BIOLOGIA
Vol. 11, No. 1
June, 1965

A Normal Table of *Bufo melanostictus* Schneider

By

MOHAMMAD SHARIF KHAN, M.Sc.

*Department of Zoology, Government College, Lahore**.

INTRODUCTION

Bufo melanostictus Schneider is the commonest toad of the Indo-Pakistan Sub-continent and occurs in almost all parts of Pakistan (Murray, 1894).

Being so widely distributed and easily obtainable, *Bufo melanostictus* could well serve as a type for classroom study, and could also provide material for experimental work on amphibian Embryology. In both cases however, it would be necessary to have a detailed account of its normal development.

Detailed description of the various stages of development of over a dozen amphibians, often arranged in the form of illustrated tables, have been published but almost no literature on the development of our common toad, *Bufo melanostietus*, is available. In this Laboratory Hashmi made a preliminary study of the early developmental stages of this toad, but his studies were aimed at getting a general picture of early ontogeny rather than at compiling a Normal Table of development. Moreover, his findings have remained unpublished so far.

The present study has been undertaken to prepare a Normal Table of *Bufo melanostictus* as a preliminary to further embryological work on this animal.

This work was done in the laboratories of the Government College, Lahore, in partial fulfilment of the requirements for the M.Sc. degree of the University of the Panjab. I wish to acknowledge my indebtedness to Dr. Ahsanul Islam who suggested the problem and under whose supervision the work was completed.

MATERIAL AND METHOD

(*a*) Collection of Material

The material for the present study was collected from the Government College lawns, the *Gol Bagh*, and other localities in the vicinity of the laboratory. In order to make sure that the eggs and the other developmental stages, on which this study is based, really belonged to *Bufo melanostictus*, it was necessary

*Present Address:
Department of Zoology, Talim-ul-Islam College, Rabwah, District Jhang, West Pakistan.

to catch the animals in the act of laying eggs. This end was achieved by following Hashmi's (unpublished) technique, whereby copulating pairs of toads are collected and kept in a large basin in the laboratory. In most cases the male and female remain in amplexus even in the collecting bag; the eggs are laid when the animals are released into the basin containing fresh-water. In other cases where the male and female go out of amplexus when disturbed, they frequently resume the process of copulation when put back in water. In either case strings of eggs can be collected as soon as laid.

In Lahore copulating toads are come across during the rainy season (July to early September) soon after a downpour, especially in the morning. The possibility of keeping a collection of live toads in the laboratory, and thus avoiding the trouble of searching for toads early in the morning, was also explored. Unfortunately the animals kept in the froggary did not go into amplexus and, thus, did not provide any material for the present study.

In the pairs which were brought to the laboratory, the entire process of egg laying was observed. To begin with the male jumps on to the back of the female and catches hold of her with the help of his fore-arms. The two remain in this position for a long time—the exact duration being different in different cases. The female may jump about from place to place, carrying the male on her back. When the female is about to lay eggs, rhythmic movements which are from infront backwards can be seen externally on her belly. Then she settles down at the bottom of the tank, and this is an indication for her mate to bring his hind-limbs together to make a "pouch". In that "pouch" the eggs are gathered and the spermatic fluid is ejaculated over them by the male. When no more eggs are coming out of the cloacal opening, the female comes up again to the surface of water. The male withdraws his hind legs and the egg strings are let loose. The eggs are laid in this way in many instalments.

As mentioned above the female continues to change her position so that the egg strings get wound round the water plants and the stones lying in the pond. The egg strings are very sticky when laid, and readily stick to every object they come in contact with. Sand particles adhere to the egg strings which, therefore, become comouflaged and are probably saved from the predators, like crows etc.

As soon as laid, the egg strings were collected in a petri dish and were transferred to a large earthen trough containing tap-water. Some plants of *Hydrilla* were also added to the trough to ensure a supply of Oxygen. In the earthen vessel the temperature of water is usually about 2°C below the room temperature. This is a distinct advantage during the hot summer afternoon.

Generally the eggs of Amphibia are studied in a saline solution, called

Holtfreter's solution, which is also known as the "standard culture medium". Previous work in this laboratory by Hashmi and Gilani (unpublished) has, however, established that development of toad (*Bufo melanostictus*) and frog (*Rana tigrina typica*) can take place normally in tap-water. The tap-water supplied to the Zoological Laboratory is pumped out by a tube-well near the college, and is stored in a reservoir. Its pH was found to be 7.0.

Unfortunately, no device for maintaining a constant temperature was available. The development was, therefore, allowed to proceed at the room temperature, which was recorded at short intervals. It was noted that fluctuations were relatively slight, and the entire development could be regarded as having taken place at a temperature between 29-32°C.

(*b*) **Fixation**

At first Bouin's fluid, rectified spirit and 5% formalin were used as fixatives, but it was seen that early embryos, particularly from gastrula Stage to tail bud Stage, were not preserved adequately in these fixatives and the material was damaged in shape and form. Bouin's fluid diluted with an equal volume of water was seen to be a good fixative. It was also seen that if the eggs are dipped in boiling water and then preserved in 3% formalin, they keep their shape and form. So both these methods were employed for fixation.

It was also noticed that coats of jelly could be removed easily from those eggs which had been fixed by immersion into hot water. After a treatment with boiling water those eggs were kept in 3% formalin overnight, and then the jelly was removed with the help of needles or fine forceps.

For storage, at first a mixture of equal quantities of 70% Alcohol and Glycerine was used, but it was seen that embryos from gastrula to tail bud Stage tended to be deformed. So, these stages were stored in 3% formalin. The rest of the stages were, however, stored in 70% Alcohol and Glycerine.

(*c*) **Study of material**

In the course of the present study only the external morphology of the various stages of development has been taken into consideration.

The observations have been recorded in drawing. All the drawings have been made with the help of camera lucida (Abbe model).

For measuring the eggs and early stages, a disc eye-piece micrometer was employed, while the later embryos were measured with the help of a mechanical stage.

The diameter of the eggs and early cleavage stages was recorded along the equatorial plane. These stages are completely spherical. Each stage was measured at least twice to ensure against any possibility of error.

For advanced stages the following lengths were measured:—

(*i*) The whole length of the animal from anterior tip of the body to the tip of the tail.

(*ii*) The length from anterior tip of body to anus.

(*iii*) The length from anus to the tip of the tail.

As mentioned above no thermostatic control of the water in which the embryos were developing could be ensured. Consequently, the age of various stages in hours could not be regarded as a valid criterion for recognizing the stages of a Normal Table. Some observations regarding the age of early developmental stages were, however, made and are recorded in Table I.

TABLE I

Stage		Time	Temperature
Oviposition	..	8-8.30 am.	29.5°C
2-Cell	..	9-9.15 a.m.	29.8°C
Blastula	..	10.00 a.m.	30.8°C
Formation of Blastopore	..	11.45-12.00 a.m.	31.2°C
Constriction of Blastopore	..	2.00-4.00 p.m.	31.85°C
Closure of Blastopore	..	6.00-6.30 p.m.	32.0°C
Neural plate	..	6.00 p.m.	31.5°C
Neural folds	..	6.30 p.m.	30.3°C

(*d*) **"Staging"**

A reference to the previous literature shows that there is no standardized method of dividing the early ontogeny into stages. Different authors have adopted their own schemes or systems with the result that there is no guarantee of the comparability of similarly numbered stages of different species.

In the course of the present study an attempt has been made to follow the pattern of the Normal Table of *Xenopus laevis* (Daudin) edited by Nieuwkoop & Faber (1956), in so far as the external characters can be used for the purpose.

Only the distinct, unmistakable and easily recognizable stages of development have been numbered, and full care has been taken to avoid multiplication of stages due to variations in the structures. In all 43 stages have been recognized in the development of *Bufo melanostictus*. It has not been found feasible to recognize a pre-metamorphosis period and a period of metamorphosis, because all changes, however sudden they may appear to be, are gradual in their final analysis. It may however be mentioned that stage 25 is relatively stable and if suitable conditions for metamorphosis are not available, the animal may continue in this stage.

THE NORMAL TABLE OF *BUFO MELANOSTICTUS* SCHNEIDER

Stage 1. Unfertilized egg. (Plate 1).

Average diameter: 1.3 mm.

Animal (A.P.) and vegetal poles (VEG. P.) are distinct. The animal hemisphere is deeply pigmented, while the vegetal pole is without pigment and appears to be almost white. The pigment extends up to midway between the equator of the egg and the vegetal pole.

Stage 2. Fertilized egg. (Plate 1).

Average diameter: 1.3 mm.

This stage differs from stage 1 in having, between the animal and vegetal hemispheres, a crescentic area which is neither black nor white, but is intermediate (grey) in colour (CRES.).

The pigment is just touching the equator of the egg, but does not extend beyond it towards the vegetal pole.

The vitelline membrane (VIT.) which adhered very closely to the egg at Stage 1, becomes rather loose after fertilization and can be distinguished readily.

Stage 3. 2-Cell stage (Plate 1).

Diameter: 1.25 mm. to 1.3 mm.

The first cleavage is vertical and divides the fertilized egg into two almost equal blastomeres (BL.). The cleavage furrow is equally distinct and deep throughout its length. The animal pole (A.P.) is deeply pigmented whereas the vegetal pole (VEG. P.) is cream-coloured. The vitelline membrane is clearly indicated. The ends of the blastomeres are rounded. The grey crescent is visible. It is broader on the future dorsal side than on the opposite side. It is like a pigmented oblique area extending on the future dorsal side between the animal pole and the middle of the egg. The yolk appears to be homogeneous.

Stage 4. 4-Cell stage (Plate 1).

Diameter: 1.25 mm. to 1.3 mm.

The second cleavage is also vertical and lies almost at right angles to the first one. The cleavage furrows start from the animal pole but fail to meet at the vegetal pole. The furrows passing through the two blastomeres do not make an angle of 180° with each other at the animal pole. This cleavage therefore, results in the formation of 4 blastomeres two of which are smaller than the others. The smaller blastomeres are cut towards the future dorsal side. This orientation can be deduced with reference to the grey crescent area: the smaller blastomeres have a smaller pigmented area (and, hence, a larger share of grey crescent area) than do the larger blastomeres.

Stage 5. 8-Cell stage (Plate 1)

Diameter: 1.25 mm to 1.3 mm.

The third cleavage is horizontal and is almost equidistant from the animal pole and the equator of the egg. It results in the formation of 4 cap-like micromeres (MI.) placed on top of 4 macromeres (ME.)

The two larger blastomeres seen on the (future) ventral side at Stage 4, cut off micromeres which are larger and more pigmented than those of the smaller blastomeres. The vitelline membrane is discernible, though with some difficulty.

Stage 6. 16-Cell stage (Plate 1).

Diameter: 1.25 mm to 1.3 mm.

The 4 micromeres and the 4 megameres have divided once to give rise to 8 micromeres and 8 megameres. Cleavage furrows make their appearance simultaneously in micro-and megameres. These furrows do not pass exactly through the animal pole but along both of its sides thus cutting the respective megameres in such a way that the portion which is cut outside is smaller than that which is cut on inner side.

The animal pole is darker than the vegetal pole which is almost white. The vitelline membrane is discernible to some extent. The grey crescent area has become divided up into cells, and is not easily recognizable.

Stage 7. Early cleavage (Plate 1).

Diameter: 1.168 mm. to 1.204 mm.

The distinction between the micromeres and megameres is quite sharp as the former are much darker than the latter. All the micromeres are not alike: the micromeres near the animal pole are smaller than those lying close to the middle of the egg. Similarly the megameres near the vegetal pole are larger than those lying near the marginal zone. The ends of the blastomeres are round. The cleavage furrows are not pigmented. The vitelline membrane is discernible.

The micro- and megameres are not delimited very clearly so that there is a gradual increase in the size of the blastomeres from the animal to the vegetal pole.

Stage 8. Mid-cleavage (Plate 1).

Diameter: 1.25 mm. to 1.3 mm.

This stage can be distinguished from the previous one by its much smaller micromeres whose area is clearly delimited, The pigment is confined only to the micromeres which occupy a region in the animal hemisphere, above the equator. The yolk cells also are much smaller and more numerous than at Stage 7.

The megameres are more compactly arranged than at the previous stage where the furrows were quite prominent. The vitelline membrane is discernible.

Stage 9. Beginning of gastrulation (Plate 1).

Diameter: 1.25 mm. to 1.3 mm.

The micromeres (MI.) are very small and have spread over the equator of the egg towards the vegetal pole. The area so covered by the micromeres is darker. The descending border of the micromeres is less dark like a band around the egg just below the equatroial line of the egg.

Megameres (ME.) white and large are now visible only at the vegetal pole and in some area around it. The whole area occupied by megameres is called vegetative field.

The vegetative field extends up to ¼th of the distance between animal pole and the middle of the egg.

Stage 10. Dorsal lip of blastopore (Plate 1).

Diameter: 1.25 mm. to 1.4 mm.

The micromeres can be seen to have spread still further towards the vegetal pole. In the vegetal hemisphere—a short distance below the equator, there appears an aggregation of pigment which ultimately marks the dorsal lip of the blastopore (D.L.B.). The blastopore is usually situated midway between animal pole and equator of the egg.

Stage 11. Crescent-shaped blastopore (Plate 1).

Diameter: 1.288 mm. to 1.304 mm.

At this stage the lateral lips of the blastopore (L.L.B.) have also come into being besides the dorsal lip (D.L.B.). Thus the blastopore has become horse-shoe shaped; and the micromeres reach up to it.

The dorsal and lateral sides of the blastopore are surrounded by the micromeres and the ventral side by the megameres.

Stage 12. Yolk plug stage (Plates 1 & 2).

Diameter: 1.288 mm. to 1.304 mm.

At this stage the micromeres have approached the blastopore from the ventral side also, so that a ventral lip of the blastopore has also become recognizable. Through the blastopore (B.) white megameres can be seen like a white circular area called yolk plug (YK.PG.).

On the dorsal side, *i.e.*, anterior to the dorsal lip of blastopore, there extends a darkish area, in the middle of which there is much darker streak running to some distance. This is the region of presumptive neural plate (Plate 2, N.PL.). There is a slight flattening of the neural plate region.

This stage could, perhaps, be split into two stages depending on the size of the blastopore and the shape of the presumptive neural plate. This stage, however, varies considerably and it was not deemed feasible to base a stage merely on the size of blastopore.

Stage 13. Neural plate & Closure of blastopore (Plate 2).

Diameter: 1.345 mm. to 1.486 mm.

The lateral lips of the blastopore have met and the once rounded blastopore has constricted to form a short dark slit (CL.B.), which lies dorsally in a dark groove (N.G.), yolk plug disappears.

The medullary folds (N.F.) (bordering the neural plate) are quite prominent in the rostral region but less so caudally. There is further flattening of the neural region on either side of the groove.

Stage 14. Early neural fold or early neurula stage (Plate 2).

Diameter: 1.427 mm. to 1.496 mm.

The cerebral part of the neural plate bends down. The neural folds (N.F.) are now distinct all along their length enclosing a groove, called the neural groove (N.G.). The neural folds in the cerebral or cranial part have a blackish area at their inner margins.

The dorsal part of the neurula, *i.e.*, the region of the neural plate, shows elongation in the antero-posterior direction, while the ventral part remains of the same dimentions.

On the antero-ventral side there is a crescentric black thin line formed by the aggregation of pigment lying transversely, it marks the position of the cement gland (CE.GL.). The position of blastopore is still recognizable like a dark streak.

Stage 15. Mid neurula stage (Plate 2).

Diameter: 1.96 mm. to 2.1 mm.

In the cerebral region the neural folds are prominent and show a tendency towards meeting in the mid-dorsal line. The anterior part of the neural plate is roundish.

In caudal region the neural folds (N.F.) are quite prominent though not to the same extent as in the cerebral region.

The darkish line observed on the antero-ventral side, in Stage 14, which heralded the formation of the cement gland has become more prominent and thick (CE.GL.).

There is still further elongation in the antero-posterior direction on the dorsal side of the embryo.

Stage 16. Late neurula or neural tube (Plate 2).

Diameter: 2.0 mm. to 2.3 mm.

At this stage the neural groove has closed to form the neural tube (CL.N.T.). The cerebral part of neural tube is much more dilated than the caudal part.

There is an overall increase in the length of the embryo in the antero-posterior direction. So the embryo is more or less rod like with both ends blunt and rounded.

The crescentic band observed in stage 14 & 15 is now "V" shaped. The anterior ends of its limbs are becoming thick. This is the cement gland (CE.GL.).

The embryo shows movements in the jelly and uses cement gland for attachment with the egg membrane.

On either side of the head in median lateral plane, two slight protuberances marking the position of the external gills are to be seen.

Lying in the mid-ventral line anterior to the cement gland, there is a slight depression called the stomodaeal pit (ST.PT.) which marks the position of the future mouth.

The pronephric region (PRO.) appears between the belly and head region on the dorso-lateral side.

Stage 17. Tail bud stage (Plate 3).

Total length : 2.2 mm. to 3.00 mm.
Length from anterior end to the end of belly: 2.9 mm.
Length of Tail bud (from the end of belly to the tip of tail) : 0.1 mm.

On the posterior dorsal end of the embryo a small bud like outgrowth, the tail bud (T.B.D), makes its appearance. Dorsal fin (D.F.) is only slightly delimited.

The head region is marked off from the trunk region. The head region is more massive than the general body.

The V-shaped cement gland (CE.GL.) shows a groove in each limb which is darkly pigmented, dividing the arms into a stomodaeal and a caudal ridge. The stomodaeal pit (ST.PT.) is darkly pigmented and prominent.

The myotomes are clearly visible. The embryo shows muscular movements so that when the embryo is shaken it hatches out of the egg membranes. The position of the eye (E.) can be marked just below the middle part of the brain as a round area on each lateral side of the head. These areas are pigmented like the general body surface. The gill areas (G. AN.) and pronephric reions are more prominent than at Stage 16.

There is an aggregation of pigment on the posterior border of the belly marking the position of future embryo.

Stage 18. Fin formation (Plate 3).

Total length : 3.1 mm. to 3.8 mm.
Length from anterior tip to the end of belly: 2.9 mm.
Length from the end of belly to the tip of tail : 0.28 mm.

Dorsal and ventral fins are clearly seen. The dorsal fin (D.F.) extends anteriorly almost to the level of the pronephric region. Posteriorly it bends round the tip of the tail (T.) towards the ventral side and extends up to the posterior border of the belly thus forming the ventral fin (V.F.). The fins are transparent.

The stomodaeal pit (ST.PT.) is more prominent, a groove extends from it to the middle of the V-shaped cement gland (CE.GL.). A pair of depressions marking the position of nasal openings appear on the anterior medio-lateral sides of the head (OLF. PT.). There is a considerable increase in the number of the myotomes (MYO.). The tail bud is distinct from the belly. The aggregation of pigment at the site of the future anus is prominent.

Hatching takes place spontaneously at this stage. The embryo wriggles out of the egg-membranes by making powerful movements which are restricted to the region between the head and belly. This region is bent alternately to the right and left sides. So the embryo can swim to some extent and can change its position while lying on the bottom of the container or sticking to its sides with the help of the cement gland.

Stage 19. Gill bud stage (Plate 3).

Total length	: 3.1 mm. to 3.5 mm.
Length from anterior tip to the tip of anal tube	: 2.98 mm.
Length from the tip of anal tube to the tip of tail	: 0.51 mm.

The area of presumptive gills (G. AN.) shows an anterior bigger and a posterior smaller outgrowths on the median lateral sides of the head, thus forming the 2 external gill buds. The eye position (E) seen in stage 17 is more marked. The brain region shows prosencephalic, mesencephalic and myelencephalic regions.

The stomodaeal cleft is now lying in a squarish area which is more prominent than in the previous stage. The anal opening is not recognizable clearly, although the anal tube at which the anus will appear can be seen. The head region is sharply marked off from the trunk region. Pronephric region is quite prominent. The tail region becomes further elongated. There is also elongation in the trunk region. Dorsal fin (D.F.) extends up to the level of the junction of head and belly. Dorsal fin is more prominent than ventral fin (V.F.).

Stage 20. Early gill stage (plate 3).

Total length	: 4.0 mm. to 4.2 mm.
Length from anterior tip to anus	: 3.20 mm.
Length from anus to the tip of tail	: 0.88 mm.

The first or the anterior most gill (G. BD.) is better developed than the posterior one, and has its free margins produced into papillae. The posterior or second gill also shows papillae on its margin which are less developed than the papillae of the first gill.

The areas where eyes would appear are quite prominent and bulge out. The stomodaeal region has assumed triangular form. One angle of this triangle continues as a groove into the two arms of the cement gland. The two arms of the V-shaped cement gland (CE.GL.) become separate from each other because the middle portion, which joins the two arms has become very inconspicuous. The belly becomes shorter than in the previous stage, but the tail elongates. The posterior part of belly is narrower than the anterior part. The future position of the anus is marked by pigmented granules. Anal pit (AN.PT.) is also discernible. The dorsal fin (D.F.) is better developed and extends up to the posterior border of the head.

Stage 21. Gill filament stage (Plate 4).

Total length	: 4.6 mm. to 5.0 mm.
Length from anterior tip to anus	: 3.5 mm.
Length from anus to tip of tail	: 1.1 mm.

At this stage the first or anterior gill (G1.) is attached to the side of the head at a slightly lower level than the second gill (G2). Posterior to the second gill there is a slight protuberance marking the position of the third gill. The first and second gills have their margins produced into finger like processes—these are the gill filaments. The first gill has long gill filaments (Plate 8). The nasal pits (OLF.PT.) come to possess distinct boundaries which are pigmented. The eye region becomes well demarcated and definite. The stomodaeal cleft (ST.CL.) is bounded by four ridges, two along the antero-lateral side and two along the postero-lateral side. These ridges lie obliquely to the transverse axis of the body. The stomodaeal depression is deeper than that of previous stages.

The two arms of the cement gland (CE.GL.) have become separate and their ends toward the mouth are swollen. The anus (AN.PT.) is indicated at the junctions of the tail and the belly. The pronephric region (PRO.) is prominent. The dorsal fin has extended up to the level of the first gill. Due to the loss of pigment in the superficial regions, the outer wall of the head becomes almost transparent.

Stage 22. 3-gill stage or tail piece stage (Plate 4).

Total length	: 5.3 mm. to 5.5 mm.
Length from anterior tip to anus	: 2.8 mm.
Length from anus to tip of tail	: 2.6 mm.

The third gill bud which was observed in Stage 21 has developed into a gill, the free margins of which are produced into papillae which are smaller than those of the first and second gills (Plate 8). The eye cups (Plate 4 E.C.) are 'C'—shaped as the ventral sides are not yet been formed.

The cement gland (CE.GL.) had divided into two at Stage 21. Each moiety of the cement gland now is beadshaped and is placed obliquely below the ventro-lateral margins of the mouth. The mouth (MTH.) has assumed a transverse slit-like shape at this stage. The formation of intestine begins at this stage. At the anterior or rostral end of the mass of yolk cells there appears a transverse groove which is called the rostral furrow (ROS.FU). There is also a caudal furrow (CA.FU.) on the right side in the yolk mass. Posteriorly the belly tapers into an anal tube (AN.T.) which opens at anus (AN.). The anus is situated at the junction of the ventral fin with the belly. The tail has elongated greatly, the belly and the tail are almost of equal length, the tail fins are also greatly developed. The portion of the skin extending from the posterior part of the belly up to the anus is called tail piece (T.PE.). In this stage the tail piece is formed. The pronephric region (PRO.) is still prominent. The head has become more transparent than in the previous stage. A lateral line (LAT.LI.) can be recognized as a dark line running on either side from the pronephric region to the posterior end of the tail.

Stage 23. Opercular fold stage (Plate 4).

Total length	: 5.5 mm. to 6.00 mm.
Length from anterior end to anus	: 2.8 mm.
Length from anus to tip of tail	: 3.2 mm.

At this stage from the region of the head just in front of the first gill on either side arise two fold-like extensions of the body wall called opercular folds. (OP.F.). They progressively elongate towards the posterior region covering the gills. The eye cup (E) is completely circular, though still the ventral portion of it is thin. The orbit and nasal openings (NA.OP.) are clearly recognizable. The mouth is surrounded above and below by fleshy areas or lips which unite together to form the oral cup. The mouth is bounded pre-orally by a crescentic horny dark brown ridge. On the post-oral side also there is a horny ridge. Both these ridges form the beak (BE.). The margins of these ridges are not provided with the teeth-like projections as yet. Dental formula at this stage is 1/0. The pre-oral fleshy lip has one complete row of denticles and the post-oral lip has not developed denticles yet.

The cement gland (CE.GL.) has become greatly reduced and the remnants of its two bead-shaped moieties are losing their dark colour. Fourth gill bud appears just behind the third gill. Pronephric region which was quite prominent in previous stages has become indistinguishable here (Plate 8). The demarcation between the head and belly has become further reduced than at Stage 22. The region of head in front of gills shows considerable lateral expansion, so that the head appears to be darso-ventrally compressed. The region of belly however is round ventrally. The head and belly have become almost transparent so that many internal structures can be seen. Melanophores are present on the dorsal side of the head, belly and tail. The lateral line (LAT.LI.) is clearly indicated along the lateral sides of the trunk and tail of the tadpole.

Stage 24. Late opercular stage (Plate 5).

Total length	: 6.0 mm. to 6.8 mm.
Length from anterior tip to anus	: 3.0 mm.
Length from anus to tip of tail	: 3.8 mm.

At this stage the operculum (OP.F.) almost touches the belly on the right side and fuse with it. Only the gill filaments (G.F.) of the right gills are to be seen. (For gills see Plate 8). The eyes are better formed than in the previous stage but still the ventral side of the eyes is thin.

The pre-oral and post-oral ridges of beak (BE.) are provided with sharply serrated margins. The pre-oral fleshy lip bears a complete and uninterrupted row of denticles. The post-oral fleshy lip has two complete rows of denticles. These denticles take the form of sharp, spine like, dark brown processes, used in scrapping the plant or flesh on which the tadpoles feed. The dental formula is 1, 1+1/2. The whole structure constitutes a sucker (S.). The cement gland (CE.GL.) is progressively in the process of reduction. It is now represented by merely small crescentic dark spots.

There is further elongation in the length of the body. Tail exceeds the length of the trunk. The distinction between belly and head is growing less and less. The intestinal coil (INT.CO.) can be seen through the transparent wall of belly. The anal tube and the cloacal tail piece also become further elongated.

Stage 25. Spiracle stage or Hind limb bud stage (Plates 5 & 8).

Total length	: 6.8 mm. to 7.0 mm.
Length from anterior tip to anus	: 3.2 mm.
Length from anus to the tip of tail	: 3.6 mm.

The opercular fold has fused completely with the body wall on the right side, but on the left side the fusion is incomplete so that a spiracle (SP.) is formed through which the gill filaments (G.F.) protrude out. The spiracle is situated on ventro-lateral side of the body in the region where the first outer intestinal coil turns inwards to the mid-ventral line.

The pre-oral fleshy lip has an outer complete row of denticles and a second interrupted row. The post-oral lip has got 3 complete rows of denticles, the dental formula being 1,1+1/3. The denticles are long, spine-like and dark-brown. On the lateral sides of the lips there appear papillae on each side. Now the oral disc is called a sucker (S.). Mouth (MTH.) occupies sub-terminal position. The beak (BE.) is well-formed (Plate 8). The two moieties of the cement gland (CE.GL.) have become reduced merely to aggregations of dark granules. These spots lie near the two ends of mouth on the anterior ventro-lateral sides of the body.

Small hind limb-buds (H. L. BD.) make their appearance at the junction of the belly and the tail on the dorso-lateral side of the anal tube. The intestinal spiral (INT.CO.) is fully formed at this stage. Faecal matter can be seen to come out from the anal tube through anus (AN.) in many specimens at this stage. Melanophores have developed in large numbers on the dorsal and lateral aspects of the body. In the belly region, however, melanophcres are present on the ventro-lateral part also. Ventrally the belly region is flat.

Stage 26. Length of hind limb bud less than its breadth. (Plates 5 & 6).

Total length	: 7.1 mm.
Length from anterior tip to anus	: 3.1 mm.
Length from anus to tip of tail	: 4.0 mm.

The operculum is complete. The spiracular opening (SP.) has no gill filaments coming out of it. The hind limb buds (H.L.BD.) are still very small, and are shorter in length than in breadth. The dorsal surface of the tadpoles is almost black due to the large number of melanophores. The melanophores are seen to spread along the outer intestinal coil. The aggregation of pigment, marking the position of the moieties of cement gland are faintly represented. There is further increase in the length of the tadpole. Other characters are the same as at Stage 25.

Stage 27. Length of hind limb bud greater than breadth (Plate 6).

Total length	: 6.95 mm. to 7.2 mm.
Length from anterior tip to anus	: 3.7 mm.
Length from anus to tip of tail	: 3.68 mm.

The hind limb buds at this stage are longer than broad. There is still further aggregation of the melanophores on the dorsal side as well as along the outer border of the outer coil of the intestine. Other characters are the same as at Stage 25.

Stage 28. Length of hind limb bud $1\frac{1}{2}$ times its breadth (Plate 6).

Length from anterior tip to anus	: 3.6 mm.
Length from anus to tip of tail	: 3.7 mm.
Total length	: 7.0 mm. to 7.4. mm.

The length of the hind-limb bud $1\frac{1}{2}$ times its breadth. The free end of the bud is rounding off.

Stage 29. The length of hind limb bud $1\frac{3}{4}$ times its breadth. (Plate 6).

Length from anterior tip to anus	: 3.8 mm.
Length from anus to tip of tail	: 4.0 mm.
Total length	: 7.1 mm. to 7.5. mm.

The length of the hind limb bud is $1\frac{3}{4}$ times its breadth. Its tip is broad and is flattening up.

Stage 30. Length of hind limb $2\frac{1}{2}$ times its breadth (Plate 6).

Length from anterior tip to anus	: 3.0 mm.
Length from anus to tip of tail	: 4.1 mm.
Total length	: 7.3 mm. to 7.8 mm.

The hind limb bud is $2\frac{1}{2}$ times longer than it is broad. It is round near its base (stump), but its greater part is flattened from side to side (paddle). The free end is no longer round but somewhat narrow and conical and foreshadows the 4th toe. The constriction between paddle and stump marks the position of the ankle.

Stage 31. 5th toe stage (Plate 6).

Length from anterior tip to anus	: 3.2 mm.
Length from anus to tip of tail	: 4.0 mm.
Total length	: 7.6 mm. to 7.8 mm.

The stump of the hind limb becomes somewhat elongated. At the same time its breadth becomes as great as that of the paddle of the hind limb. The middle region (Ankle constriction) is however narrower. On the dorsal (or post-axial) aspect of the foot (paddle) there appears an indentation marking the 5th toe. Melanophores have appeared on the dorsal side of the limb stump. They extend up to its middle.

Stage 32. 3rd toe stage (Plate 6).

Length from anterior tip to anus	: 3.2 mm.
Length from anus to tip of tail	: 4.8 mm.
Total length	: 8.3 mm. to 8.5 mm.

In the hind limb the paddle-like foot (FT.) is clearly marked off from the stump. The latter is slightly longer than the paddle. On the lower or pre-axial side of the 4th toe another indentation appears in the free margin of the paddle and marks the position of the 3rd toe. Some melanophores appear on the 4th toe.

Stage 33. 2nd toe stage (Plate 6).

Length from anterior tip to anus	: 3.4 mm.
Length from anus to tip of tail	: 5.6 mm.
Total length	: 8.9 mm. to 9. 3 mm.

Below the indentation of the 3rd toe (on pre-axial side) on the paddle-like foot there appears another indentation marking the position of the 2nd toe. The distance between the 5th, 4th and 3rd toes increases. The area in between becomes much thinner. The basal portion of the stump is broader than the rest of the limb. There are aggregations of melanophores, one on the dorsal side of the basal part of the stump, foreshadowing the thigh; another aggregation between the thigh and the paddle marking the position of the shank; and the third aggregation on the paddle marking the position of the foot. Melanophores also appear between 4th and 3rd toes.

Stage 34. 1st toe stage (Plate 6).

Length from anterior tip to anus	: 4.8 mm.
Length from anus to tip of tail	: 5.9 mm.
Total length	: 9.4 mm. to 10.3 mm.

On the pre-axial or ventral side of the paddle-like foot appears another indentation marking the position of the first toe, below the indentation for the second toe. The various digits (2-5) are better demarcated and separated from each other than at Stage 33.

The three parts of the leg, thigh or stylopodium (TH.) shank or zeugopodium (SH.) and the foot or autopodium (FT.) are clearly demarcated. The limb is bent downwards at the knee joint. There is an increase in the pigmentation of the thigh, shank and dorsal side of foot. The area between the second and third toes has also been invaded by melanophores.

Stage 35. Full tail piece stage (Plates 6 & 7).

Length from anterior tip to anus	: 6.5 mm.
Length from anus to tip of tail	: 8.5 mm.
Total length	: 1.2 Cm. to 1.5 Cm.

The hind limbs become further elongated. The undersides of the feet are applied to the ventral fin on either side. The knees are bent outwards. The tail piece (T.PE.) extends up to the ankle. All the toes are separated from one another, the fourth toe being the longest. On the dorsal side of this digit there are three aggregations of melanophores showing the position of phalanges.

Stage 36. Full anal tube stage (Plates 6 & 7).

Length from anterior tip to anus	: 7.0 mm.
Length from anus to tip of tail	: 7.5 mm.
Total length	: 1.4 cm. to 1.6 cm.

The anal tube has extended up to the ankle. The tail piece (T.PE.) is fully developed. The palmar region of the foot is closely applied to the ventral fin on lateral sides of it. Eyes are almost black. The fore-limb rudiments can be seen through the transparent body wall on ventral side. The shank makes an angle of 30° with the longitudinal axis of the body. Melanophores can be seen on the dorsal side of all the 5 toes, and the outer side of the limb. Melanophores can be seen aggregated in a circle round the outer border of the intestinal loop. Small groups of melanophores are present on the dorsal fin also. The length of the tail (frcm anus to tip of tail) and that of the trunk (from anterior end to the anus) are almost equal.

Stage 37. Mid anal tube stage (Plates 6 & 7).

Length from anterior tip to anus	: 7.0 mm.
Length from anus to tip of tail	: 9.0 mm.
Total length	: 1.8 cm. to 1.9 cm.

The anal tube extends up to the level of the knee. Shank makes an angle of 60° with the longitudinal axis of the body. The tail piece (T.PE.) is in the process of reduction.

The toes are turned slightly outwards, and only the metatarsal region remains applied to the side of the ventral fin. The fingers of anterior limb with melanophore are discernible through the anterior limb atrium. On the ventral side of the foot two tubercles (TUB.) appear. Groups of melanophores are seen on the fins. The melanophores on the dorsal side of nasal openings are spreading downwards.

Stage 38. Disappearance of anal tube stage (Plates 6 & 7).

Length from anterior tip to anus	: 6.0 mm.
Length from anus to tip of tail	: 9.0 mm.
Total length	: 1.4 cm. to 1.5 cm.

The anal tube has disappeared. The tail piece (T.PE.) is very narrow. The cloacal opening lies on the ventral side behind the origin of the two hind limbs. The fore limbs (F.L.) with their fingers can clearly be seen through the limb atrium. The eye shows a whitish area in the middle. This area indicates the position of the lens. The hind legs make an angle of 75° with the longitudinal axis of the body. The toes are directed outwards from the axis of the body: only the metatarsal region is in contact with the ventral fin. The intestinal spiral cannot be seen as clearly as in the previous stage, because it has been covered over by the abdominal muscles. The skin on the antero-ventral part of the trunk becomes slightly opaque.

Stage 39. Left fore-limb stage (Plates 6 & 7).

Length from anterior tip to anus	: 6.0 mm.
Length from anus to tip of tail	: 7.0 mm.
Total length	: 1.35 cm. to 1.45 cm.

The left fore-limb (LFT.H.) has come out through the spiracle (SP.). It is small and bent downward so that when the tadpole is on its ventral side its palmar side is towards the ground. The fingers and arms are covered over by melanophores. The tail piece (T.PE.) has become greatly reduced. The abdominal muscles cover the intestine completely. The skin of the body is translucent or opaque. On some individuals guanophores make their appearance.

Matatarsal portion of the hind limb is still applied to the ventral fin. The thighs (femurs) do not come out at right angle to the longitudinal axis of the body but make an angle of 75° with it as in Stage 38.

Stage 40. Complete fore-limb stage (Plates 6 & 7).

Length from anterior tip to anus	: 5. 1 mm.
Length from anus to tip of tail	: 7.0 mm.
Total length	: 1.2 cm. to 1.35 cm.

The right fore-limb has broken through the limb atrium. Thighs make a right angle with the longitudinal axis of the body. There is complete loss of denticles and the horny beak. The mouth (MTH.) is slit like. It extends backwards up to a point lying midway between the nasal opening and the eye on either side. The eyes protrude to some extent. The tail piece has disappeared. On the dorsal side an area possessing the characteristic adult skin can be recognized.

Stage 41. Beginning of regression of tail (Plates 6 & 7).

Length from anterior tip to anus	: 5.0 mm.
Length from anus to tip of tail	: 2.5 mm.
Total length	: 7.0 mm to 8.00 mm.

The tail has considerably been reduced. The dorsal and ventral fins are rudimentary. The corners of the mouth approach the anterior end of the eyes. The head region of the body is more massive than the belly part. Nasal openings come to lie at the tip of the snout. The body is rendered c mpletely opaque.

Eyes protrude to a greater extent than at Stage 40. The adult skin area has spread considerably on the dorsal side of head and the trunk.

Stage 42. Stumpy tail stage (Plate 6).

Length from anterior tip to anus	: 4.5 mm.
Length from anus to tip of tail	: 0.7 mm.
Total length	: 5.2 mm. to 6.0 mm.

The gape of mouth widens up, so that the corners of the mouth lie at the level of middle of eye. The tail is like a stump. The dorsal and ventral fins cannot be made out. The tip of the tail is crumpled and black. The adult skin is recognizable on the whole of the body.

Stage 43. Tail-less stage (Plate 6).

Length from anterior tip to anus : 6.5 mm.

Length of tail: tail is completely absorbed.

Total length : 6.5 mm.

The tail has completely been absorbed. The gape of the mouth widens up so that it extends backwards up to the posterior end of the eye, or even beyond it.

The fore-limbs and hind-limbs become further elongated. The eyes protrude in the characteristic fashion. The skin shows warts.

COMPARISON WITH NORMAL TABLES OF OTHER AMPHIBIA

An attempt has been made in this part to compare the Normal Table of *Bufo melanostictus*, as described above, with the Normal Tables of some other Amphibians. A similar attempt has been made by Nieuwkoop & Faber (1956) who have compared the Normal Table of *Xenopus laevis* with that of other Amphibians; and by Rossi (1958), who has compared the Normal Table of *Bufo bufo* with that of *Rana pipiens*. In the present comparison help has been taken freely from the comparative chart prepared by Nieuwkoop & Faber.

In the above-described Normal Table of *Bufo melanostictus* the unfertilized egg has been taken as Stage 1. Similarly Del Conte & Sirlin (1952) in *Bufo arenarum*; Weisz (1945) in *Xenopus laevis*; Shumway (1940) in *Rana pipiens*; Pollister & Moore (1937) in *Rana sylvatica*; and Milller (1939) in *Rana pipiens* recognize the unfertilized egg as the first Stage. However Rossi (1959) in *Bufo bufo*; Adler (1901) in *Bufo vulgaris*; Eakin (1946) in *Hyla regilla*; Nieuwkoop & Faber (1956) in *Xenopus laevis*; Gallien & Houillon (1951) in *Discoglossus pictus*; Kopsch (1952) in *Rana fusca*; Cambar and Marrot (1954) in *Rana dalmatina* and Moser (1950) in *Rana temporaria*; do not recognize the unfertilized egg as a Stage. There is something to be said for this view also, because, after all, the unfertilized egg cannot possibly be regarded as a developmental stage. In the present study the first view has been adopted because the female extrudes unfertilized eggs which differ markedly from fertilized eggs. In order to facilitate description and identification of stages, the unfertilized egg must be taken into account.

The fertilized egg (Stage 2) has not been recognized as a distinct stage by workers on *Bufo vulgaris, Hyla regilla, Rana fusca, Rana dalmatina* and *Rana temporaria*. As regards the Normal Tables of *Bufo vulgaris, Hyla regilla*, and *Rana temporaria*, work has been done only on advanced stages of development.

In some cases, *e.g.* in the Normal Table of *Xenopus Laevis* (Nieuwkoop & Faber, 1956) some "half-stages" have also been recognised. Thus Stage 7 of the present study is represented in their Table by Stages 6½ and 7. This has been done by relying on very fine characters some of which are not readily visible. Similarly for Stage 11 (Crescent-shaped blastopore) of the present study, Nieuwkoop & Faber recognize two Stages and name them as Crescent-shaped blastopore (Stage 10½) and horse-shoe shaped blastopore (Stage 11). For mid-neurula Stage (15) of *Bufo melanostictus* these authors recognize 3 Stages, *viz.*, No. 15-18 in *Xenopus laevis* depending upon minute changes in the neural folds. Such distinctions are not feasible in *Bufo melanostictus.* A Normal Table does lose some of its usefulness if it involves the recognition of obscure characters.

It is not easy to compare the Normal Tables of different species because any particular organ may appear at one stage in one species and at quite a different stage in another species. For example opercular folds appear at Stage 23 in *Bufo melonostictus* but at Stage 42 in *Xenopus laevis* (Nieuwkoop & Faber, 1956). Similarly hind-limb buds appear when opercular fold is covering the gills at Stage 46 in *Xenopus laevis*, but in *Bufo melanostictus* they appear at Stage 25 when the gill filaments are to be seen coming out of the spiracle and the operculum is complete. The hind limb buds in *Bufo bufo* appear at Stage 23 (Rossi 1959). This stage of *Bufo bufo* is comparable in almost all characters to Stage 23 of *Bufo melanostictus* except that the latter has not yet acquired the hind limb buds. Taylor & Kollros (1946) record the appearance of the hind limb buds in *Rana pipiens* at a stage when the operculum is complete and there is no trace of gill filaments coming out of the spiracle. This Stage corresponds to Stage 26 of *Bufo melanostictus.*

The development of hind limb takes place gradually in *Bufo melanostictus.* The appearance of toes is also systematic. Firstly the foot stump becomes paddle like (Stage 30). The tip of this paddle forms the 4th toe. Then on its post-axial side appears the 5th toe (Stage 31). Then on pre-axial side appear the 3rd, 2nd and 1st toes respectively, characterizing the Stages 32, 33 & 34. The same sequence has been noted by Taylor & Kollros (1946) in *Rana pipiens*, and by Rossi (1959) in *Bufo bufo*, marking the Stages VI, VII, VIII, IX, X and Stages III, IV, V, VI, VII respectively. However in *Xenopus laevis* Nieuwkoop & Faber (1956) recognize Stage 53 marking the appearance of the 4th and 5th toe; and Stage 54 when all the 5 toes are represented. It means that a criterion which helped in recognizing the different stages of *Bufo melanostictus* was useless in *Xenopus laevis*. Most probably in the latter the toes do not appear one after another otherwise Nieuwkoop & Faber (1956) would surely have made use of this criterion.

In *Rana pipiens* Taylor & Kollros (1946) based 7 stages (XI-XVII) on such apparently insignificant characters as the appearance of melanophores on the toes or the length of the toes and the distance between them. Rossi (1959) however, does not recognize such stages in *Bufo bufo*, and has compressed the above-mentioned 7 stages of *Rana pipiens* into only 2 (VII & VIII) in *Bufo bufo*. In *Bufo melanostictus* where toes are short, the 7 stages recognized in *Rana pipiens* correspond to one stage (Stage 35).

The next 3 stages (36, 37 and 38) in *Bufo melanostictus* are based upon the length of the anal tube. Similary stages (IX, X & XI) have been recognized by Rossi (1959) in *Bufo bufo*. Taylor & Kollros (1946) recognized two stages XVII-XVIII, on the basis of the tail piece (anal tube) in *Rana pipiens*. In *Xenopus laevis* Nieuwkoop & Faber (1956) do not base any stage upon anal tube, but base the corresponding stages upon the fore limb. In *Bufo melanostictus* tadpoles which are heavily pigmented and in which the development of the fore-limb takes place in the limb atrium, it is not possible to use the fore-limb for recognizing stages.

In *Bufo melanostictus*, and in *Bufo bufo* (Rossi 1959) the left arm comes out of the spiracle marking Stage 39 in the former and Stage XII in the latter. Taylor & Kollros (1946) speak of the appearance of one or both of the fore-legs when they record this event in Stage XX in *Rana pipiens*. In *Bufo melanostictus* and *Bufo bufo* both the fore-limbs are out at Stage 40 and XIII respectively.

The next 3 stages (Stages 41, 42 & 43) in *Bufo melanostictus* are recognized on the basis of different stages of the regression of the tail. In *Bufo bufo*, Rossi, (1959) records only two stages (XIV & XV) on this basis. In *Bufo melanostictus* the processes of increase in the gape of the mouth and regression of tail go on side by side during Stages 40-43. Nieuwkoop & Faber (1956) records the gaping of mouth accompanied by reduction in the length of tail in Stages 62-64. However, at Stage 65 the mouth has been formed but tail is still there. So the next two stages (6o & 66) are based on the tail. In *Rana pipiens* the regression of the tail (Stages XXIII-XXV) takes place after the mouth has been formed fully (Stages XXI & XXII). Hence the toadling comes into being in *Bufo melanostictus* at Stage 43, in *Bufo bufo* at Stage XV, and in *Xenopus laevis* at Stage 66. In Rana pipiens the frogling is formed at Stage XXV.

From the above account it is clear that the development of *Bufo melanostictus* resembles very closely with that of other anuran Amphibia. The differences in the number of stages and method of staging in different species is due primarily to the fact that the various organs or parts of organs do not arise in precisely the same sequence. Such differences, however, are very minor, and it can safely be asserted that the epigenetics of *Bufo melanostictus* is essentially the same as in other ranids and bufonids.

SUMMARY

1. Normal development of the common toad *Bufo melanostictus* Schneider has been studied, from the egg to the stage when definitive body form has been acquired.

2. The ontogeny has been divided up into stages. For this purpose only the external morphological criteria have been used.

3. The stages in the Normal Table of *Bufo melanostictus* have been compared with those of a large number of toads and frogs.

4. The Normal Table of *Bufo melanostictus* is seen to resemble the Normal Tables of other Anura in all essential respects.

Name of the Stages		*Bufo melanostictus* (Present study)	*Bufo bufo* (Rossi, 1958)	*Bufo arenarum* (Del conte & Sirlin, 1952)	*Bufo vulgaris* (Adler, 1901)	*Hyla regilla* (Eakin, 1946)
Unfertilized egg	..	1	..	1	..	..
Fertilized egg	..	2	1	2	..	..
2-cell stage	..	3	2	3	..	..
4-cell stage	..	4	3	4	..	..
8-cell stage	..	5	4	5	..	..
16-cell stage	..	6	5	6	..	..
Early cleavage stage	..	7	6	7	..	..
Mid cleavage stage	..	8	7	8	..	..
Beginning of gastrulation stage	..	9	8	9	..	..
Dorsal lip of blastopore stage	..	10	9	10	..	..
Crescent-shaped blastopore	..	11	10	11	..	..
Yolk plug stage	..	12	11	12	..	..
Neural plate stage	..	13	12	13	..	..
Early neural fold stage	..	14	13	14	..	..
Mid neurula stage	..	15	14	15	1	15
Late neurula stage	..	16	15	16	2	16
Tail bud stage	..	17	16	17	3 }	17
Fin formation stage	..	18	17, 18	18	4 }	
Gill bud stage	..	19	19	19	5	18
Early gill stage	..	20	20	20	6	19
Gill filament stage	..	21	21	21	7	20
3-gill stage	..	22	22	22	8,9	21, 23

Xenopus laevis (Nieuwkoop & Faber, 1956)	*Xenopus laevis* (Weisz, 1945)	*Discoglossus pictus* (Gallion & Houillon, 1951)	*Rana pipiens* (Taylor & Kollros 1946; Shumway, 1940)	*Rana fusca* (Kopsch, 1952)	*Rana dalmatina* (Cambar & Marrot, 1954).	*Rana temporaria* (Moser, 1950)	*Rana sylvatica* (Pollister & Moore, 1937)	*Rana pipiens* (Miller, 1939)
..	1	..	1	..	..	..	1	1
1	2	1	2	..	..	..	2	2
2	3	2	3	1	1	..	3	3
3	4	3	4	..	2	..	4	4
4	5	4	5	2	3	..	5	5
5	6	5	6	..	4	..	6	6
6½, 7	7	6	7	3	5	..	7	7
8	8	7	8	..	6	..	8	8
9	9	..	9	..	..	..	9	9
10, 10¼	10	8	10	4	..	..	10	10
101, 11	11	9	11	5	7—10	..	11	11
12, 12½	12	10—12	12	6	11—14	..	12	12
13	13		13	..	15	..	13	13
14	14	13	14	7	16	..	14	14
15—18	..	14, 15	15	8	17	..	15	15
19, 20	15	..	16	9	18	..	16	16
21—24	16	16	17	10	19	..	17	17
25—33	17	17—19	18	11	20—22	1	18	18
34	18	20	19	12	..	..	19	19
35, 36	19	21	20	13	23	..	20	20
37—40	20	22—25	21	14	24—26	2—4	21	..
41	21	26—28	22	15	27—29	5	22	..

Name of the Stage		*Eufo melanostictus* (Present study)	*Bufo bufo* (Rossi, 1958)	*Bufo areuarum* (Del conre & Sirlin, 1952)	*Bufo vulgaris* (Alder, 1901)	*Hyla regilla* (Eakin, 1946)
Opercular fold stage	..	23	23	23	10	24
Late opercular stage	..	24	24	24	11	..
Spiracle stage (hind-limb bud stage)		25	25	25	12	..
Length of hind-limb bud less than its breadth	..	26	23	..	..	..
Length of hind-limb bud greater than breadth	..	27	24	..	..	..
Length of hind-limb $1\frac{1}{2}$ times its breadth	..	28	25	..	13	..
Length of hind-limb $1\frac{3}{4}$ times its breadth	..	29	1	..	..	..
Length of hind-limb $2\frac{1}{2}$ times its breadth	..	30	II	..	..	..
5th toe stage	..	31	III	..	..	..
3rd toe stage	..	32	IV	..	..	..
2nd toe stage	..	33	V	..	..	..
1st toe stage	..	34	VI	..	..	..
Full tail piece stage	..	35	VII, VIII	..	..	..
Full anal tube stage	..	36	IX	..	..	..
Mid anal tube stage	..	37	X	..	14	..
Disappearance of anal tube stage	..	38	XI	..	..	..
Left fore-limb stage	..	39	XII	..	..	..
Complete fore-limb stage	..	40	XIII	..	..	..
Beginning of regression of tail	..	41	XIV	..	..	..
Stumpy tail	..	42	..	..	15	..
Disappearance of tail	..	43	XV	..	..	..

Xenopus laevis (Neuwkoop & Faber, 1956)	*Xenopus laevis* (Weisz. 1945)	*Discoglossus pictus* (Gallion & Houillon, 1951)	*Rana pipiens* (Taylor & Kollros 1946. Shumway, 1940)	*Rana fusca* (Kopsch, 1952)	*Rana dalmatina* Cambar & Marot, 1954)	*Rana temporaria* (Moser, 1950)	*Rana sylvatic* (Pollister & Moore, 1937)	*Rana pipiens* (Mille, 1939
42	..	29	23	16	30, 31	6	23	21
43—45	..	30	24	17	32, 33		..	22
46—48	22, 23	31, 32	25(5); I (T & K)	18	34—40	7—10	..	23
49	..	..	II	19	41	11	..	..
50	..	..	III		..	12	..	..
51, 52	..	..	IV	20	42	13	..	..
53	..	..	V	21	..	..	..	..
..	..	..	VI	22	43	..	..	..
..	..	..	VII	..	44	..	..	..
..	..	..	VIII	23	45	..	..	..
..	..	..	IX	..	..	..	..	..
45, 55	..	..	X	..	46	..	..	..
56	..	..	XI—XVII	24, 25	47—50	..	..	..
57	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..
..	..	..	XVIII	..	..	..	..	..
58	..	..	XIX	..	..	..	..	..
62	..	..	XX	27	..	..	..	..
63	..	..	XXI	..	51	..	..	..
64	..	..	XXII	28	52	..	..	..
65, 66	..	..	XXIII—XXV	29, 30	53, 54	..	..	..

Xenopus laevis (Neuwkocp & Faber, 1956)	*Xenopus laevis* (Weisz. 1945)	*Discoglossus pictus* (Gallion & Houillon, 1951)	*Rana pipieus* (Taylor & Kollros 1946. Shumway, 1940)	*Rana fusca* (Kopsch, 1952)	*Rana dalmatina* Cambar & Marrot, 1954)	*Rana temporaria* (Moser, 1950)	*Rana sylvatic* (Pollister & Moore, 1937)	*Rana pipiens* (Mille, 1939
42	..	29	23	16	30, 31	6	23	21
43—45	..	30	24	17	32, 33		..	22
46—48	22, 23	31, 32	25(5); I (T & K)	18	34—40	7—10	..	23
49	..	..	II	19	41	11	..	..
50	..	..	III		..	12	..	..
51, 52	..	..	IV	20	42	13	..	..
53	..	..	V	21	..	..	..	..
..	..	..	VI	22	43	..	..	..
..	..	..	VII	..	44	..	..	..
..	..	..	VIII	23	45	..	..	..
..	..	..	IX	..	..	..	..	..
45, 55	..	..	X	..	46	..	..	..
56	..	..	XI—XVII	24, 25	47—50	..	..	..
57	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..
..	..	..	XVIII	..	..	..	..	..
58	..	..	XIX	..	..	..	..	..
62	..	..	XX	27	..	..	..	..
63	..	..	XXI	..	51	..	..	..
64	..	..	XXII	28	52	..	..	..
65, 66	..	..	XXIII—XXV	29, 30	53, 54	..	..	..

LIST OF ABBREVIATIONS

AN.	Anus.
ANK.	Ankle.
AN.PT.	Anal pit.
AN.T.	Anal tube.
A.P.	Animal pole.
A.V.	Anterior view.
B.	Blastopore.
BE.	Beak.
BL.	Blastomere.
CA.FU.	Caudal furrow.
CE.GL.	Cement gland.
C.G.	Cleavage groove
CL.B.	Closed blastopore
C.L.N.T.	Closed neural tube
CRES.	Grey crescent
C.T.P.	Cloacal tail piece
DEN.	Dentical
D.F.	Dorsal fin
D.L.	Dorsal lip
D.L.B.	Dorsal lip of blastopore
D.V./DOR.	Dorsal View
E.	Eye
E.C.	Eye cup
FI.	Finger
F.L.	Fore-limb
FT.	Foot
G1—G4.	First gill—Fourth gill
G.	Gill
G.AN.	Gill analage

G.BD.	Gill bud
G.F.	Gill filament
H.L.BD.	Hind limb bud
INT.CO.	Intestional coil
K.	Knee
LAT.LI.	Latral line
LFT.H.	Left fore-limb
L.LP.	Lower lip
L.L.B.	Latral lip of blastopore
L.V.	Latral view
ME.	Megameres
MEL.	Melanophores
MI.	Micromeres
MTH.	Mouth
MYO.	Myotomes
NA.OP.	Nasal opening
N.F.	Neural folds
N.G.	Neural groove
N.PL.	Neural plate
N.T.	Neural tube
OLF.PT.	Olfactory pit
OP.F.	Opercular fold
OR.SUC.	Oral Sucker
POST.DEN.	Post-oral denticles
PRE. DEN.	Pre-oral denticles
PRO.	Pronephric region
S.	Sucker
SH.	Shank
SP.	Spiracle
ST.CL.	Stomodaeal cleft
T.	Tail
T.BD.	Tail-bud
TH.	Thigh

T.PE.	Tail piece
TUB.	Tubercle
U.LP.	Upper lip
VEG.P.	Vegetable pole
V.F.	Ventral Fin
VIT.	Vitelline membrane
V.L.	Ventral tip
V.V./VEN.	Ventral view
YK.PG.	Yolk plug.

REFERENCES

Adler, W. Die Entwicklung der aussereu Korperform und des Mesoderms be *Bufo vulgaris*. *Inter-natl. Monatscher Anat. Physiol.*, 18 (1901).

Boulenger, G. A. *Fauna of British India, Reptiles and Batrachia*, London: Taylor and Francs. (1890).

Cambar, R. & Marrot, B. Table chronologique du development de la grenoville agile (*Rana dalmatina* Bon.) *Bull. Biol. Franco-Belg.*, **88,** 168-177 (1954).

Del Conte, E. & Sirlin, J. L. Pattern series of the first embryonary stages in *Bufo arenarum*. *Anat. Rec.*, 125-135 (1952).

Eakin, R. M. Determination and regulation of polarity in the retina of *Hyla regilla*. *Univ. California Publ. Zool.*, **51,** 245 (1946).

Eakin, R. M. & Harris, H. Incompatability between amphibian hosts and xenoplastic grafts as related to host age. *J. Exp. Zool.*, **98,** 35-64 (1945).

Gallien, L. & Houillon, G. Table chronologique du developement chez *Discoglossus pictus*. *Bull. Biol. Franco-Belg.* **85,** 373-375 (1951).

Gilani, S. H. (unpublished). The early development of *Rana tigrana* Daudin. M.Sc. Research. Punjab University, Department of Zoology, 1957.

Harrison, R. G. Experiments on the development of fore-limb of *Amblystoma*. *Journ. Exper. Zool.* **25,** 413 (1918).

Hashmi, T. H. (unpublished) The Amphibia of Lahore with description of the early development of *Bufo melanontictus* Schneider. M.Sc. Research. Punjab University Department of Zoology, (1955).

Kopsch, F. Die Entwicklung des laraunen Grasfrosches *Rana fusca* Roesel (dargestellt in der Art der Normentafeln zur Entwicklungsgeschichte der Wirbelthiere) Stuttgart, (1952).

Miller, D. C. A table for the normal development of *Rana pipiens*. *Proc. Indiana Acad. Sci.* **49,** 209-214 (1939).

Moser, H. Ein Beitrag zur Analyse der Thyroxineinwirkung in Kaulquappenversuch und zur Frage nach dem Zustandekommen der Fruhbereitschaft des Metamorphose-Reaktionssystems. Thesis, Basel; *Rev. Suisse Zool*, 57 (Suppl., 2), 1-144 (1950).

Murray, J. A. *'The vertbrate Zoology of Sind'* London: Richardson (1884).

Nieuwkoop, P. D. & Faber, J. *Normal Table of Xenopus Laevis* (Daudin). North-Holland Publishing Company, Amsterdam (1956).

Pollister, A. W. & Moore, J. A. Tables for the normal development of *Rana sylvatica*. *Anat. Rec.*, **68,** 489 (1937).

Rossi, A. Tavole cronologiche dello sviluppo embrionale elarvale di "*Bufo bufo*" (L); *Monitare Zool. Ital.* **66,** 133-145 (1958).

Schreiber, G. La definizione degli stadi della Metamorphosi del *Bufo*. *Rend. R. Acad. Naz. Linci*, **25,** 342-348 (1937).

Shumway, W. Stages in the normal development of *Rana pipiens*. 1, External forms. *Anat. Rec.* **78,** 139-147 (1940).

Taylor, A. C. & Kollros, J. J. Stages in the normal development of *Rana pipiens* larvae. *Anat. Rec.*, **94,** 7-23 (1946).

Tschernoff, N. D. Zur Embryonalentwicklung der hinteren Extremitatten des Frosches. *Anat. Anaz.*, **30,** 593-612 (1907).

Weisz, P. B. The normal stages in the development of the South African clawed toad, *Xenopus laevis*. *Anat. Rec.* **93,** 161-169 (1945).

Winterbert, M. P. Zur l'ordre d'apparition des orteils et el premier developpement des members chez les Anaures. *Compt. Rend., Soc. d. Biol.* **59,** 576-578 (1905).

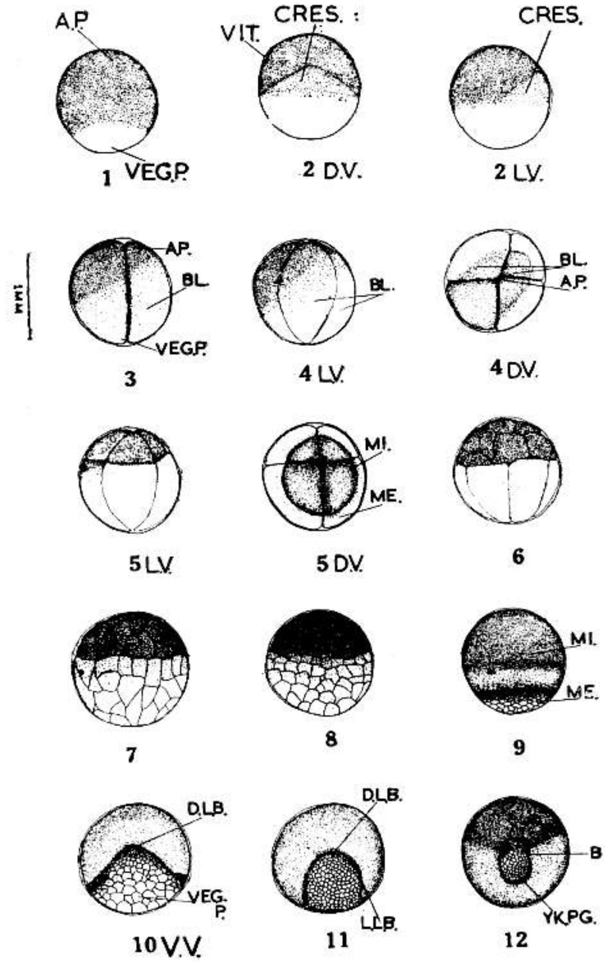

A.P.
VIT.
CRES.
CRES.
1 VEG.P.
2 D.V.
2 L.V.
A.P.
BL.
VEG.P.
BL.
BL.
A.P.
3
4 L.V.
4 D.V.
MI.
ME.
5 L.V.
5 D.V.
6
MI.
ME.
7
8
9
D.L.B.
VEG. P.
D.L.B.
L.L.B.
B
YK.PG.
10 V.V.
11
12

PLATE I

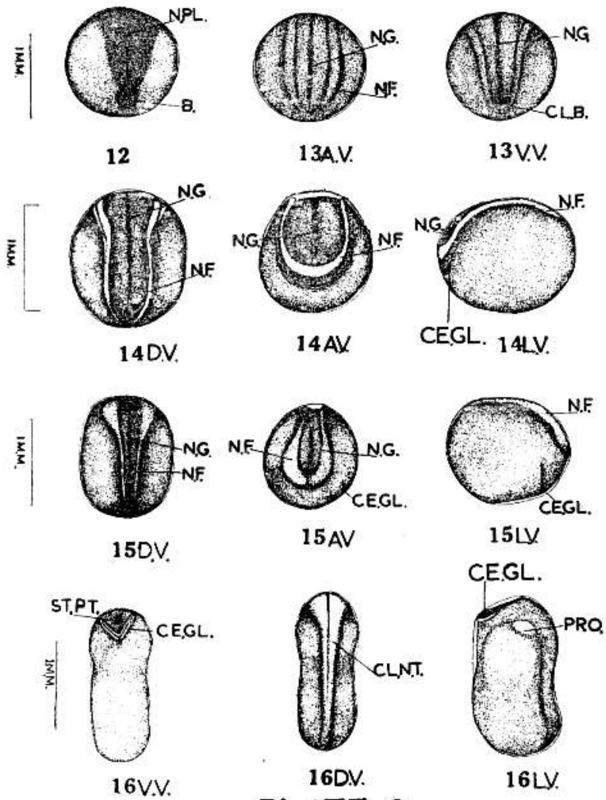

1M.M.
N.PL.
B.
12
N.G.
N.F.
13A.V.
N.G.
C.L.B.
13V.V.
N.G.
N.F.
14D.V.
N.G.
N.F.
14A.V.
N.G.
N.F.
CE.GL.
14L.V.
N.G.
N.F.
15D.V.
N.F.
N.G.
CE.GL.
15A.V.
N.F.
CE.GL.
15L.V.
ST.PT.
CE.GL.
16V.V.
CL.N.T.
16D.V.
CE.GL.
PRO.
16L.V.

PLATE 2

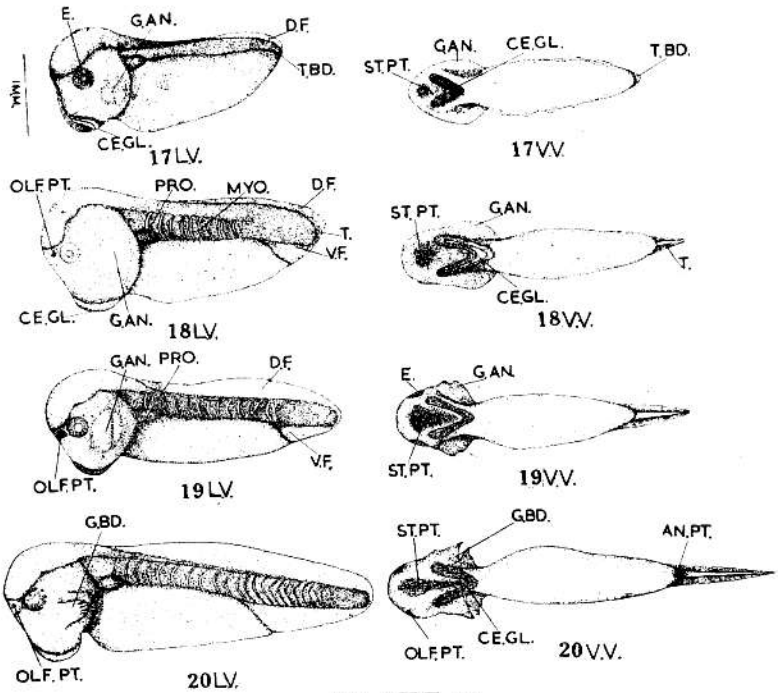

E.
G.AN.
D.F.
T.BD.
1MM.
CE.GL.
17 L.V.
G.AN.
CE.GL.
T.BD.
ST.PT.
17 V.V.
OLF.PT.
PRO.
MYO.
D.F.
T.
V.F.
CE.GL.
G.AN.
18 L.V.
ST.PT.
G.AN.
T.
CE.GL.
18 V.V.
G.AN. PRO.
D.F.
V.F.
OLF.PT.
19 L.V.
E.
G.AN.
ST.PT.
19 V.V.
G.BD.
OLF. PT.
20 L.V.
ST.PT.
G.BD.
AN.PT.
OLF.PT.
CE.GL.
20 V.V.

PLATE 3

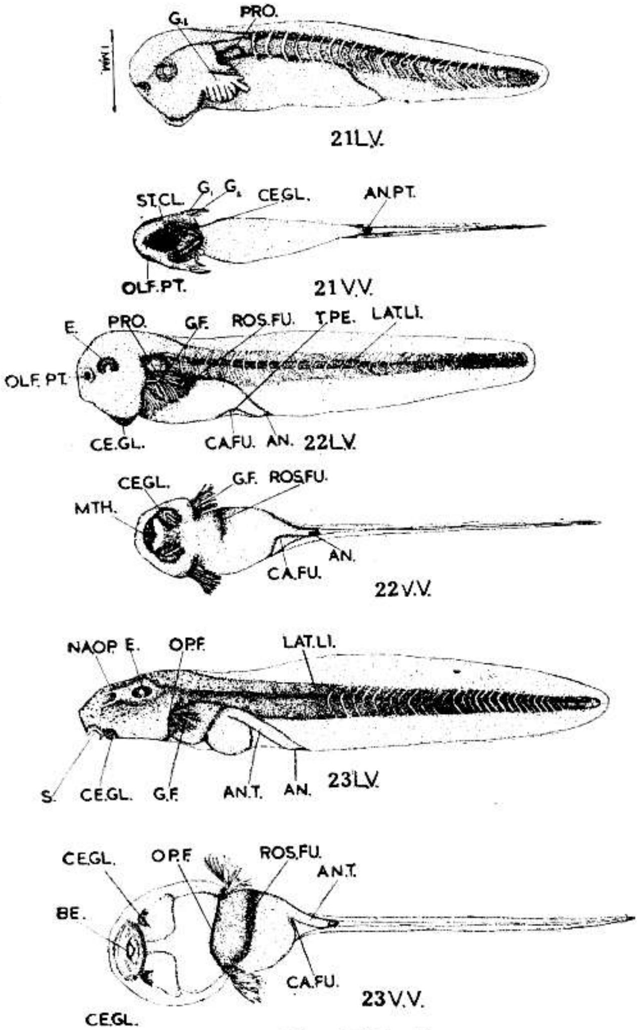

G1
PRO.
1 mm
21L.V.
ST.CL.
G1 G2
CE.GL.
AN.PT.
OLF.PT.
21V.V.
E.
PRO.
G.F.
ROS.FU.
T.PE.
LAT.LI.
OLF. PT.
CE.GL.
CA.FU.
AN.
22L.V.
CE.GL.
G.F.
ROS.FU.
MTH.
AN.
CA.FU.
22V.V.
NAOP.
E.
OP.F.
LAT.LI.
S.
CE.GL.
G.F.
AN.T.
AN.
23L.V.
CE.GL.
OP.F.
ROS.FU.
AN.T.
BE.
CA.FU.
23V.V.
CE.GL.

PLATE 4

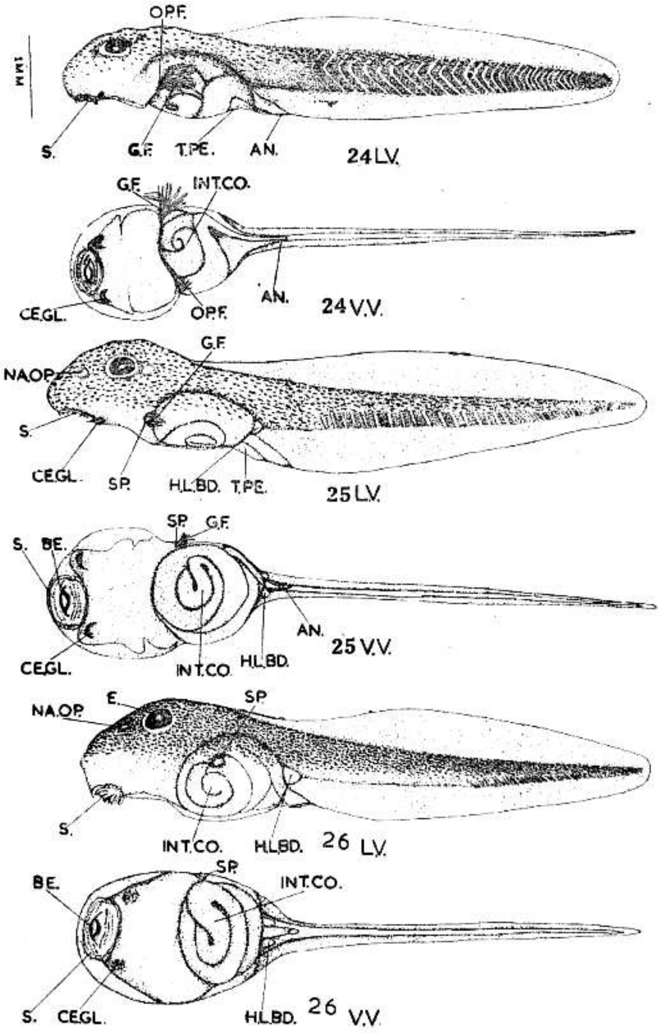

OPF.
1MM
S.
G.F.
T.PE.
AN.
24 LV.
G.F.
INT.CO.
CE.GL.
OPF.
AN.
24 V.V.
G.F.
NAOP.
S.
CE.GL.
SP.
HL.BD.
T.PE.
25 LV.
SP.
G.F.
S.
BE.
CE.GL.
INT.CO.
HL.BD.
AN.
25 V.V.
E.
SP.
NA.OP.
S.
INT.CO.
HL.BD.
26 LV.
SP.
BE.
INT.CO.
S.
CE.GL.
HL.BD.
26 V.V.

PLATE 5

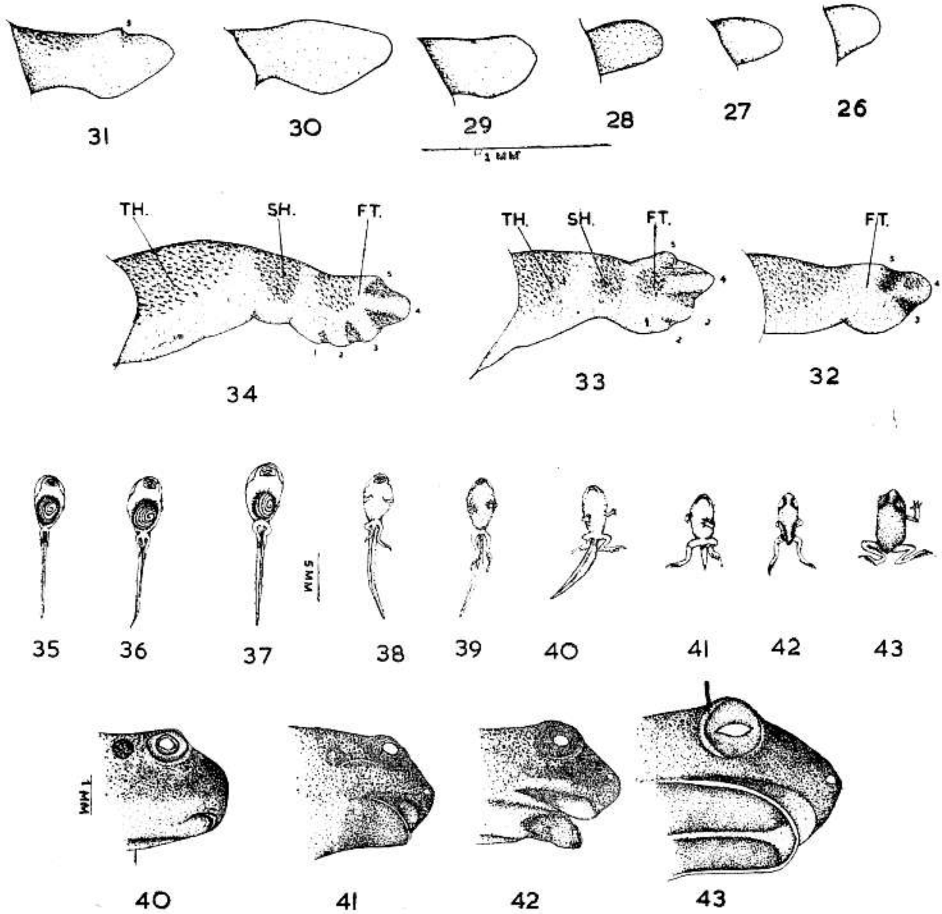

31
30
29
28
27
26
1 MM
TH.
SH.
FT.
34
33
32
5MM
35
36
37
38
39
40
41
42
43
1 MM
40
41
42
43

PLATE 6

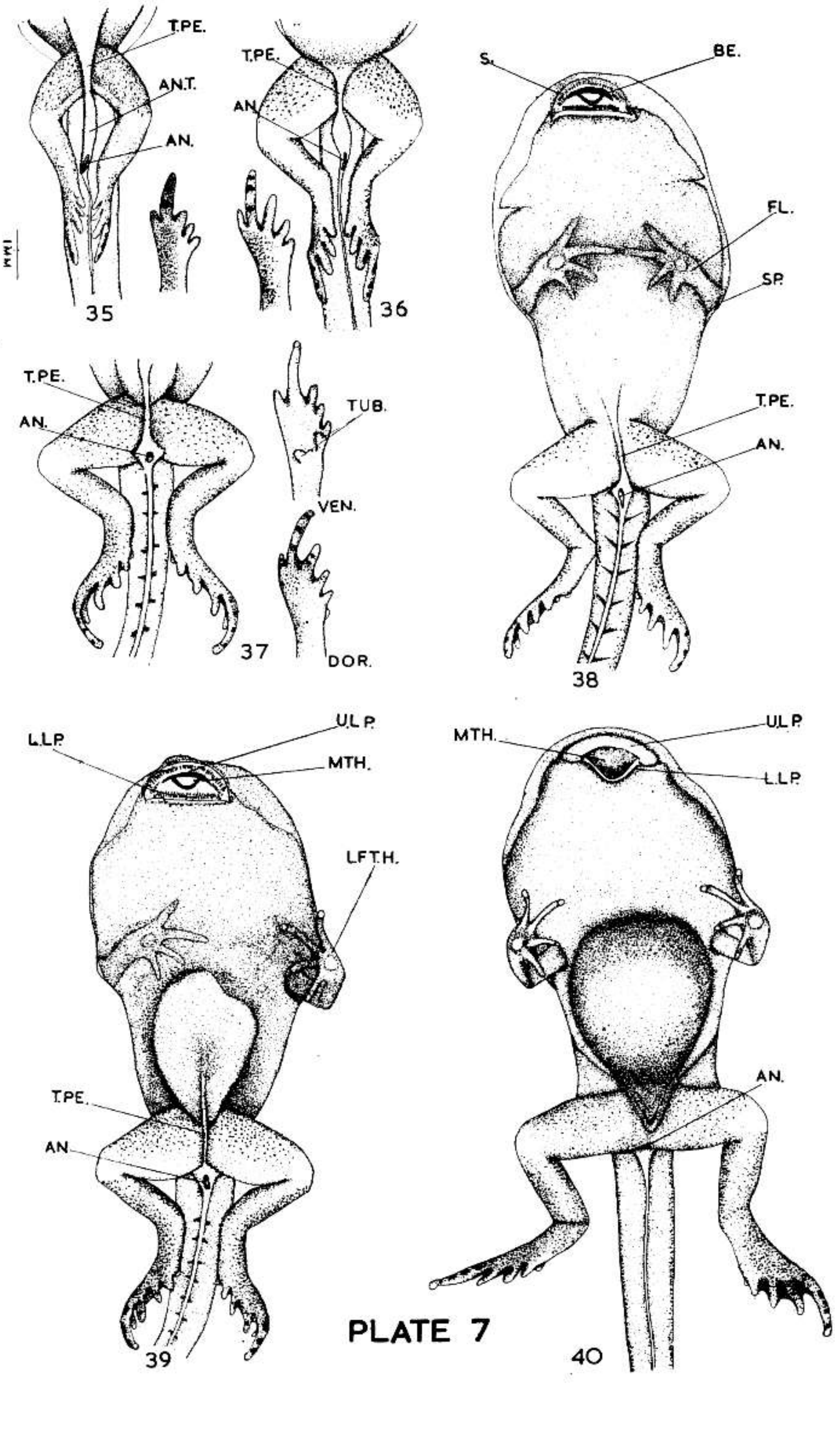
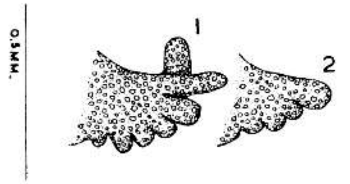
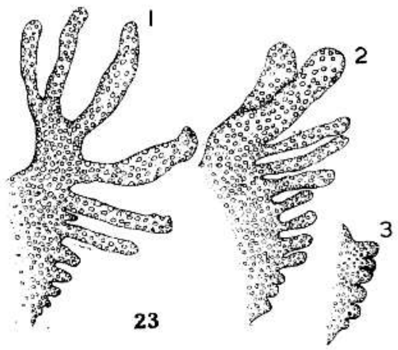

T.PE.
AN.T.
AN.
1 MM
35
T.PE.
AN.
36
S.
BE.
FL.
SP.
T.PE.
AN.
38
T.PE.
AN.
TUB.
VEN.
DOR.
37
U.L.P.
L.L.P.
MTH.
LFT.H.
T.PE.
AN.
39
MTH.
U.L.P.
L.L.P.
AN.
40

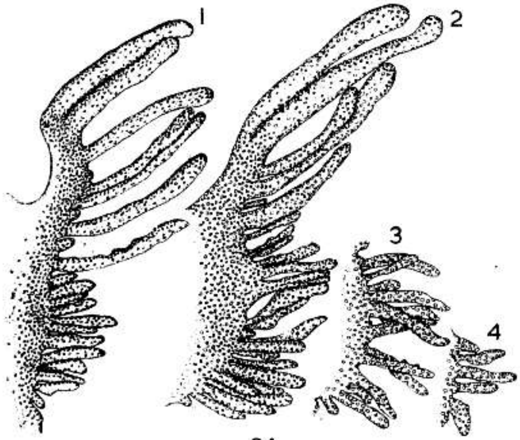
PLATE 7



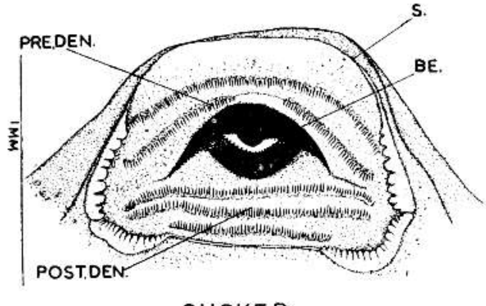
21



23



24



SUCKER

PLATE 8