THE BREEDING HABITS AND DEVELOPMENT OF RANA CHALCONOTA (SCHLEG.) (AMPHIBIA)

by

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Introduction

In the present work I have endeavoured to give a fuller account of the development of *Rana chalconota* than one can gather from the literature. It is remarkable that the development and biology of a frog which is common in Java is practically unknown. Several previous papers (SARASIN, VAN KAMPEN 1923) appear to contain errors; these are corrected and some new facts are given in this contribution.

During the study of much embryological material of this frog I came to the conclusion that data concerning age and length are of very little value. The ten basic stages as proposed by LIEBERKIND (1937) prove to be more practical and are therefore followed. The known synonyms for *Rana chalconota* are:

Hyla chalconotus.
Polypedates junghuhnii.
Rana everetti.
Rana mocquardi.
Rana tytleri.
Rana labialis.

BOULENGER (1920), SMITH and SCHIJFSMA (1932) consider *Rana labialis* as a variety of *Rana chalconota,* while VAN KAMPEN (1932) considers them as two allied but different species.

Materials and methods

Daily observations have been made on a population of *Rana chalconota* from January till July 1958. The animals were found in a circular ditch about 1½ m deep, on the campus of the technical faculty in Bandung, Indonesia.

To obtain embryological material several pairs in copula were captured and placed separately in an aquarium in the laboratory till the eggs were id. The eggs were then put in aquaria filled with water taken from the .tch where the frogs live. In order to reduce the mortality the water was ɜrated till the larvae reached stage VIII. The highest mortality was found uring the yolk plug stage.

The tadpoles were fed with algae (*Phormidium*, Cynophyceae) and the quaria cleaned regularly. These conditions, however, seem not to corres-ond with the natural condition, since the development in the laboratory 'as much slower than in nature. In addition to the food there are of course ıany other factors influencing the speed of development and the size of he tadpoles. It may be that one of the factors is the lack of space in the quarium. The tadpoles in nature perform very violent up and down move-nents in the deep ditch which cannot be done in the aquarium. It was ıbserved that they remained quiet in the aquarium. M. H. HINCKLEY (1884) ound that the tadpoles from *Rana sylvatica* and *Rana catesbeiana* are ;maller in shallow water than in deep water. Another factor may be that .he Oxygen content of the aquarium water is less than the running water n the ditch. It is a well known fact that a rich O_2 supply accelerates the levelopment of frog larvae (GODLEVSKI 1901). The temperature of the water was checked regularly. The different embryonic stages were fixed in 4% formalin. The drawings were made with a camera lucida. It will be more convenient to refer some minor techniques in the appropriate sections of the text with which they are individually concerned.

Breeding habits

From daily observations during January till July 1958, it may be stated that there is no certain breeding season and that the eggs can be found throughout that half year (table I).

TABLE I.

Month	Total number of batches	Average number of batches per day
January	174	6
February	176	6
March	198	6
April	174	6
May	186	6
June	204	7

SCHIJFSMA (1932) recorded that a great many tadpoles of different sizes were collected from the end of October till the end of January and in March, April and July. From this statement, combined with my own observations, we may accept that the eggs are laid throughout the year.

The data collected during the six months show no relation between the number of batches laid and rainfall. This fact agrees with VAN KAMPEN's statement (1910) that the Javanese Amphibians do not lay their eggs at certain periods but at any time when there is sufficient water, which is the case in the ditch on the campus of the technical faculty, where the water is supplied artificially throughout the year.

The water in the ditch is not stagnant, but slowly running. The tadpoles were found together with *Bufo melanostictus* tadpoles, *Cyprinus carpio* and *Tilapia mosambica*. Copulating pairs can be found at all times of the day. The different stages of the eggs taken from different batches, compared with the speed of development of the eggs in the laboratory show that eggs are laid not only during the night but also during daytime and that there is a certain preference to lay the eggs at night or early in the morning. The female of a pair in copula in the laboratory laid her eggs at 12.30 a.m.. During copulation the male frog clasps the female just behind her front legs. It was observed several times that a pair copulate during more than 24 hours; in some cases there were 2-3 males on the back of one female; when the female moves or jumps the male starts to call.

In more than 900 observed cases the eggs were always laid in a thin surface layer in the water, attached to water plants, drifting objects or against the wall of the ditch, but never as SARASIN, VAN KAMPEN (1923) and DELSMAN (1950) described that the eggs are laid in a mass of mucilage, forming a sort of nest out of the water. It is true that these frogs have more or less the same life habits as the genus *Rhacophorus*, living on the leaves of shrubs and even high in trees, but they do not lay their eggs in a nest out of the water. A batch taken out of the water will die in the very first stages.

In order to estimate the number of eggs in a batch and the size of the eggs, ten batches were collected in the last week of June. A batch was dried roughly, a part of it (50 eggs) weighed on a Mettler balance which gave a certain value (m). Then the whole batch was weighed (b), $\frac{b}{m} \times 50$ gives an estimate of the number of eggs in a batch which after some tests, proved to be very close to the actual number. From every batch, twenty eggs were drawn with a camera lucida and the diameter measured. The mean value with its standard deviation was then calculated.

TABLE II.

Batch	Number of eggs/Batch	Mean diameter of the eggs from one batch.	Standard deviation	The biggest egg	The smallest egg
A	2044	1,5 mm	± 0,067	1,6 mm	1,4 mm
B	1002	1,4 mm	± 0,097	1,6 mm	1,4 mm
C	1225	1,5 mm	± 0,081	1,6 mm	1,4 mm
D	3250	1,7 mm	± 0,081	1,8 mm	1,5 mm
E	2236	1,6 mm	± 0,050	1,7 mm	1,6 mm
F	1646	1,5 mm	± 0,055	1,6 mm	1,5 mm
G	1292	1,6 mm	± 0,071	1,8 mm	1,6 mm
H	1004	1,8 mm	± 0,055	1,8 mm	1,7 mm
I	1000	1,3 mm	± 0,098	1,6 mm	1,3 mm
K	1050	1,5 mm	± 0,097	1,6 mm	1,4 mm

Mean number of eggs per batch = 1575 ± 221,8. The number of eggs varies from 1000 to 3250 per batch. The mean diameter of the eggs = 1,5 mm ± 0,047.

The greatest variation of the size of the eggs within a batch is 0,3 mm. The eggs from the different batches vary in size between 1,3 — 1,8 mm.

Similar variations are recorded for:

Rana temporaria, 1,5 mm — 2,1 mm } CHAMBERS (1908).
Rana esculenta, 1,17 mm — 1,72 mm }
Bufo viridis, 1,00 mm — 1,50 mm AHL (1930).
Rana platyrrhinus, 1,8 mm — 2,5 mm LIEBERKIND (1937).

Statistical calculations show that there is no correlation between egg size and the number of eggs in a batch.

DEVELOPMENT

Eggs from the same batch, reared under the same conditions, developed at quite different rates. Up to the yolk plug stage there is not much difference in the speed of development of the different eggs, but after the yolk plug stage the rate of development varied so much that it was of very little value to indicate the different stages of development by the age length of the embryo. Bigger embryoes can be in a much earlier stage than smaller ones, embryoes of the same age can differ very much in respect to their stage of development even if they are taken from the same batch and reared under identical conditions.

Because of these facts I followed the ten basic stages from LIEBERKIND (1937). The length and the age of the embryo are also mentioned but the data given apply only to a particular case, under certain conditions; they are not generally applicable.

The uncleaved egg (fig. 1)

After fertilization the egg rotates freely within the perivitelline space, oriented with the dark brown pigmented animal hemisphere upwards.

Cleavage (fig. 2, 6)

Temperature of the water: 26° C.

The first cleavage occurs one hour and ten minutes after fertilization, the second 35 minutes after the first, the third 18 minutes after the second, the fourth 35 minutes after the third and the fifth 15 minutes after the fourth, giving rise to the 32 cells stage with 16 pigmented micromeres and 16 cream white macromeres. From now on the regularity of the cleavage is lost, the micromeres divide faster than the macromeres.

The blastula (fig. 7-8)

With advancing cleavage the blastula is formed. In the later blastula stage (2 hours after the 32 cells stage) pigment accumulations around the nuclei of the cells become visible with a magnification power of 50 (Leitz binocular microscope with illumination), especially in the macromeres (fig. 8).

The nuclei in the cells are visible as brown spots, and even the nuclear divisions are very clearly to be followed and even the spindle is visible as fine brownish threads of pigment running from one daughter nucleus to the other.

The gastrula (fig. 9-14)

The beginning of gastrulation appears as a crescent shaped invagination (fig. 9). This happens 5 hours and 25 minutes after fig. 8. One hour and forty minutes after the dorsal lip is formed the lateral lips appear (fig. 10) and thirty minutes later the ventral lip is formed (fig. 11). The crescent shaped dorsal lip of the blastopore which appeared at the beginning of gastrulation becomes converted finally into a ring. The numerous yolk cells (fig. 12, 1 hour and 50 minutes after fig. 11) are crowded into the blastopore and form the yolk plug.

Stage A - (fig. 13, temperature of the water 25° C)

Three hours later the yolk plug has decreased in diameter, but gastrulation is not yet completed.

Stage B - (fig. 14)

Three and a half hour after stage A the gastrula is completed and the blastopore has diminished in size to a small pear-shaped outline.

Stage C - (fig. 15, temperature of the water 24,5° C)

Two hours after stage B, the neural folds are just formed and are visible as two faint elevations; the lateral lips of the blastopore are fused completely to form a very small longitudinal groove, the primitive streak.

Stage I: (fig. 16 b, 16 c)

Two and a half hours after stage C, also called the early neurula stage the embryo has elongated in its antero-posterior axis and has a more or less ovoid shape. The neural folds appear as two clearly visible bands widely separated by the neural groove.

Posteriorly the neural folds become narrower and anteriorly they broaden out and converge in the midline, so that a horseshoe-shaped structure is formed. The anterior neural folds thicken in their anterolateral region, forming the sense plate. The gill plate is not yet visible at this stage. Just below the sense plate on the anteroventral side of the embryo the first traces of the oral suckers appear as two elongated dark lines. These are more prominent laterally and narrower towards the midline of the embryo where they seem to meet each other very indistinctly. One gets the impression that the organ consists of two halves.

The first faint elevations around these pigmented lines start to develop.

Stage II: (fig. 17 a - 17 b)

Two hours after stage I.

Fig. 17 a and 16 b do not fit quite well in this stage which is characterized by LIEBERKIND as follows: Neural tube closed, oral suckers indicated by pigmented areas, not yet surrounded by distinct swellings. The gill Anlage not yet or vaguely indicated.

I have placed fig. 17 in stage II based on the shape of its oral sucker and absence of the gill Anlagen though the neural tube is not yet closed. At the time that the neural tube is closed swellings of the oral suckers are very prominent, so that the embryo does not fit in stage II in respect to the shape of the oral suckers (fig. 18).

It would be more practical, at least for this particular case, to eliminate the factor "Neural tube closed". The two pigmented lines from both sides meet each other in the midventral line of the embryo, so that the Anlage of the organ becomes a crescent-shaped pigmented band vaguely elevated.

Stage III: (fig. 19 a-c - 20 a-b)

Twenty eight to thirty hours after stage II. The contour of the back of the embryo has changed from a concave curve to a straight line. There is a distinction between head, neck, and tail regions. In this stage (fig. 19 a) the three primary differentiations of the brain become apparent. The location of the mesencephalon is easily visible since it forms the cranial flexure. Anterior to the mesencephalon is the prosencephalon and posterior the rhombencephalon. The gill plate appears as an elevation behind the optic bulge which is still in formation and which can be seen laterally by its more or less circular outline on a slightly higher level.

In front of the optic bulges the nasal pits are situated as two small depressions. At the posterior side of the embryo the tail-bud becomes visible.

The oral sucker is divided into separate identical portions (fig. 19 b-c). Each part consists of a furrow, which first runs more or less parallel to the long axis of the embryo; this furrow is rather narrow and ends anteriorly in a widening in which is situated a plug of dark substance. This furrow is surrounded by very prominent elavations (swellings), which are horseshoe-shaped. It is clear from the above description that the oral sucker from this frog belongs to LIEBERKIND's "Esculenta typus". His second type for *Rana* embryoes, the "Platyrrhinus type", is mainly characterized by:

1) Division into two parts happens in stage IV.
2) The furrow is narrow and it has a widening on the posterior end.
3) The first Anlage is a broad dark band.

At the end of this stage (fig. 20 a-b) the gill Anlagen begin to appear. Behind the narrow neck region of the embryo, on both sides of the body, the pronephric bulges appear and for the first time the embryo shows muscular responses.

Stage IV (fig. 21 a-b, 22 a-b)

Ten hours after stage III. The swelling around the furrows becomes more and more prominent, so that any of the original connection between the halves disappears completely. In this stage the oral suckers reach their highest development (fig. 22 a-b).

The mouth of the larva starts to develop, and the Anlagen of the external gills grow rapidly into plume-like structures. At the end of this stage (fig. 22a) the pronephric bulge disappears.

LEMBAGA PERPUSTAKAAN ... RAYA INDO...

Stage V: (fig. 23, a-b)

Eight and a half hour after stage IV. Length of larva: 6 mm. The external gills are fully developed (fig. 23), and the mouth bordered by an upper and lower lip (fig. 24).

The suckers start to reduce gradually. A reduction begins on the posterior side of the organ when the two swellings which first surround the furrow in a horseshoe-shape meet and fuse together at the same time it flattens and rounds itself off (fig. 24). At the end of this stage the opercular fold starts to grow from the hyoid arch backwards over the external gills.

Stage VI: (fig. 25)

One day after stage V, 8 mm larva. The external gills are nearly completely covered by the opercular fold except on the left side where the gills are still visible underneath the operculum (fig. 25). The oral suckers are reduced to two faint elevations and are heavily pigmented. The jaws are edged with black. On the upper lip the Anlage of the first row of labial teeth is indicated by a brownish ridge. On the lower lip three such brownish tooth ridges are visible. The lower border of the lower lip carries one row of small knobs, the Anlagen of the papillae.

Stage VII: (fig. 26)

8 hours after stage VI. The oral suckers are clearly indicated by pigment accumulations; the organ is nearly flat. On the lower border of the lower lip a second row of papillae appears and the sides of the upper lip are now also bordered with papillae.

The gills are completely covered by the operculum; on the left side the spiracle is formed. In this stage the first granular patches make their appearance; an oblong one at each side on the belly.

Stage VIII: (fig. 27 - 28)

Two days after stage VII. The remnants of the oral suckers are indicated by some traces of pigment.

The mouth is rather well developed. On the upper lip there is one row of labial teeth; On the lower lip two rows are present.

Stage IX: (fig. 29 - 34)

The remnants of the oral suckers have completely disappeared. The second pair of granular patches arises in the 1,6 cm tadpole. The patches are found one at each side of the base of the tail dorsoventrally and they are oblong in shape.

The limb buds appear (fig. 29-30) 4 months after stage VIII. At this stage the mouth is well differentiated. The upper lip has three rows of labial teeth of which the two inner rows are broadly interrupted. On the lower lip three rows of labial teeth, almost equal in length, are present. The inner row is sometimes very narrowly interrupted. The lower lip and the sides of the upper one are now bordered with papillae which are arranged in 2 - 3 rows. The papillae of the outer row of the lower lip are not or but slightly longer than those of the inner row in this stage. A month later the tadpole has grown to a length of 2,5 cm (fig. 31).

On the upper lip the 4th row (the innermost one) of labial teeth is developed. It is the shortest one and it is broadly interrupted. All ten tadpoles with fully developed hindlimbs, examined by me, possess four rows on the upper lip. All tadpoles with the hind limbs only slightly developed (fig. 29 - 30) possess three rows of labial teeth on the upper lip; while in still younger tadpoles there are only 2 rows (fig. 28). This is again a fact which shows that teeth formulae for the identification of tadpoles can only be applied when the hind limbs have appeared, all the granular patches are present at this stage:

1. an oblong one at each side on the belly.
2. dorsoventrally an oblong one at each side of the base of the tail.
3. dorsally a round one behind each eye.
4. two patches beside each other behind the mouth have just appeared on the ventral side but are not yet fully developed.

Two weeks later (fig. 33 - 34), 3,2 cm tadpole, the papillae of the outer row of the lower lip become long and prominent.

In all tadpoles of this stage and the following stages the papillae of the lower border of the lip are long and prominent. One of the characters which distinguishes the tadpoles of *Rana labialis* from those of *Rana chalconota*, according to VAN KAMPEN (1923), is that *Rana labialis* has long and prominent papillae on the lower border of the lip. while *Rana chalconota* has not. But according to my observations the older tadpoles (with fully developed hind limbs) of *Rana chalconota* have long papillae on the lower border of the lip; the length of these papillae changes during development, short at the beginning, long and prominent in the older stages.

Stage X.

The left fore leg becomes visible extending through the spiracle on that side. At this stage the tadpole was five cm (tip of the snout 6 — tip of he tail) in length; its thighs are granular. From now on the tail starts its regression which takes about 5 - 6 days.

In a 4.6 cm tadpole with both front legs extended, the larval skin is cast off and with it the horny jaws of the mouth. The frilled lips start to disappear; on the upper lip the outer row of labial teeth has disappeared completely and from the three inner ones only remnants are visible. So the row which has first appeared during the development will disappear first during metamorphosis. This fact has already been described by LIEBERT (1894) for *Rana esculenta*. On the lower lip only the traces of the innermost row are visible, the other two outer rows have disappeared completely. In the upper lip the rows of labial teeth appear during the development, from the outer edge of the lips towards the mouth and they disappear during metamorphosis also in that succession. In the lower lip the first row that appears is the most inner one, the other two arise later towards the edge of the lip, but the rows disappear in the opposite succession, from outside towards the mouth.

Contrary to VAN KAMPEN's statement (1910) that the granular patches become indistinct in the older tadpoles, the granular patches in ten observed cases were always still very clearly visible, and that remained for at least a week after metamorphosis.

In microscopical sections (fig. 35) these glands appear as an accumulation of glands which are very similar, at least morphologically, to granular glands. A piece of skin containing a granular patch of a recently metamorphosed frog was fixed in an alcohol-formalin mixture, embedded in paraffine, cut at 7 μ and stained with Delafields haematoxylin and eosin.

The glands are multicellular and are 100 - 300 μ in diameter, extending into the corium. Each gland opens by a minute duct of about 5 μ in diameter to the outside. The lumen of the gland is filled with granules stained deeply red with eosin. There are also some blackish brown granules, mostly more concentrated in the part toward the epidermis.

The lumen of the gland is lined by cells the shape of which depends on the activity of the cell. In this case the lining cells are very flat. The glands are enveloped by a connective tissue capsule and smooth muscles. This assemblage of glands is enmeshed in a network of capillaries.

It is rather obvious that these glands are poison glands, a reason why the tadpoles are not attacked by carnivorous fishes as e.g. *Tilapia mossambica*, and *Monopterus albus*, with which they live.

Conclusions

1. There is no breeding season, the eggs are laid throughout the year.
2. Contrary to the statements of SARASIN VAN KAMPEN and DELSMAN, the eggs are always laid in a thin surface layer in the water attached to drifting objects.
3. There is no correlation between egg size and the number of eggs in a batch.
4. The ten basic stages from LIEBERKIND are more practical than data concerning age and length of the tadpoles.
5. In the blastula and gastrula stages the nuclei of the cells are visible as pigment accumulation.
6. It is advisable to eliminate one of the characteristics for stage II: "neural tube closed".
7. In respect to the adhesive organ *Rana chalconota* belongs to the "Esculenta type".
8. The granular patches appear after each other: first one at each side of the belly, then at the base of the tail, after it one behind the mouth. They remain till at least one week after metamorphosis.
9. Teeth formulae can only be used for tadpoles with fully developed hind limbs. *Rana chalconota:* 4 rows in the upper lip, 3 inner ones broadly interrupted. Three in the lower lip.
10. In the upper lip the rows of labial teeth appear from the upper border inwards, and disappear during metamorphosis in the same succession.
11. In the lower lip the rows of labial teeth appear from the mouth towards the lower border of the lip but disappear in the opposite succession.
12. The papillae of the outer row on the lower lip become long and prominent in the older tadpoles.

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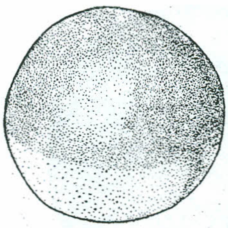


Fig. 1.

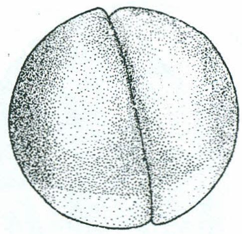


Fig. 2.

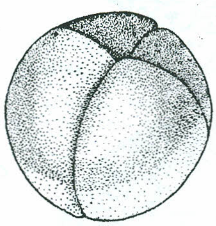
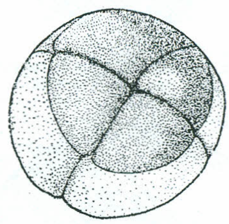


Fig. 3.



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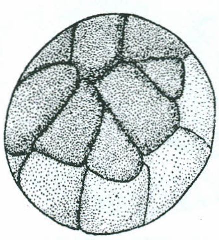

Fig. 5.

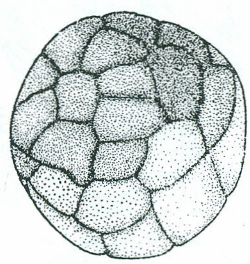

Fig. 6.

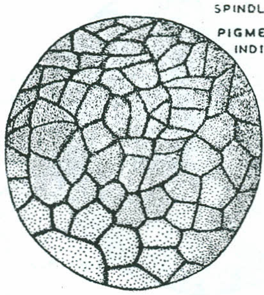

Fig. 7.

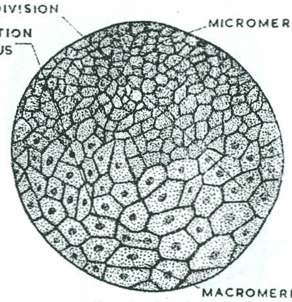

Fig. 8. BLASTULA

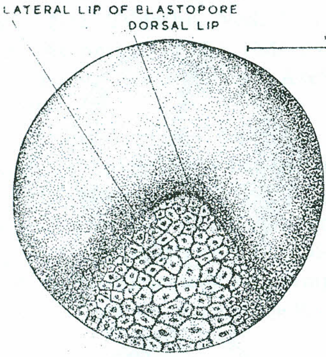

FIG. 10 GASTRULATION.
CRESCENT-SHAPED BLASTOPORE
SEEN FROM POSTERIOR OR BLASTOPORAL SIDE

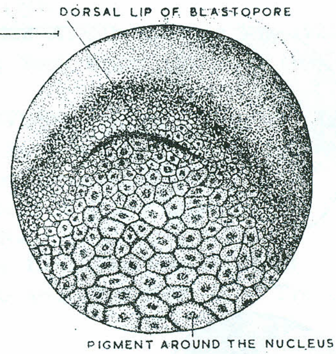

FIG. 9 BEGINNING OF GASTRULATION

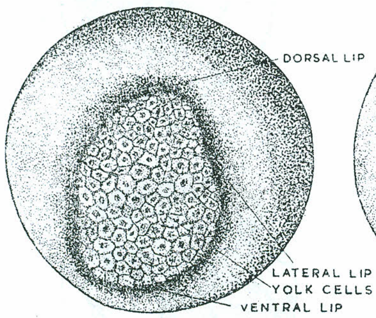

FIG 11 RING-SHAPED BLASTOPORE
SEEN FROM THE POSTERIOR
OR BLASTOPORAL SIDE.

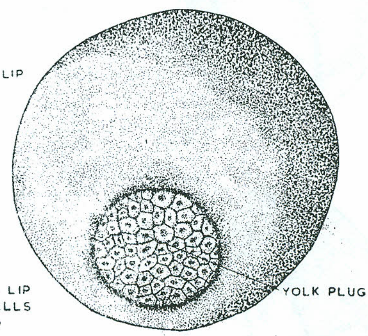

FIG 12 YOLK PLUG STAGE.
SEEN FROM BLASTOPORAL SIDE

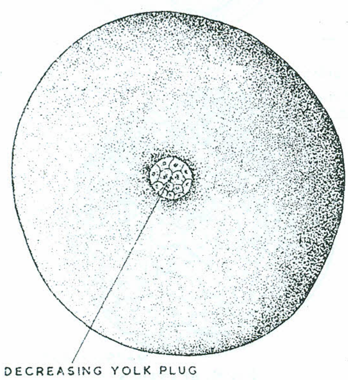

FIG. 13 STAGE A. DECREASING YOLK PLUG
SEEN FROM BLASTOPORAL SIDE

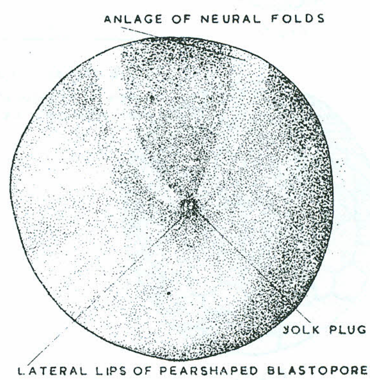

FIG. 14 STAGE B. PEARSHAPED BLASTOPORE
SEEN FROM BLASTOPORAL SIDE

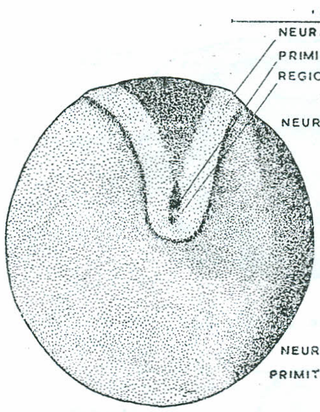

FIG. 15 STAGE C. PRIMITIVE STREAK. POSTERIOR VIEW

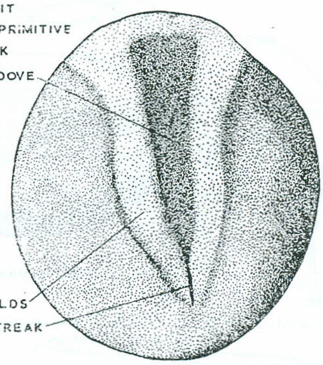

FIG. 16A STAGE I. DORSAL VIEW NEURAL-FOLDS FORMING A HORSE SHOE-SHAPED STRUCTURE

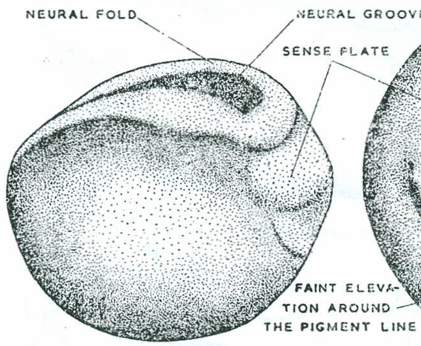

FIG. 16B STAGE I. DORSOLATERAL VIEW

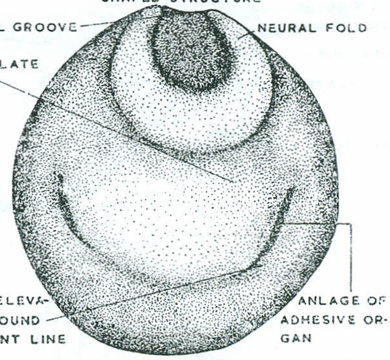

FIG. 16C STAGE I. ANTERIOR VIEW

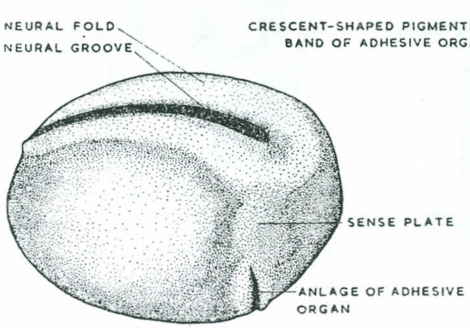

FIG. 17A STAGE II. DORSOLATERAL VIEW

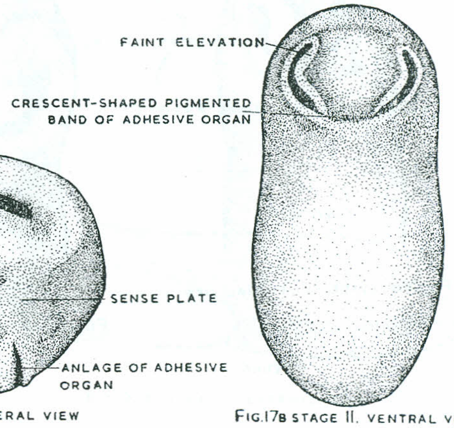

FIG. 17B STAGE II. VENTRAL VIEW

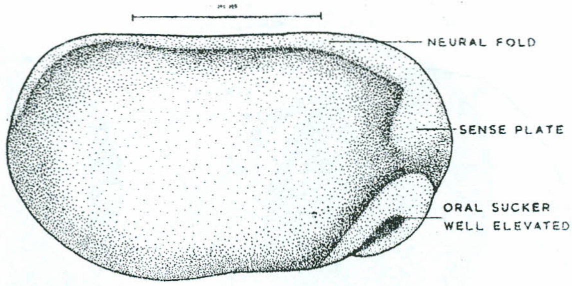

FIG. 18. CLOSED NEURAL TUBE. LATERAL VIEW

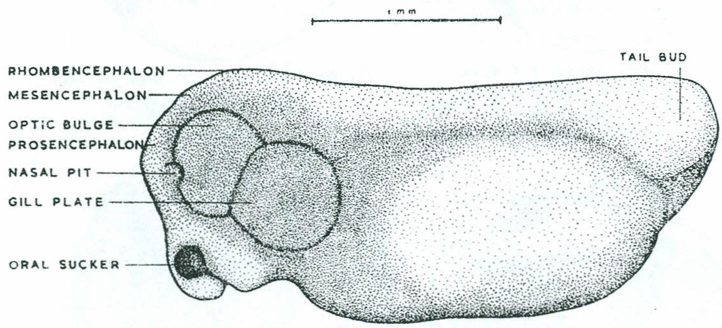

FIG. 19A STAGE III LATERAL VIEW

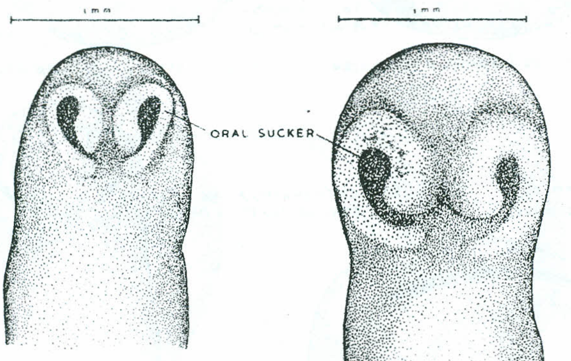

FIG. 19B. STAGE III VENTRAL VIEW

FIG. 19C. STAGE III VENTRAL VIEW

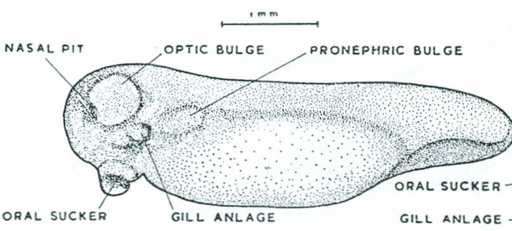

FIG. 20A STAGE III LATERAL VIEW

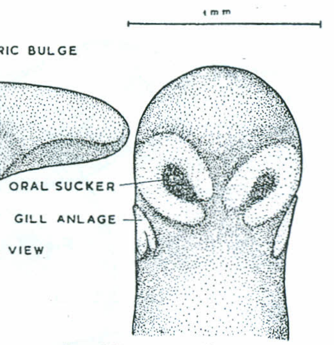

FIG. 20B STAGE III VENTRAL VIEW

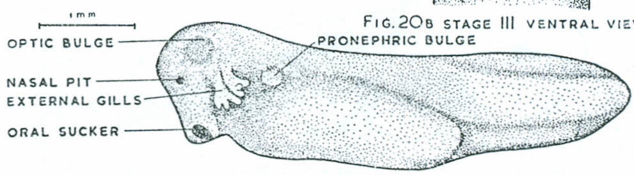

FIG. 21A STAGE IV LATERAL VIEW

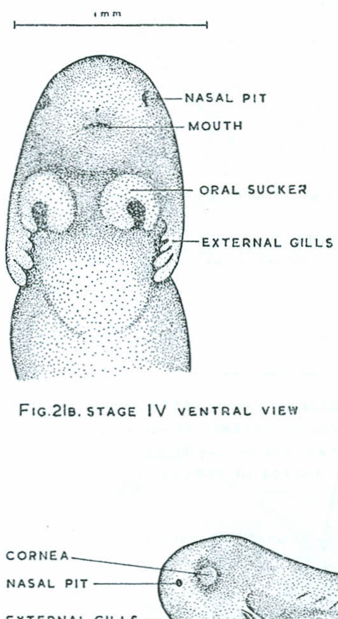

FIG. 21B. STAGE IV VENTRAL VIEW

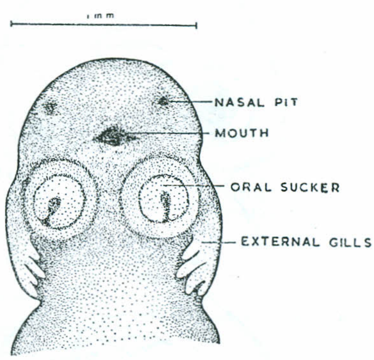

FIG. 22B STAGE IV VENTRAL VIEW

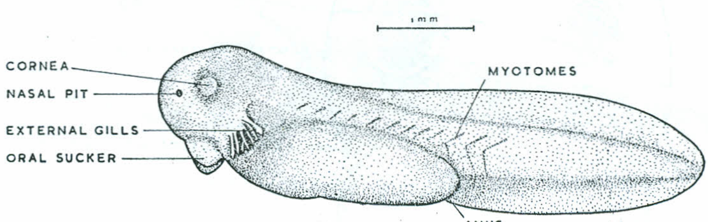

FIG. 22A STAGE IV LATERAL VIEW

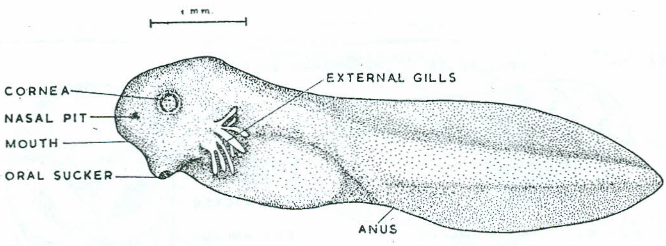

FIG. 23. STAGE V. LATERAL VIEW

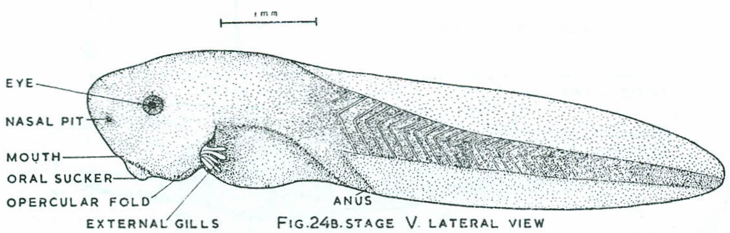

FIG. 24B. STAGE V. LATERAL VIEW

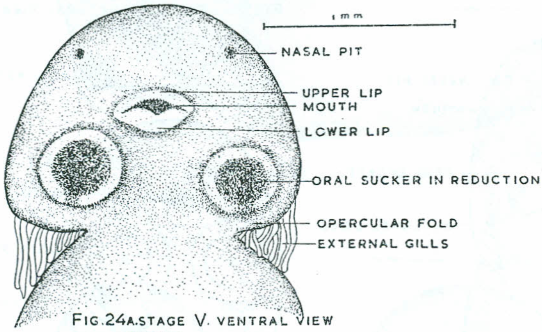

FIG. 24A. STAGE V. VENTRAL VIEW

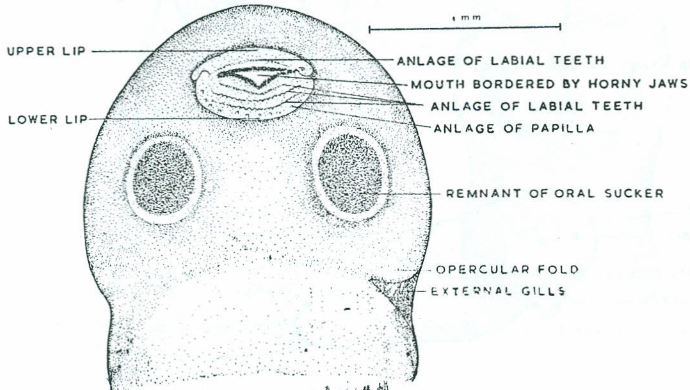

FIG. 25 STAGE VI. VENTRAL VIEW

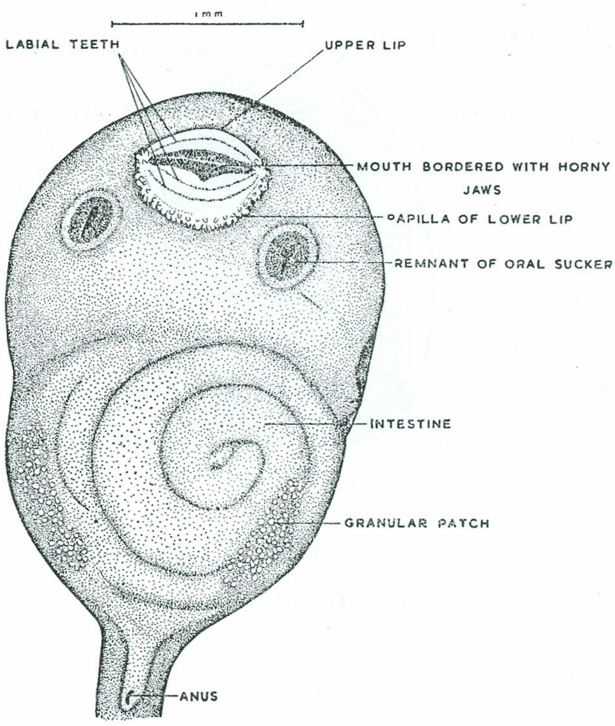

FIG. 26. STAGE VII VENTRAL VIEW

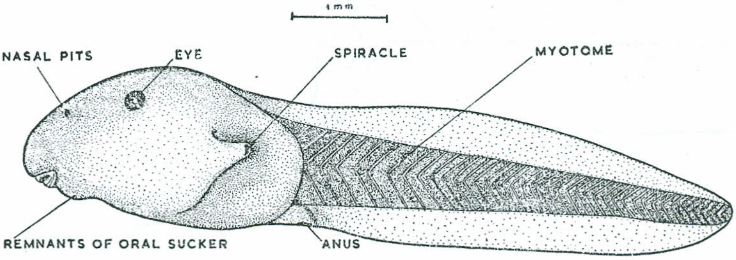

FIG. 27. STAGE VIII LATERAL VIEW

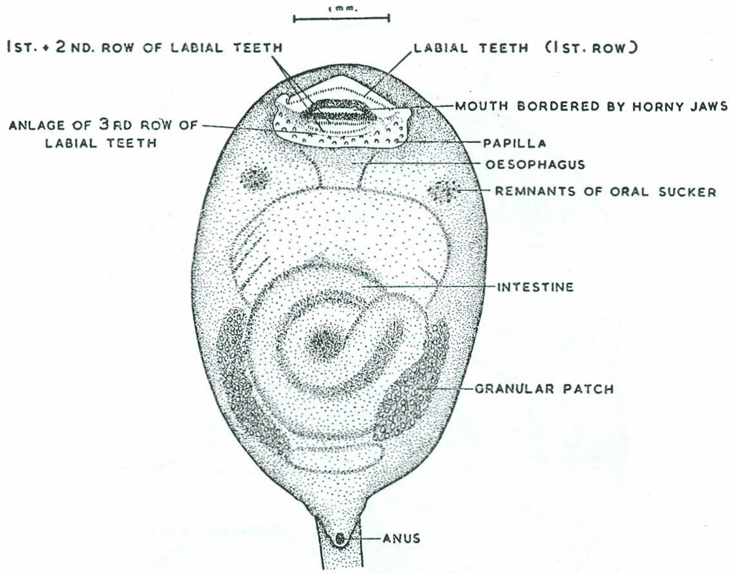

FIG. 28. STAGE VIII VENTRAL VIEW

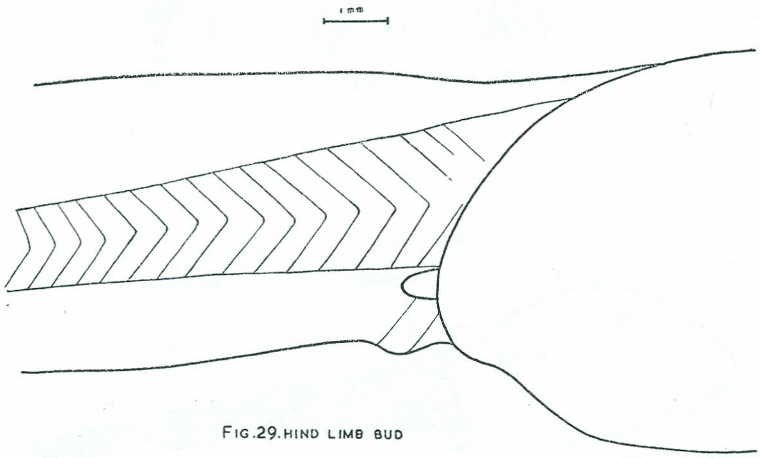

FIG. 29. HIND LIMB BUD

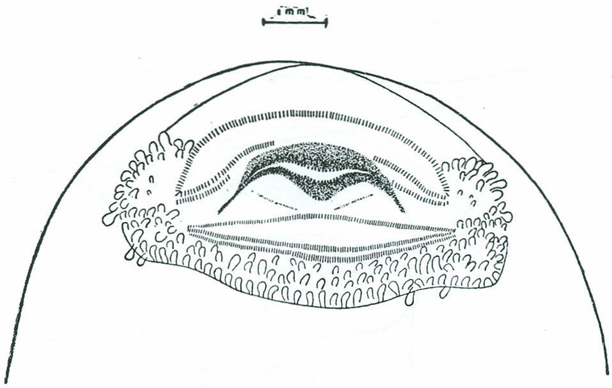

FIG. 30. MOUTH OF A TADPOLE WITH A LIMB BUD

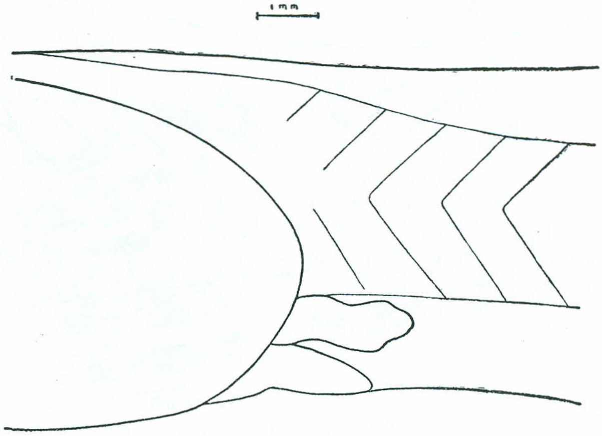

FIG. 31.

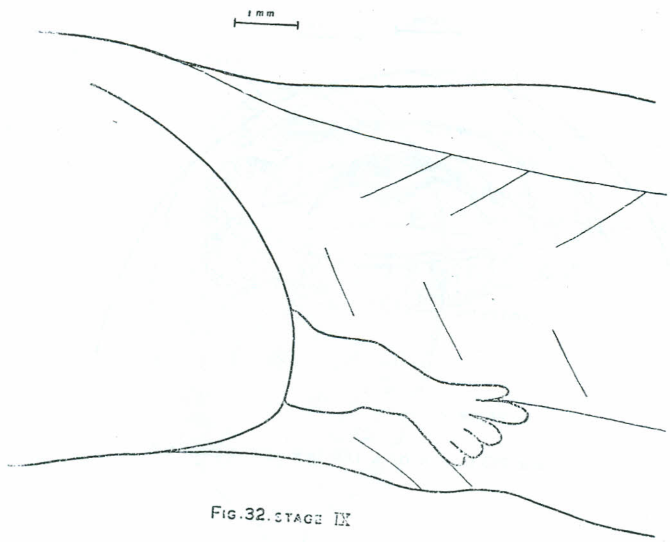

FIG.32. STAGE IX

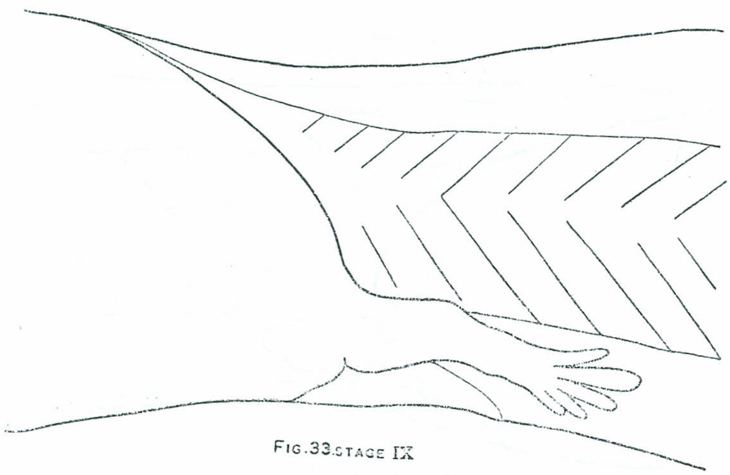

FIG.33. STAGE IX

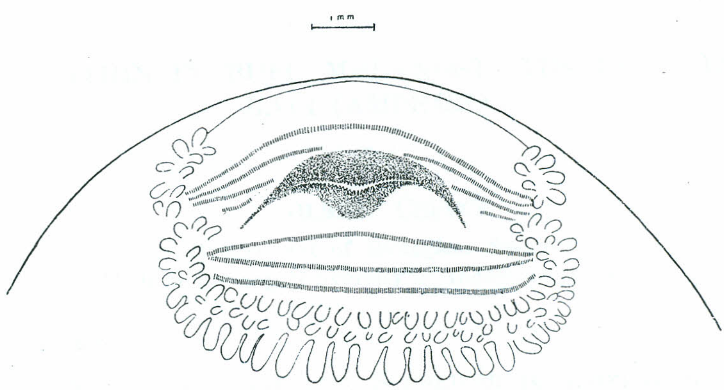

FIG. 34. STAGE IX

MOUTH OF A TADPOLE WITH FULLY DEVELOPED HINDLIMBS

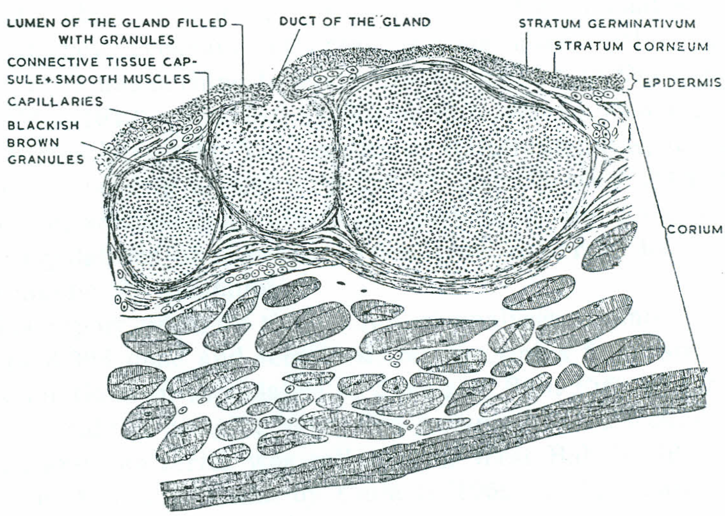

FIG. 35. SECTION THROUGH

A GRANULAR PATCH OF A JUST METAMORPHOSED FROG

H. E. STAINING, FIXATION ALCOHOL-FORMALINE MIXTURE