits is derived are enclosed by or are possibly included in this dark area; it is not possible to identify them at this stage.

The medullary folds have arched over and their edges have met along their whole length in the next stage figured (fig. 9). The ectoderm is raised as a gentle ridge over the closed neural tube, and there is a line of deeply pigmented ectoderm cells along the median external line of junction of the folds, and at the bottom of the shallow groove formed by the rounded edges of the folds. The anterior end of this pigmented groove marks the position of the neuropore. A short distance posterior to the neuropore, the pigmented groove is intersected by a crescentic band of pigmented ectoderm, the early rudiment of the frontal gland. Below the position of the neuropore is a large pigmented patch of ectoderm, the rudiment of the cement organ. It shows some indication of a paired nature in the presence of a more deeply pigmented patch at each end of the transversely elongated area. This figure (9), compared with fig. 8A, shows that the greater part of the pigmented area in front of the medullary folds at the earlier stage becomes

cement organ at the later stage. And the postero-lateral horns of this crescentic area (fig. 8A) are carried inwards towards the median dorsal line as the roof of the fore-brain closes in and give rise to the transverse band of cells I have called the *frontal gland*. This will be dealt with in detail later. We shall see that the area shown in fig. 9 enclosed by the line of the frontal gland and the upper edge of the cement organ is the part from which the anterior organs of the face develop, viz.—the stomodæum and the nasal pits. At this stage the egg has just begun to elongate in the direction of the future axis of the body.

In fig. 10 an early stage in the elongation of the larva is represented, and it will be at once observed that the dorsal concavity which is so marked a feature of corresponding stages in all such forms as *Rana*, *Bufo*, *Hyla*, *Bombinator*, and *Discoglossus* is not only absent here, but there is a distinct convexity of the dorsal contour in side view—the two ends of the embryo are slightly bent ventralwards, so that there is a shallow ventral concavity. It is this feature in its general appearance which in early embryonic stages of *Xenopus* recalls rather a Urodelan embryo than one of the familiar Anuran embryos. The difference is due mainly to the fact that while the embryo of the typical *Phaneroglossa* owe their increase in length from the beginning to the elongation of the tail and sometimes of the head also, the abdominal region remaining short, the embryo of *Xenopus* for sometime grows in length in the trunk region, the tail remaining short and stunted. Connected with the checked growth of the trunk in the forms mentioned above is the persistence of the anus in its position high up on the posterior surface of the egg, which involves the outgrowth of the tail, so as to make it sprout not directly backwards but obliquely upwards at an obtuse angle with the long axis of the body. In *Xenopus*, on the other hand, the growth takes place in such a way that the anus is swung round from its equatorial position on the spherical egg to a ventral position in the elongating embryo, as indicated in fig. 10. As indicated in figs. 10 and 12–14, the tail remains a mere stump, while the trunk elongates considerably. A form of embryo results, bearing considerable resemblance in the proportions of its main regions to Urodele larvæ and also to young Dipnoan larvæ, where the anus is placed close to the posterior end of the body at the root of a very short tail. This short-tailed, long-bodied phase of the development of *Xenopus* may, with some confidence, be looked upon as primitive.

Returning to stage 10, it will be seen that the region of the spinal cord is pinched up, as it were, from the ventral part of the trunk, and the fore-brain swells out the tip of the head. Behind the fore-brain is an elevation of the whole branchial region. There is an accumulation of yolk at the posterior end producing a swelling out of that part. The fin-fold has not made its appearance. The cement organ is beginning to assume its characters, showing a compact group of cells with densely pigmented inner ends and outer ends filled with a clear mass of secretion. At the extreme anterior end is placed the frontal gland, seen better in the frontal view (fig. 10A). This shows how the frontal gland has become continuous with the pigmented band of cells along the sagittal line.

The position of the neuropore is indicated by a few pigmented cells forming a short longitudinal streak exactly at the extreme anterior end of the animal. Between the neuropore and the cement organ is the region of the stomodæum.

The next change in the external appearance of the embryo is due to the outgrowth of the median fin-fold of the tail. An early condition is shown in fig. 12. The trunk is slightly longer than before (stage of fig. 10), and the back of the embryo has become straighter.

The growth in length of the embryo has stretched out the vitelline membrane into an ellipsoidal form. In the particular embryo drawn the cement organ happened to be larger than it is usual to find it at this stage. The line of the frontal gland is well defined; it extends ventrally as far as the edge of the cement organ, and between it and that edge is enclosed a patch of deeply pigmented ectoderm. The portion of the median fin-fold to develop first is that bordering the tail region; in fact, the extent of the tail region is fairly well defined by the limits of the fin-fold at this stage. The fold is deepest in the post-anal ventral part, extends round the posterior end of the embryo, and fades away just before reaching the dorsal surface.

When the embryo has reached a length of 3·8 millimetres (fig. 13) the fin-fold has grown up along the dorsal surface and extends forwards as far as the part over the hind-brain, practically marking out the whole length of the organ. The fin is still deepest in the post-anal ventral portion. It is necessary at this stage to distinguish between a ventral post-anal abdominal part of the tail into which the posterior end of the yolk mass is drawn out, and a dorsal part which, it will be seen, grows out more vigorously and gives rise to the segmented, muscular part of the tail. In the head there is remarkably little indication externally of the developing eye, brain, and visceral arches. The position of the eye can just be discerned by the dark patch drawn in the figure; the mandibular arch is slightly raised above the general surface, and the posterior group of visceral arches is just discernible as another broad and gentle elevation on each side of the head. The line of the frontal gland is very distinct and obviously continuous with the cement organ at its lower ends. The cells of the cement organ are filled with clear secretion at their outer ends, and thus produce an appearance of a low translucent ridge running across the dark cement organ from side to side. No indications of nasal pits or of the stomodæum are to be seen. The myotomes are just beginning to be visible externally in the hinder trunk region.

Further growth is shown in the embryo of fig. 14, drawn after removal from the egg. The tail has grown out more especially in its dorsal muscular part, and the fin-fold of the tail has now become widest along this muscular part. The post-anal abdomen has also been slightly drawn out in length by the growth of the tail. The dorsal fin has become higher, and now reaches forwards on the head as far as the hind level of the fore-brain. Numerous myotomes can now be seen in the living embryo. The eye is plainly seen and also indications of the lens thickening. A bulging behind the branchial region indicates the position of the heart; more dorsally the ear vesicle

may be seen faintly through the skin, and a swelling shows the position of the pronephros.

All the preceding stages are still enclosed within the egg. We now pass on to the larvæ at the time of hatching. Fig. 16, Plate III, shows the larva at the normal stage of hatching with the vitelline membrane now enormously swollen out. The more or less thin layer of jelly which surrounds the membrane has not been drawn. Compared with the last stage illustrated (fig. 14), the muscular portion of the tail has grown, while the abdominal portion of it has become insignificant. The length of the tail (fig. 15A) is still only about half the length of head and trunk together; the fin-fold has not yet become a powerful swimming organ. The head is now for a time divided from the trunk by a constriction, a neck, which is the more marked since the trunk is swollen just behind the constriction by the bulging of the skin over the pronephros (see fig. 16, Plate III). On the somewhat flattened anterior surface of the head the following can be made out (fig. 15, Plate III). On the ventral surface of the head is a conspicuous cement organ projecting downwards and forwards, exceedingly deeply pigmented at its base, so as to appear almost black. Running round the anterior edge of the protuberance is a clear-looking ridge with its outer end curved backwards; this is composed of the outer ends of the tall columnar cells of the cement organ filled with the cell-secretion. The oval patch behind the crescentic ridge is formed by the inner ends of the pigmented gland cells shining through the epidermis. The outline of the cement organ is thus crescentic and not circular, as BEDDARD described it in his *Xenopus* tadpoles. Running up from the anterior border of the cement organ, and passing obliquely outwards, is the pair of bands of pigmented cells connecting the frontal gland with the cement organ. The cells of all three structures are found to be essentially similar when examined in sections, so that there is a continuous line of mucus-secreting cells enclosing an area shaped roughly like an inverted trefoil on the anterior surface of the head. The base of the trefoil above contains the paired rudiments of the nasal pits, and the apex contains the stomodæum. The lateral bands of mucus cells are narrow and meet the ends of the broad transverse band which forms the frontal gland just internal to the level of the outer edge of the nasal pits, so that the nasal pits are bordered dorsally by the frontal gland. By comparing fig. 15 with fig. 10A (Plate I), it will be seen that the frontal gland is dorsal to the neuropore, and that the neuropore, if it persisted or if traces of it remained until this stage, would lie between the centres of the nasal pits. Another point worth notice is brought out by a comparison of the frontal view of these two stages, and that is the very close proximity of the neuropore to the small area from which the stomodæum will be formed later in development. Taking into account the thickness of the ventral wall of the fore-brain, it will be seen how little space is available between the cement organ and the brain for the potential mouth at the earlier stage (fig. 10A).

Hatching.--The larva of *Xenopus* hatches forty-eight hours after the egg is laid, when the eggs are kept undisturbed in a constant temperature of 22° C. I found, for instance, that a batch of some hundreds of eggs, laid on the night 24th-25th August 1903, had all hatched on the morning of 27th August, with the exception of twenty or thirty. As the larvae might be expected to emerge from these during the course of the day, they were placed for observation in a little tank kept at a temperature of 22° and were watched from the side through a horizontal binocular microscope (Zeiss' Braus-Drüner model) under a low power. The view of the egg obtained in this way is represented in fig. 16, Plate III. The jelly on the surface is not shown; a very thin layer of it covers the vitelline membrane, the thinness being partly due to the way in which the vitelline membrane has swollen up, the jelly having now to cover a much larger surface. At an early stage the membrane loses its spherical form and becomes ellipsoidal (see fig. 12); it is elastic and becomes swollen up by the pressure of its fluid contents. It is easy to test this by tearing a hole in the membrane; a jet of the fluid is forced out through the hole and the membrane collapses and shrinks. The egg is, then, in a condition of turgidity, and this forms a factor in the hatching process. The larva lies on the lowest part of the vitelline membrane, the side of its body in contact with it and roughly parallel to the long axis of the egg, which is always horizontal. In this position the larva is attached by the secretion of its cement organ to the vitelline membrane about two hours before the hatching occurs. Before attaching itself it seems to have sunk down to a position of equilibrium with its centre of gravity over the lowest point in the curve of the egg membrane. This is indicated by the position of the tip of the tail, which is always at a higher level than the head. The surface of the larva is richly ciliated and the fluid in the egg membrane is kept in rapid rotation, the current over the body of the larva passing from head to tail. The larva lies perfectly still, except that every ten to fifteen minutes it turns over from one side to the other. It will be seen from fig. 16 that the head of the larva only touches the vitelline membrane where the surface of the eye rests upon it; the cement organ is not itself in contact, as a short string of secretion passes from the gland to the membrane. The first sign that hatching is about to take place is a slight bulging outwards of the vitelline membrane opposite the head of the larva. In the course of the next five minutes the membrane under the anterior part of the head softens and the head sinks into the soft place, the membrane partly moulding itself to the contours of the head and partly bulging beyond the head, as drawn in fig. 17A, Plate III. It will be observed that in this position the extreme anterior end of the head where the frontal gland is situated is not actually touching the vitelline membrane, although very close to it. In another five minutes the membrane has moulded itself by a further softening to the anterior contour of the head (see fig. 17B), and now the frontal gland touches the vitelline membrane. When this stage is reached the hatching is rapidly completed. During the next three minutes the pouch of vitelline membrane in which the head lies distends more and more, until an imaginary line drawn in the original smooth curve of the membrane would pass through the middle of the cement

organ (fig. 17c). In this position the tadpole remains for not more than thirty seconds; the stretched part of the membrane then bursts, and the larva is shot out head foremost, as shown in fig. 18. In the same instant the vitelline membrane shrivels up like a burst india-rubber toy balloon. The larva may remain motionless with its tail between the torn edges of the egg membranes for one minute; it then wriggles and frees the tip of its tail, the tail swings round through an arc of 180° and the larva is then seen hanging, as in fig. 19, by a thread of mucus to the still further shrunk egg membranes. This thread of mucus is the short thread which attached the larva to the inside of the vitelline membrane and now drawn out to the length of the larva. It is apparently pulled out when the tadpole bursts out of the membranes, but it is not shown in fig. 18, as it is hidden by the body.

The following suggestions are offered as to the method of this hatching process. The larva attaches itself and fixes its position in relation to the vitelline membrane. Each time the animal turns over it must necessarily straighten itself, and in so doing the frontal gland touches the vitelline membrane, and smears it with a little of the secretion of the frontal gland. The secretion is of a different nature to the secretion of the cement organ; it acts upon the vitelline membrane and softens it. The softened patch is distended by the pressure of the fluid in the turgid egg, and the head of the larva sinks into the pouch which is formed. When the frontal gland comes into contact with the vitelline membrane in the last stages of hatching, the softening process is hastened, and when the larva's head is pressed against the pouched-out membrane, the fluid pressure in the egg must act, not on the membrane, but on the body of the larva. The fact that the softening process only goes on opposite the anterior surface of the head is shown by the vitelline membrane retaining its normal curvature at all other points, even so close to the head as opposite the neck (see figs. 17A and B).

To test the hypothesis that the secretion of the frontal gland acts on the egg membrane, the following experiment was made. Four eggs out of six left unhatched at 12 P.M. on 27th August were hung up, so that the long axis *xy* (fig. 16) was vertical instead of horizontal, and the head of the larva uppermost. By grasping with fine forceps a little of the jelly at the pole *x*, and drawing it out of the water against the glass of the tank, the eggs could be fixed in this position. Of the four eggs slung up in this way, none were hatched at 6.30 P.M.; the two eggs left in the normal position hatched out the larvæ between 1 P.M. and 2 P.M. At 6.30 P.M. the four eggs were returned to the normal position, and within half an hour all were hatched. The larvæ were thus prevented from hatching for about five hours.

The reason appears to be this. When the egg is carefully revolved into the new position, the larva remains attached, but slides down towards the pole *y*; the head is consequently carried away from the egg membrane near the pole *x*, and when the larva moves, it is easy to see that the head apex, where the frontal

gland lies, swings out away from the membrane and never touches it. There was not the slightest bulging of the membrane opposite the head in the reversed eggs.*

Regarding the action of the secretion of the frontal gland, there seems to be reason to believe that it is digestive, and probably is due to a peptic ferment. Miss R. ALCOCK ('91 and '99) discovered that the external epithelium of the skin in the *Ammocetes* of *Petromyzon planeri* and in *P. fluvialis* produces a peptic ferment capable of digesting fibrin in a 0.2 per cent HCl solution. In the frontal gland, then, a similar secretion is probably localised in an appropriately-placed patch of epithelium, and the acid medium requisite for the action of the peptic ferment is, no doubt, supplied by the excretion from the pronephros. But it is not necessary to dwell on this point, as the specific action of the frontal gland can no doubt be tested by experiment.

It is obvious that the frontal gland is actively secreting at hatching time from the coating of secretion which hardens on the surface of the gland in larvæ preserved at this stage. The light band seen (fig. 15) running along the middle of the gland is produced by coagulated secretion. After hatching, the frontal gland begins to atrophy, and has disappeared three or four days later, before the tadpole begins to feed.

Considerable morphological importance has been attached to the frontal gland by VON KUPFFER ('93, p. 78, '94 and '03, pp. 188 and 190). He regarded it as the "unpaarige Riechplakode" of the frog and uses it as evidence of a monorhine stage in the development of an amphirhine form of Vertebrate, believing that it arises as a sensory thickening of the ectoderm at the spot (the neuropore) where the brain retains its connection with the ectoderm longest; the connection he considered to be the primitive unpaired olfactory nerve. It would not be necessary to refer to this view here if VON KUPFFER had not repeated his interpretation of the "Stirnknospe" in his chapter on "Die Morphogenie des Centralnerven-systems" in O. HERTWIG'S "*Handbuch der Entwicklungslehre der Wirbeltiere*." In the same work KARL PETER ('02), in the chapter on "Die Entwicklung des Geruchsorgans in der Reihe der Wirbeltiere," gives weighty reasons for rejecting VON KUPFFER'S fascinating theory of Mono- and Amphirhiny (PETER, '02, pp. 12-13, p. 26). PETER, in this chapter, refers to his own paper on VON KUPFFER'S theory ('01, p. 654), where he includes observations on *Bufo "cinerea"* (syn. *B. vulgaris*), showing that the "Stirnknospe" has in the toad no connection with the neuropore and that it must be placed in a different category to the sense-organs of the Anura, since it develops from the external layer of the ectoderm, while the sense-organs are all derived from the inner nervous layer (see also PETER, '01a). CORNING ('99) and HINSBERG ('01) have described the frontal gland in *Rana temporaria* and *R. esculenta*, and it can be gathered from their descriptions and PETER'S of the

* It has been possible to show by experiment on *Hyla* larvæ that the surface of the frontal gland only, and no other part of the ectoderm, has the power when touching it to soften the vitelline membrane.—9th April 1904.

toad that the gland is at the height of its development in each of the three species when the larva reaches the size at which hatching occurs, and that it rapidly atrophies after hatching. Its structure in these forms and in *Xenopus* is essentially similar.

The results arrived at on this subject may be summed up by stating that the main function of the frontal gland in the Anura is to soften by means of its secretion the tough and turgid egg membrane in order to allow the larva to escape at an early stage of development before any external hard parts have been formed which might be used for breaking out. The frontal gland is a transitory structure, like the egg-tooth of lizards, and like it again only actively functional for a few minutes in the life of each individual. Its interest is chiefly physiological, but it may serve as a warning to morphologists of the danger run in assuming that an inconspicuous organ, the function of which is not known, is vestigial.

The Larvæ after Hatching.—The first few days after hatching are spent by the tadpole attached to weeds, etc. Its abdomen contains a considerable amount of yolk which must be absorbed and the alimentary canal opened up before it can begin to swim about and feed. This does not come about for a period varying between three days and a week in an aquarium kept at 22° C. The cement organ is functional during the whole of this time. The tadpoles at this stage never lie on the bottom of the aquarium, where, owing to their pale colour, they would stand out conspicuously against the sediment. Those found on the bottom are prematurely hatched younger larvæ. The secretion of the cement gland is extremely tenacious. If a tadpole is induced to move, the mucus thread breaks away from the surface of the sucker and the animal swims with a curiously stiff flickering wriggle, remarkably like that of a young *Amphioxus*. For the first day or two after hatching a free-swimming larva always tends to assume the vertical position with its head up, and consequently the swimming movements carry it towards the surface. Another physiological character tends in the same direction. Although not clearly discernible at any given time in any particular individual, the larvæ respond to light stimuli in such a way that it could be put down to feeble positive heliotropism. If an aquarium containing some hundreds is disturbed and the larvæ scattered, the great majority of them will, in twenty-four hours, be near the surface or on the light side of the vessel; a few dozen, however, will be distributed at random. The mucus from the cement organ is both very adhesive and very tough. Whatever the cement organ touches it immediately becomes fixed to and the tadpole comes to rest. This applies to so unsubstantial an object as the surface film. A 20-inch bell-jar, four or five days after spawning has taken place in it, will have one hundred to two hundred larvæ hanging from the surface film in the position of the larva in fig. 19, Plate III. The thread of mucus is of varying length, and may either be as long as or rather longer than the larva. The surface film is, of course, drawn down into a slight dimple by the weight of the animal. I have never seen any movement in an attached larva, except when in the act of swimming away; it usually hangs down perfectly

quiescent. A strong continuous current sufficient to sway them 45° from the normal will not induce more than one in twenty or thereabouts to detach itself.

The most obvious changes during the first days of this phase are the darkening of the pigmentation both of the eye and of the nasal pit, which both become more conspicuous, the fin becoming wider, but only very gradually (cf. fig. 22A), and three short unbranched external gills appearing on the three branchial arches. The gill slits are closed. As the yolk is absorbed the larva becomes more and more transparent, and at the same time the ciliation disappears *pari passu* with the yolk, except the ciliation in the nasal pits, which persists. As it becomes more transparent the larva becomes more and more restless, and about four to six days after hatching spends more time swimming about than hanging on by its cement organ. At this time the mouth opens and the branchial current of water is set up with its rhythmic action. By this time the operculum has grown back from the hyoidean arch and fused in the mid-ventral region with the body-wall under the pericardium to form the gill-chamber. The upper, lateral ends of the folds remain free and form the ventral or outer lip of the spiracle on each side of the neck. The spiracle at all stages opens upwards and backwards and is not produced into a spout; the inner wall of the spiracle is the body-wall of the animal.

The mouth opens and the branchial respiratory current begins some hours, probably about twelve hours, before the animal takes in food. It is easy to see that the gut still contains a mass of yolk, and in sections the oesophagus is found to be solid. Exactly the same condition of things has been described in the common frog (MARSHALL and BLES, '90, pp. 223-4), with the difference that in *Rana* the branchial chamber is not completely formed and that feeding begins after a much longer interval.

Drawings of the animal at this stage are given in figs. 22 and 22A. In the front view of the head an attempt has been made to show the commencing transparency of the tissues. It brings out clearly the fact that the chromatophores of the skin are confined to the dorsal surface; the ventral is free from them except in one place to be referred to later. Covering the whole underside of the head there is a large continuous lymph space, well marked off from the other lymph spaces, which I propose to call the *submental lymph sac*. Through its translucent walls can be seen the ventral wall of the buccal cavity. The cement organ is at the extreme tip of the head (see fig. 22A), and immediately above it is the mouth or rather the lower jaw, still showing at the symphysis the junction of the mandibular arches. At this stage the lower lip protrudes in front of the upper; this condition becomes still more pronounced in older tadpoles, where the mouth opening comes to lie on the upper surface. The ventral chromatophores on the skin of the mandible then face upwards and become practically dorsal. This disposition of the mouth vanishes at the metamorphosis and is apparently adapted to the peculiar feeding habits of the tadpole. The oral tentacles have not yet made their appearance. Above the mouth are the large shallow depressions of the nasal pits, with their well-marked

raised rim incomplete behind. The median swelling behind the olfactory pits is caused by the brain. The eyes stand out prominently from the side of the head.

In the side view there are very obvious alterations to be observed in the proportions of the parts of the animal. At hatching the tail was only one-third of the total length; it is now two-thirds, while the abdomen, which was very elongated, now appears very short in proportion. The fin-fold has grown considerably, and has grown out from below the abdomen, drawing out the cloaca, so that the cloacal opening comes to lie at the edge of the fold. The pronephros tubules can be seen quite distinctly through the transparent skin.

The Lymph Hearts.—Just behind the pronephros on each side is the newly developed lymph heart. It lies in a small lymph space immediately below the skin on a level with the inner coelomic outline of the pronephros. It can be best located in the living animal by the movements of the nearest chromatophore of the skin, which, under the microscope, are more conspicuous than the pulsations of the lymph heart itself. Delicate trabeculæ run across the enveloping lymph space from the heart to the integument, and these pull down the skin at each contraction of the heart. At this stage the pulsations are very irregular; they sometimes cease for one or two minutes, and seldom continue uninterruptedly for even twenty beats, hence it is difficult to time them. When most regular they average forty beats a minute.

This early appearance of the pectoral lymph hearts is after all not very remarkable when the extent and physiological importance of the lymph spaces in the tadpole is considered. However, the find was an unexpected one, as I had already paid a little attention to the subject and found that the pelvic lymph hearts do not appear in *R. temporaria* and *Bufo calamita* before the metamorphosis.

The Tadpole.—It is not difficult to notice the commencement of feeding in a batch of young tadpoles. Those which have not begun, only swim about fitfully and then hang by the cement organ, the breathing movements continuing while they hang. Those which have begun to feed are suspended in mid-water, making little or no progress, and are steadily gulping away; the fæces appear in the cloaca within twenty minutes or half an hour afterwards; thus the time at which the alimentary canal is open to the passage of food can be easily and definitely fixed. This is important, because it makes the following interesting fact easy to determine by watching at the correct time, namely, that within two hours after beginning to feed the tadpoles rise to the surface for air and begin to use their lungs as breathing organs. BEDDARD observed that *Xenopus* does not develop "internal" gills ('94, p. 106) and concluded that respiration was carried on through the blood-vessels of the "filters" placed on the internal side of the branchial arches. His observations are correct, but as he paid no attention to the use of the lungs, he was led to a conclusion which turns out to be of subsidiary importance. It is possible that a certain small amount of oxygenation of the blood does go on in the processes of the filtering apparatus. And until feeding commences respiration is carried on by the external gills. But as the tadpoles are constantly rising to the surface for air

and do so more frequently the warmer the water is, it follows that the lungs are not only hydrostatic in function but also respiratory. Reducing the quantity of water has the same effect as raising the temperature. It is difficult to follow an individual tadpole under normal conditions in a large aquarium, but isolated ones in small aquaria will rise every five to ten minutes.

The tadpole shown in fig. 23 should be glanced at as an intermediate stage before proceeding to the typical and final form. This specimen is in the condition reached after about two days spent in feeding. During the first few hours feeding may be interrupted now and then for a few minutes while the animal suspends itself by its cement organ, then this organ begins to atrophy and by this stage has completely disappeared. The snout has not yet assumed the characteristic shape, which is seen one or two days later. Here it is rounded; but it is important to note that there is no trace in this transitional stage of lips like those bearing the horny teeth in ordinary tadpoles. W. K. PARKER and BEDDARD have already drawn attention to the total absence of horny teeth. The pronephros and the lymph heart are very clearly seen at this stage; the transparency of the tissues is still increasing. The contents of the coelom are, however, beginning to disappear from view, as the chromatophores are rapidly increasing in the abdominal wall. The hind limb is just forming as a rudimentary bud. The tail has lengthened and its shape is quite typical.

A day or two later the appearance of the tadpole has undergone a very obvious change. The shape of the snout becomes like that of the advanced tadpole shown in fig. 24, Plate IV. It may be described as wedge-shaped, with the lower lip forming the slightly curved edge of the wedge. The tentacles sprout exactly at the angles of the mouth and soon become long slender processes. This stage is one which persists for about two and a half months without any marked changes, apart from the great increase in size and the growth of the hind limb. It is, therefore, the typical larva of *Xenopus*, but for various reasons I must omit its full description here and reserve it for a future communication. The chief reason for so doing is inherent in the tadpole itself. It is so transparent in the head region that almost all the complicated structure of the vertebrate head can be studied in the living animal, and it would be necessary to give accurate figures to make all this clear in a description. The difficulty lies in my inability to produce such figures, and, as fig. 24 shows, the dead specimen allows very little internal structure to be seen. I will confine myself to giving a brief account of the extraordinary feeding habits. The tadpoles I have reared (one male was brought to maturity) were fed exclusively until the metamorphosis on pure culture of the green Flagellate *Chlamydomonas*. They thrive best in water which is thick with the Flagellates. In this they float almost vertically in mid-water, rapidly undulating the posterior third of the tail and at the rate of forty to fifty a minute take in gulps of the water. The water passes out through the spiracle; the *Chlamydomonas* are retained by the filters in the buccal cavity and drawn into a ciliated groove on either side of the pharynx. In this groove the green mass

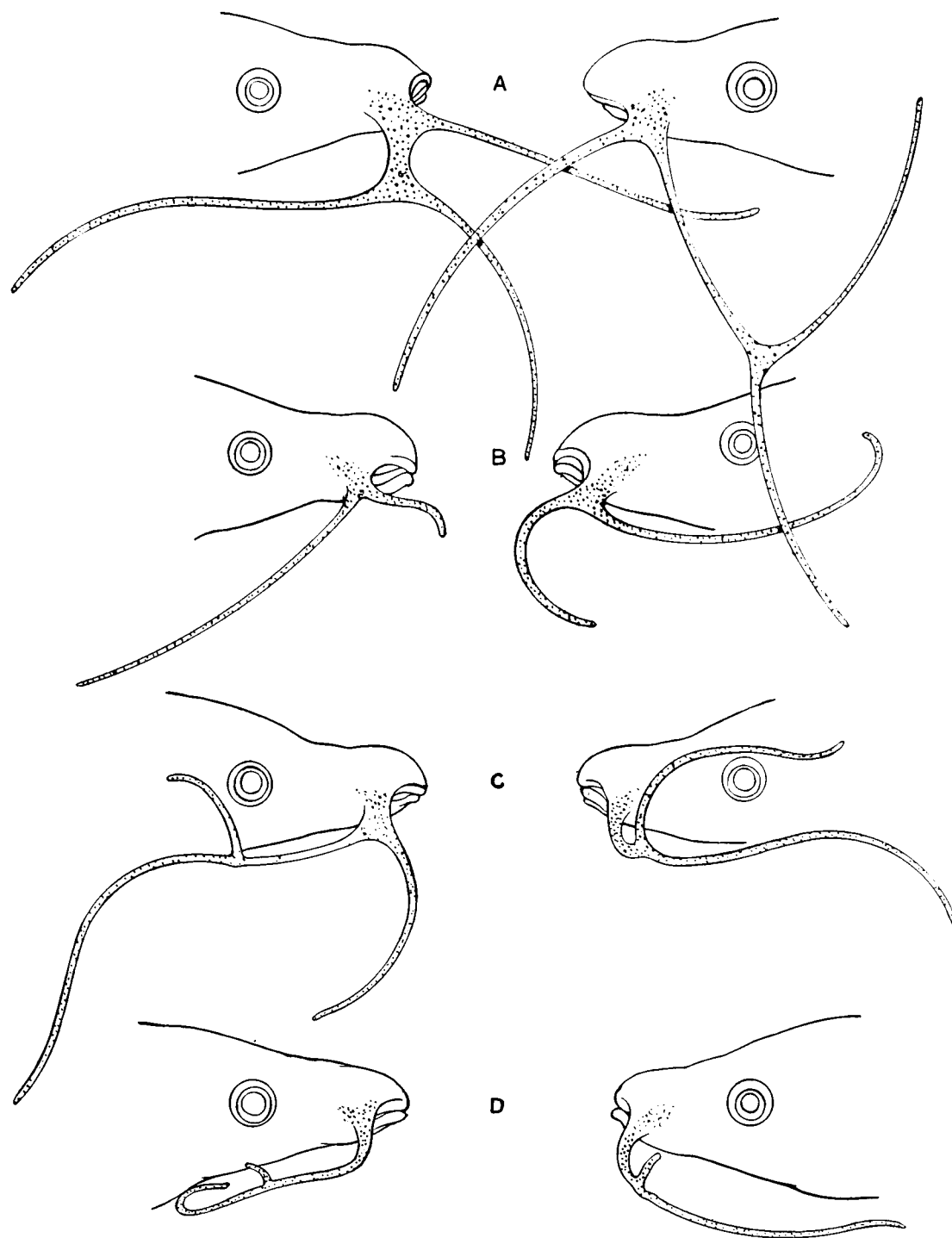
can be seen passing back in a kind of helicoid vortex towards the oesophagus, where the two green currents converge and disappear. Every day for ten weeks fresh culture must be added to the aquarium water; even so few as ten large tadpoles will clear twenty gallons of water in a single day of one added gallon of thick culture. When the water is clear they swim restlessly about like fish, as though searching for food, taking a gulp every now and then, as if to test the water, and then swimming on. As soon as fresh culture is poured in they immediately suspend themselves in mid-water and commence gulping regularly. That the current of water through the gill-slits is not kept up except when feeding confirms the statement made above that it is not a respiratory current. This method of feeding is so remarkable that it is desirable to find out whether it is normal in the natural habitat. It is interesting that the only Craniate known to feed in a similar way is *Ammocetes*; according to A. SCHNEIDER its chief food is *Euglena*. In both cases the food is filtered out and then collected in a ciliary current. *Xenopus* tadpoles kept in *Euglena* culture were starved to death, however. Mr BEDDARD's tadpoles fed on Cyprids and nothing else; mine invariably rejected any small Crustacean which entered their mouths and starved amid an abundance of Ostracods and Cladocera. The movements of the tadpoles, their way of taking in water, the ciliated bands, the dorsal position of the mouth and the shape of the lips, all point to micro-plankton being a staple item of their diet, quite apart from the fact that they thrived on it. I would like to suggest that the swarms of Cyprids in the Zoological Gardens Tank were feeding on micro-organisms which also formed the staple food of the *Xenopus* tadpoles, the Cyprids being swallowed incidentally.

The Mature Tadpole.—Fig. 24 is introduced to supply a more detailed figure than that published by W. K. PARKER and copied in so many text-books. At the same time it should be observed that this tadpole in one important respect, which is probably diagnostic, differs from PARKER's. It will be seen that the long tentacles in fig. 24 are given off from the angles of the mouth; in fact a groove from the junction of upper and lower lip is often continued up the base of the tentacle. Now, in all PARKER's figures the tentacles are given off above the mouth, from behind the upper lip. The species to which PARKER's specimens belong is, most probably, *Xenopus calcaratus* from Lagos.

The attitude of the tadpole in fig. 24 is that taken when swimming rapidly in a vertical position to the surface of the water for air. The hind leg is stretched back, as in a swimming Urodele, and the resistance of the water as it shoots up seems to sway back the slender tentacles, usually directed straight forwards, into the position figured.

There are three points in connection with this stage still to be mentioned, regarding the tentacles, the coloration, and the fore limb.

BEDDARD mentions that he "more than once observed the tentacle of one side to be bifid." This I found to be quite frequent among a limited number which reached this stage (length of 60 mm.); out of eight specimens, six had both tentacles branched. Four of these, picked at random, are reproduced here. It will be seen that in A, B, and



RIGHT SIDES

LEFT SIDES

TEXT FIG. 3.—A, B, C, and D. Tentacles of the right and left sides of four Tadpoles (*Xenopus laevis*) about 60 mm. long.

D the right and left tentacles are symmetrical as far as the general arrangement of the branches is concerned, but the position and sizes of the branches differ on the two sides, so that the symmetry is very imperfect. In C the right tentacle has a secondary branch on its backwardly directed fork which is not represented on the left side. Branched tentacles have only been found in the late tadpole stages over 50 mm. long.

As BOULENGER pointed out (footnote to LESLIE, '90), the tentacle of *Xenopus* may be homologised with the balancers of Urodele larvæ. These must be the representatives on the mandibular arch of the external gills on the branchial arches. If these homologies are correct, the tentacle in *Xenopus* is an external gill, and this conclusion is supported by the fact that from its very earliest appearance it has a capillary loop doubling into it, supplied from the dorsal end of the first branchial aortic arch. The branches figured above are, on this hypothesis, the result of a tendency to branch in a persisting gill similar to that found in all external gills which persist for some time during the life or, as in *Proteus* and *Siren*, for the whole of the life of the individual. In the last two animals the tendency to form branches and secondary branches is especially well marked. If the branching is put down to regeneration after injury, how is the bilateral symmetry to be accounted for?

Coloration.—A most remarkable feature in the behaviour of the chromatophores is found in tadpoles of 15–18 mm. and onwards. As is not uncommon among tadpoles, the dark chromatophores on the head and trunk contract at night into spherical masses, but what is most unusual, if not at present unparalleled in any vertebrate, is the fact that other chromatophores, apparently of the same nature, namely, those in the distal half of the tail, expand at dusk as the others are contracting.

The end of the tail, which in the day-time is so transparent that the presence of chromatophores would never be suspected, becomes, to the naked eye, jet black after nightfall. The expansion takes place in the chromatophores in the fin-fold, but not in those on the myotomes of the same (distal) part of the tail; the latter contract at night in harmony with those in the trunk region and anterior myotomes of the tail. The general effect is well shown in fig. 24, although the contrast is much stronger in the living animal, where the pale regions become of glassy transparency. The explanation is, I believe, due to a need for protective colouring in the transparent part of the tail tip. It is kept undulating constantly; in the daylight it needs no pigmentation—it is protected by its transparency; but the refractive stellate cells of the mesenchyme in the fin-fold would at night be liable to catch and reflect any stray light rays, and the expanded chromatophores effectively prevent this. Moreover, they are absent in the part of the fin-fold which does not move actively. But when the physiology of the case is considered it seems to make the solution of the problem of control over the pigment cells more difficult than ever. Taking tadpoles from daylight into a darkened room has the same effect as the changes from day to night.

In figs. 20 and 21, Plate III, the appearance of the skin is shown under the microscope of the part of the tail marked with a cross in fig. 24, in a specimen killed in the daytime

(fig. 20) and one killed during the night (fig. 21). The pale brown network in fig. 20 suggests, by the sharp double outlines occurring in many places, the presence of inter-cellular passages into which the chromatophores expand radially and leave pigment granules behind adhering to the walls of the passages.

The Fore-limb.—In fig. 24 the fore-limb is seen lying under a transparent patch of integument, and in fig. 25 this region is shown magnified in a slightly older larva. Here the arm has burst through the thin wall of the sac, the edges of which are still present, and it can be seen that the wall of the branchial chamber immediately in front of the arm-sac is intact, nor is the spiracle affected in any way. This is probably what PARKER was referring to when he stated, "The fore-limbs are not hidden beneath the opercular fold" (PARKER, '76, p. 626). The explanation of the difference between *Xenopus* and the more familiar tadpoles is that in the latter the fore-limb develops in a diverticulum of the gill-chamber which remains in communication with it, so that the developing arm protrudes into the branchial space; in *Xenopus* a similar diverticulum is formed, which becomes completely shut off from the gill-chamber, and the arm cannot encroach on that space. The arm emerges in the common frog by breaking through the wall of the branchial chamber on the right side and by passing through the spiracle on the left side, blocking up this passage completely. The appearance of the arms therefore in typical tadpoles sharply marks the abrupt cessation of branchial respiration. In *Xenopus* the arm appears by the rupture of what may be called the "brachial sac." This event in no way interferes with the habits of the tadpole. It remains floating in mid-water in the same position as before, taking in water at the mouth and passing it out by the spiracles, these being, as shown in fig. 25, Plate IV, quite unaffected by the protrusion of the arms. In fact the branchial current is used here not for respiration, but for nutrition, and is not interfered with during the metamorphosis. The main part of the change into the adult condition is very gradual, and feeding can be continued almost without a break while it is going on.

Metamorphosis.—The metamorphosis is completed ten to twelve weeks after fertilisation, in a constant temperature of 20° to 22° C. At this temperature the whole change from the mature tadpole into the tailless frog is passed through in about 15–20 days, but I have no doubt that in its native African pools the temperature in the late spring will be much higher and the metamorphosis correspondingly much more rapid. The commencement of the metamorphosis is well marked by the protrusion of the arms, the beginning of co-ordinated swimming movements of the legs, and the first appearance of blood-vessels across the width of the fin-fold of the tail. It is a remarkable fact that until this time the whole of the fin-fold is completely non-vascular. The usual sub-vertebral vessels are of course present and supply the tail-myotomes, but they give off, until this period of development, no vessels into the fin. The space between the two bounding layers of integument is filled with a loose trabecular tissue composed of stellate mesenchyme cells, the interstices of which seem to be filled either with fluid or

with a gelatinous matrix. It was not possible to make out any circulation of lymph or movement of lymph-corpuscles in the tail fin. The first capillaries appear near the tip of the tail, and they spread towards the proximal parts, becoming more and more numerous as the time for the resorption of the tail approaches; thus obviously raising the suggestion that the process of resorption is carried on by methods connected with this vascularisation. The tail is not used for the respiratory function.

The limbs now grow rapidly. The arm rotates from the shoulder-joint through 90° , and about forty-eight hours after protrusion has left the position shown in fig. 25, Plate IV. (where the arm hangs down in a plane at right angles to the long axis of the animal) for the adult position: namely, directed forwards towards the mouth and lying in a horizontal plane parallel to the long axis. At first the arms are ridiculously out of proportion to the size of the head; they have lagged behind in development very considerably. The deficiency is made up in three weeks, and by the end of the metamorphosis the length of the arms is such as to allow of the fingers just meeting in front of the head. The legs are developed in the primitive fin position, and, apart from the bending of the limb, are kept in this position throughout life. Soon after the stage of fig. 23, Plate IV, where the leg is still in the primitive position, the thigh is swung round at the hip-joint until it stands out at right angles to the body; the knee-joint is bent so that the tibia remains parallel to its original position, and the ankle is bent so as to turn the foot out through 90° , the sole facing backwards instead of inwards. These changes go on during the three days after the arms emerge; at the same time the black claws appear on the three preaxial digits, some weeks before they come into use. The legs now assist the tail quite efficiently in swimming, and grow rapidly, especially the feet.

The colour of the skin changes gradually in character. Fig. 24 still shows the tadpole coloration; two to three weeks later the adult coloration had been assumed, and then the whole creature becomes a strange mixture of larva and adult. The whole habit is larval; the creature still swims in mid-water in an upright position; there is a long tail, mouth and tentacles are unchanged, spiracles are present, but the body itself, in shape and colour, and also the appendages, are adult in character. In two days after this condition is reached (about fourteen days after the arms emerge) the tentacles have almost disappeared, and then the mouth very quickly transforms from the larval to the adult state. The whole process is finished in four to six hours. When the tentacles are very much shrunken, the angle of the mouth, where they were attached until now, seems to grow back under them; the gape of the mouth is consequently widened, and at the same time the stumps of the tentacles become dorsal to the mouth. A minute basal part of the tentacle persists throughout life in *Xenopus laevis*. As the mouth metamorphoses the spiracles close up.

Immediately after the mouth has transformed, the tailed frog ceases to keep

constantly to its larval free-swimming habits, and spends more and more time lying on the bottom. At this time the tail has begun to atrophy, the blood-vessels in the fin spread, the notochord at the tip becomes wavy, and the pigmentation darkens. At the end of a week the greater part of the tail is absorbed; about one-third of it is left, very deeply pigmented, and the young frog has thus reached the stage at which the typical *Planeroglossan* lands and becomes terrestrial. There is not the slightest tendency to land in the case of *Xenopus*. It swims about actively in search of food, and for some weeks lives on small, free-swimming Crustacea. Seven specimens reared to this stage consumed enormous quantities of *Daphnia pulex*; a great swarm of these vanished every twenty-four hours, and the frogs thrive.

Their hands are at once used in the grown-up manner to cram the food into their mouths; the arms are not used for progression at all, except to push aside water-weeds—hence one of their functions as limbs has almost disappeared. The size of the arm is altogether out of proportion to the size of the leg, which is an extremely powerful swimming organ. The limbs of *Xenopus* as a frog are paralleled by the limbs of *Macropus* as a marsupial.

When W. K. PARKER ('76) described the skull in larval *Xenopus*, he laid stress upon what he considered Chimæroid features in the chondrocranium, and was naturally led to attach morphological importance to the lash-like tail end of the *Xenopus* tadpole. Now, although this close resemblance does not exist, there is a certain degree of resemblance which suggests similarity of function. The end of the tail of the *Xenopus* tadpole has a very narrow dorsal and ventral fin-fold (see figs. 23 and 24, Plate IV), and it is easy to see in the living animal that the constant undulatory movement of this narrow membrane has very little propelling power. The suggestion is, then, that the *Xenopus* tadpole, *Chimæra*, and such fishes with a narrow lash-like tail end as the Mormyridæ, use that part for suspending themselves either in mid-water or, in the case of bottom-feeders or mud-feeders, just over the bottom, by means of a rapid undulatory movement.

Sexual maturity appears to be reached at an early age. One male was kept until two years old, when it began to pair.

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EXPLANATION OF PLATES.

PLATE I.

- Xenopus levis*, Daud. Segmentation stages, blastopore formation, medullary plate, and early embryo.
- All figures on this plate numbered alike are drawn from the same egg, and figs. 1-9 are magnified $\times 28.5$.
- Fig. 1. Unfertilised egg.
- Fig. 2. Egg with eight blastomeres, seen from the right side.
- Fig. 2A. Seen from below.
- Fig. 3. Egg with about thirty-two blastomeres, seen from anterior side.

- Fig. 3A. Seen from below.
 Fig. 4. Early blastula stage.
 Fig. 5. Late blastula stage.
 Fig. 6. Egg with early stage in the formation of the blastopore. The egg is in its natural position, and is seen from the right side.
 Fig. 6A. The same stage seen from below.
 Fig. 7. Stage showing circular blastopore with large yolk plug.
 Fig. 8. Stage with open medullary groove, seen from behind.
 Fig. 8A. The same stage seen from before.
 Fig. 9. Stage after closure of medullary groove.
 Fig. 10. Embryo, 3 mm. long, taken out of egg. ($\times 25$.)
 Fig. 10A. Anterior end of the same embryo viewed in the direction of the arrow in fig. 10. ($\times 48$.)

PLATE II.

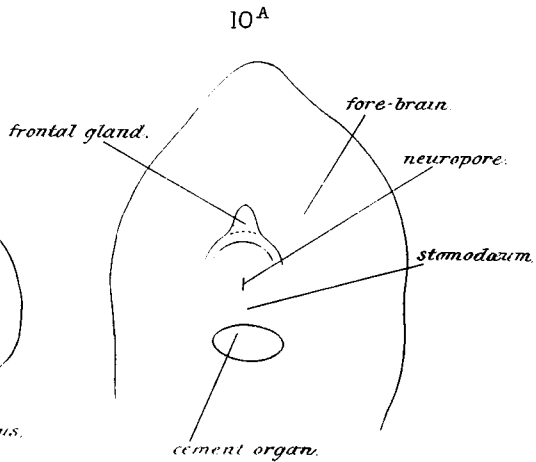
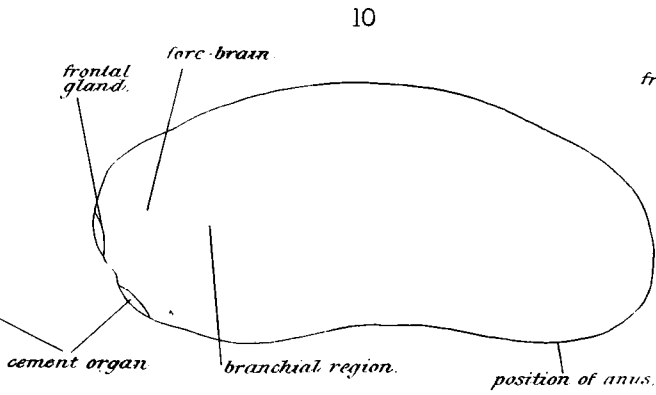
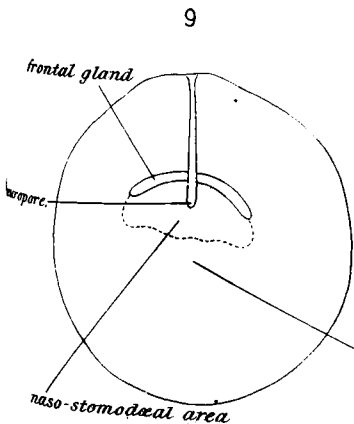
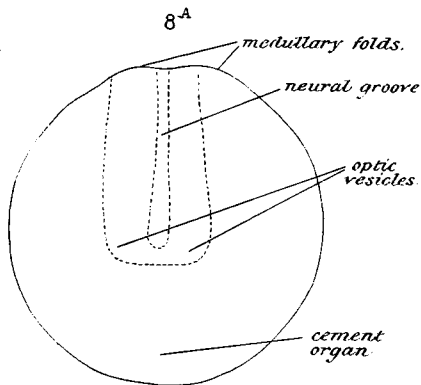
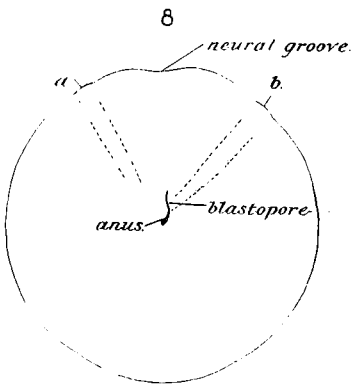
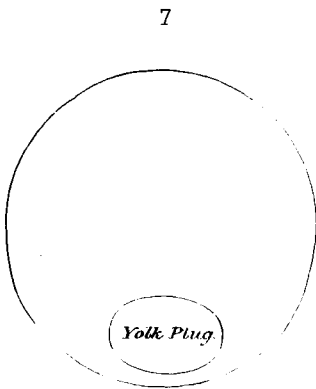
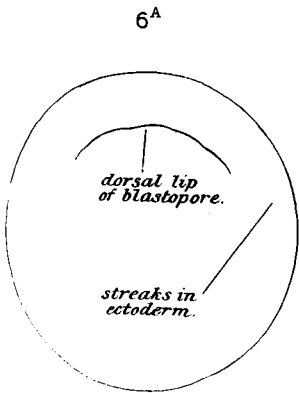
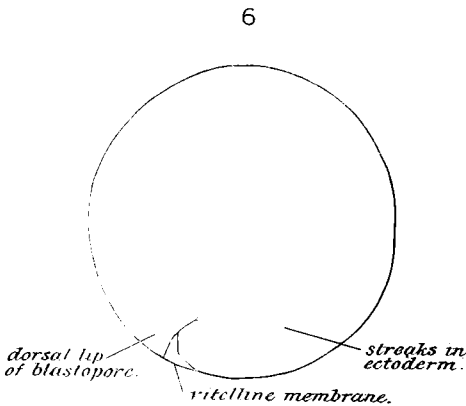
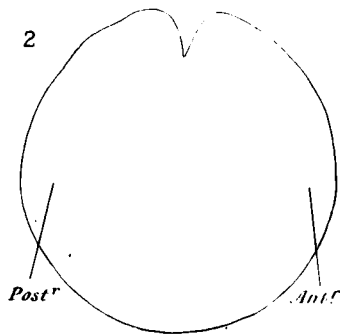
- Fig. 11. Egg deposited on a leaf of *Myriophyllum proserpinuacoides*. ($\times 6$.)
 Fig. 12. Embryo, 3.2 mm. long, in vitelline membrane; the jelly surrounding this has been stripped off. Drawn from life. ($\times 25$.)
 Fig. 13. Embryo, 3.8 mm. long, taken from egg. Drawn from life. ($\times 25$.)
 Fig. 14. Embryo, 5 mm. long, taken from egg. Drawn from life. ($\times 25$.)

PLATE III.

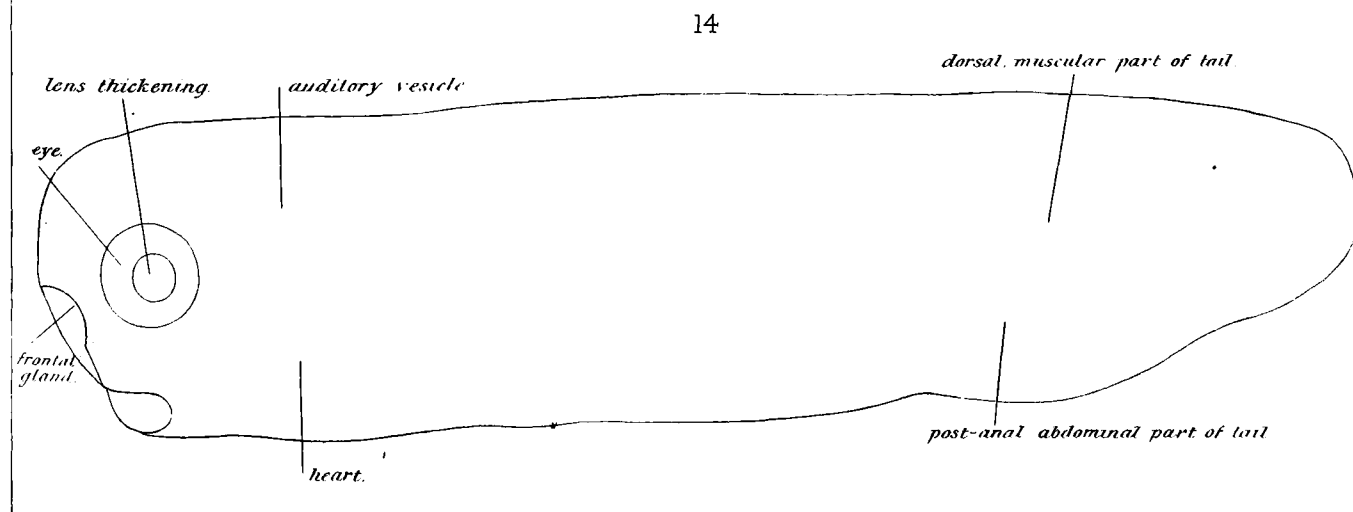
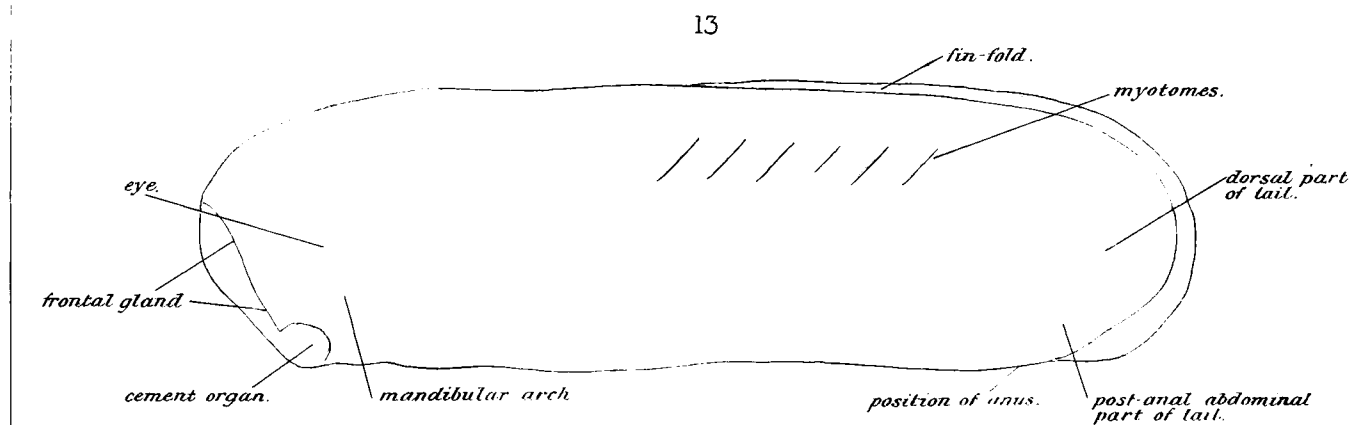
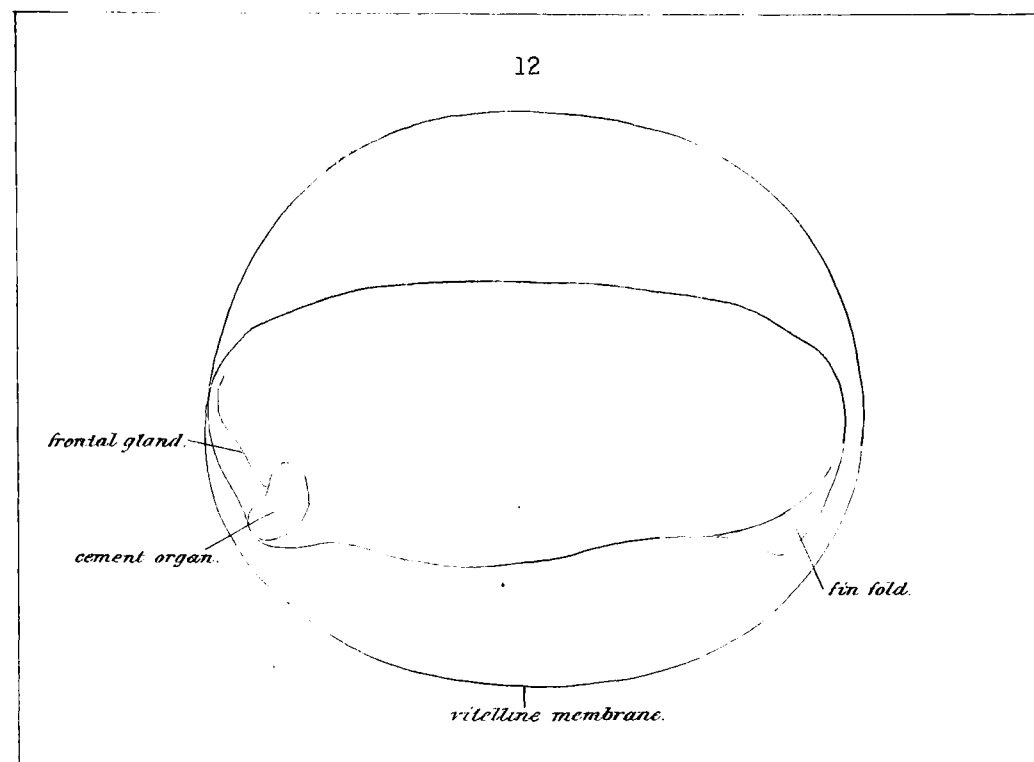
- Fig. 15. Head of larva just hatched, seen from before, in the direction of the arrow in fig. 15A. ($\times 60$.)
 Fig. 15A. Outline of larva, 5 mm. long, just hatched, seen from side. ($\times 15$.)
 Fig. 16. Larva, 4.5 mm. long, lying in the egg attached to the vitelline membrane by the cement organ before hatching commences; the larva is seen from the ventral side. The layer of jelly outside the vitelline membrane is omitted. ($\times 11$.)
 Fig. 17. A, B and C. Head of larva during process of hatching, to show the yielding vitelline membrane. A., 8' 15" before hatching. B., 3' 15" before hatching. C., 15" before hatching. ($\times 12$.)
 Fig. 18. The position of the same larva when it emerged from the egg. The vitelline membrane has shrunk and caught the tip of the tail. ($\times 10$.)
 Fig. 19. The same larva as in figs. 17 and 18, as seen a few seconds later, hanging by a thread of mucus to the further shrunken vitelline membrane. ($\times 10$.)
 Fig. 20. Portion of the skin of the tail-fin of a tadpole, 62 mm. in length, killed during the daytime, from the middle of the length of the tail. ($\times 170$.)
 Fig. 21. A corresponding portion of the tail-fin of the tadpole drawn in fig. 24, which was killed at night. ($\times 170$.)

PLATE IV.

- Fig. 22. Anterior view of the head of a larva 10 mm. long. The stage reached is a few hours earlier than that at which the animal begins to feed. ($\times 38$.)
 Fig. 22A. Outline sketch of the same larva as fig. 22 in side view. ($\times 12$.) (From a formalin specimen.)
 Fig. 23. Side view of a tadpole 12.4 mm. long, which has fed for two days. ($\times 11$.) (From a formalin specimen.)
 Fig. 24. Side view of a tadpole, 60 mm. long, with the arm not yet extruded. Drawn in the position in which the rapid ascent to the surface is made to obtain air. ($\times 3$.) (From a specimen killed at night and preserved in alcohol-formalin-acetic acid mixture.)
 Fig. 25. The left pectoral region of a tadpole, 62 mm. long, slightly older than that shown in fig. 24, to show the extruded arm and the spiracle into which a style has been passed. ($\times 13$.) (Preservation as described above, fig. 24; killed at nightfall.)







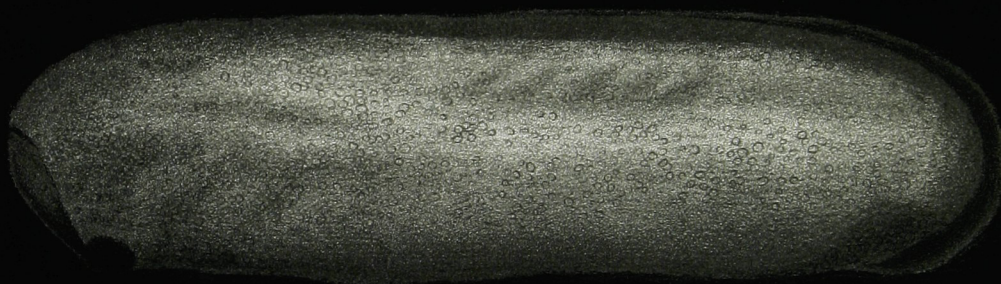


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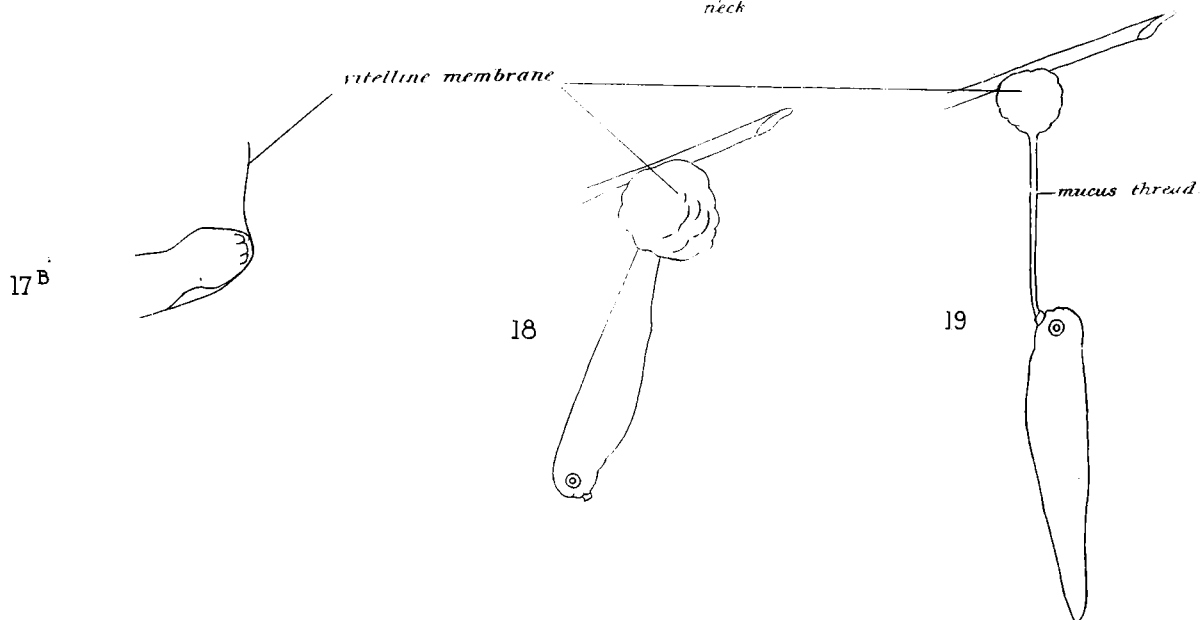
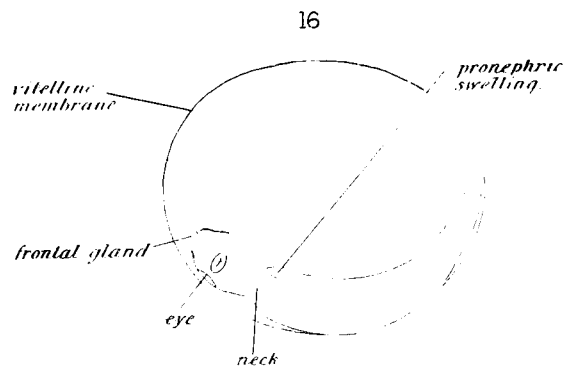
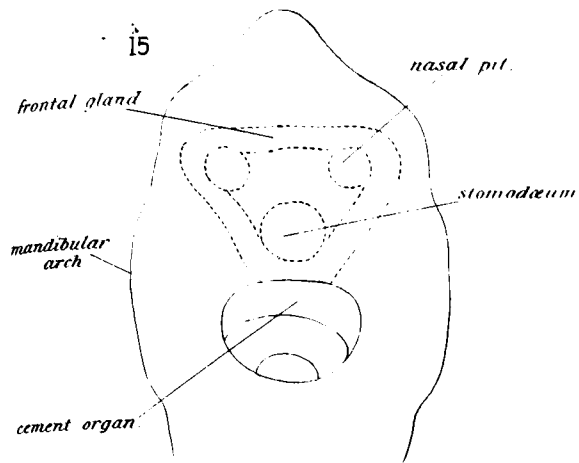


13



14





15



15^A



16

x



y

17^A



17^B



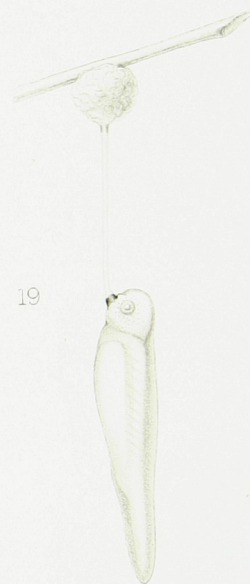
17^C



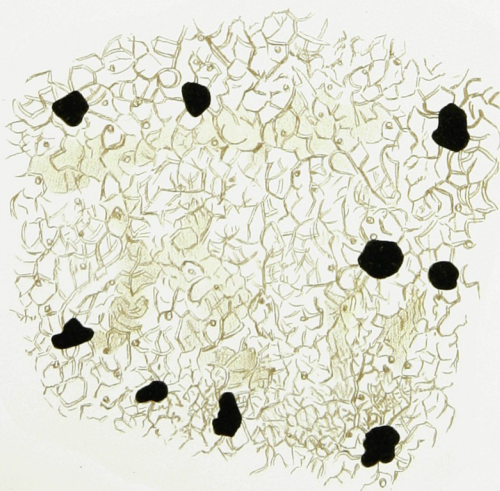
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19



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