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On the Development of the Green Frog, *Rhacophorus schlegelii* var. *arborea*

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With Plates III—V and two Text-figures

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I. EXTERNAL CHANGES

Introduction

Although the development of amphibia has been studied intensively since the end of the last century by many investigators, *Rhacophorus schlegelii* and its varieties have never been utilized as the material of such studies except in the works by IKEDA (1902) and AKAZA (1913). IKEDA worked mainly on the mode of the closure of the blastopore and the formation of the embryonic body in *Rhacophorus schlegelii s. s.*, while AKAZA observed exclusively the cleavage in its variety *arborea*. More recently, OKADA (1928) published his observations on the breeding habits of this frog and its two varieties, *arborea* and *intermedia*, as well as their geographical distribution. With regard to the allied forms, SIEDLECKI (1909) reported his ecological investigations on *Polypedates reinwaldtii* found in Java, which has essentially the same breeding habit. The eggs in all the above-mentioned forms are laid in the air in a frothy mass of secretion from the oviduct. The first discovery of this frothy mass in Japan was made by W. J. HOLLAND (1889) among the shrubs near the famous red bridge at Nikko where he had been collecting insects. Since then it has been found by many other observers in various localities, such as, Osoreyama and Hakkodasan in Mutsu Prov., Bandaisan in Iwashiro Prov., Nikkôsan, as well as in Tokyo, Kyoto and in the Provinces of Mino, Echigo, Kii, Yamato, etc.. In the vicinity of Kyoto this mass is rather commonly found. The breeding season of the frog usually begins at the end of May and ends in the middle of July, but this is subject much to the climatic conditions and differs of course according to the locality. As to the peculiar manner of oviposition and habits, AKAZA and OKADA have reported so fully that I shall confine myself to a brief statement. In the daytime the frogs usually sit still on a branch or on a leaf, but in the evening they become active and go about looking for the other sex, when they begin to croak loudly. As they meet with the other sex, the male embraces the female, while some more males, usually three or four, also assemble near the couple, and the whole group join in making the mass on the leaves. The eggs are laid mingled with the secretion from which the mass is made. It is not certain whether all these males participate in fertilizing the eggs, although this is very probable. Spawning occurs usually at the break of day ; but in cloudy weather the oviposition may take place in the

daytime, though this seldom occurs. After the oviposition, the males leave the mass one after another until there remain only the first couple. Shortly afterwards this couple also go. The newly laid mass is viscous and white, but soon acquires a firmer consistency by evaporation. The outer surface develops into a brownish-yellow crust, while the inner mass liquefies gradually with the development of the eggs. The tadpoles wringle about for a short time in this liquefied central mass, they are then brought into the water by means of the flow of the inner liquid, through the ruptured bottom of the crust. The tadpole just liberated, is active from the first; it never remains attached by its sucker to objects in the water, but swims, though comparatively slowly.

On account of the peculiar breeding habit mentioned above, it might be expected that the mode of development of this frog is more or less different from that of other frogs. It was under this supposition that the present investigation was undertaken.

Before going further with this description, I wish to express my hearty thanks to Prof. Taku KOMAI for his kind direction and also to Prof. Osamu MINOUCHI for his kind assistance in the matter of technique. I am also indebted to Mr. Yasuhiro KOBATAKE, Principal of the Bessho Primary School, for his hospitality during my stay in his village to collect materials.

Material and Methods

Materials were collected in the breeding season of 1928 at Bessho, a desolate village lying in a ravine about ten miles north of Kyoto. To observe the cleavage in its normal state, it was found necessary to handle the egg with the greatest care, because it is already known that the eggs of amphibians are apt to show abnormalities with even a slight difference of environment. In *Rhacophorus* also, the eggs laid by frogs in captivity very often show some abnormality or other in their development, as already pointed out by IKEDA who attributes this fact to the forced arrest of oviposition. Now Bessho, where I observed the normal cleavage, is an ideal place for this purpose, because everywhere around the house one can easily get any stage of the development of the egg. The uncleaved eggs were cautiously taken out of the frothy mass and observed in a watch-glass filled with water. The normal development might have been hindered to some extent by this handling. I, therefore, checked my findings with observations on the eggs left in mass. No difference in the cleavage pattern could

be detected between the two groups of eggs.

The development was observed with the help of the ZEISS monocular or binocular microscope and a LEITZ planktoscope. A ZEISS prism rotator was also used for the purpose of getting the lower and lateral views of the egg and tadpole. Above all, with the aid of the LEITZ planktoscope, I was able to watch rise and progress of the furrow over the surface of the living egg, thus avoiding the risk of disadvantages brought about by handling.

Eggs in all stages were preserved with ZENKER's mixture for about 24 hours and washed in running water for 24 hours or so, and then put in 70 % alcohol. Some part of gastrulation and the later development were observed in the laboratory of the Zoological Institute of Kyoto, because at Bessho electric light was not available.

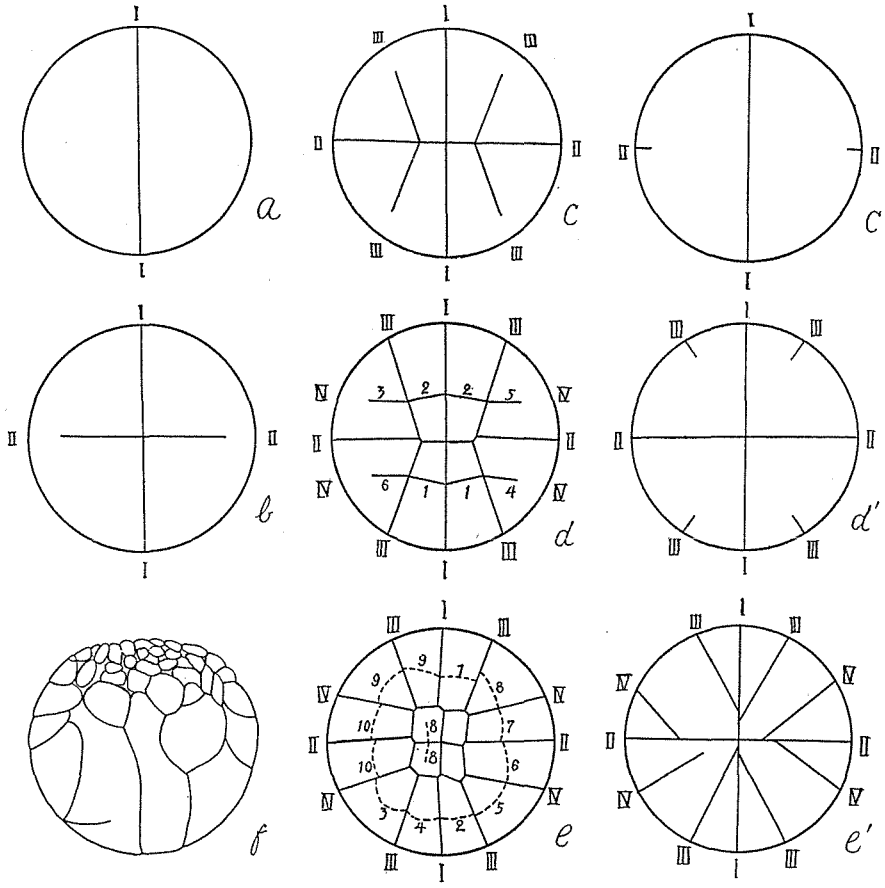
Observation

a) Cleavage Pattern

The egg is spherical and looks pale yellow in colour; more precisely, it is pale yellow at the upper pole, grading into milky white at the lower. The eggs in the same mass are nearly uniform in size, but there are slight differences among the batches of the different masses, the diameter measuring from 2.6 mm. to 3.0 mm.

The following remarks concerning cleavage are based upon a cautious examination of the surface change of both living and preserved materials. When eggs are taken out of the frothy mass and placed in a watch-glass filled with water, the outer of the two membranes enveloping the egg absorbs water rapidly and swells to a great extent, while the inner membrane expands only a little, and the so-called perivitelline space becomes conspicuous. The cleavage is initiated by the upper pole of an egg becoming flat, and the inner 'vitelline' membrane becomes visible for the first time.

The first meridional furrow is first noticeable on the flat area and gives the egg the appearance as if it were cut with a blunt knife into two approximately equal halves; then the cut becomes a narrow deep fissure or cleavage groove *(Text-figure 1, a)*. At this stage many radial wrinklings are recognized on each side of the fissure *(Pl. III, Fig. 1)*. The progress of this groove over the protoplasmic part of the egg—which might well be termed 'blastodisc' for the sake of convenience—is rather rapid, requiring only one or two minutes to pass the distance from the pole to the margin of the disc. Beyond



Text-figure 1. (*a*—*e'*, diagrams; *f*, camera drawing) *a*, view of the upper hemisphere; the first meridional groove dividing the egg into two approximately equal halves. *b*, view of the upper hemisphere; the first cleavage groove has extended a little below the equator and the second cleavage groove intersected the first at the middle of the latter at right angle. *c*, view of the upper hemisphere of the egg at the stage in which the third cleavage grooves extend to the margin of the blastodisc. *c'*, view of the lower hemisphere of the same stage; the first groove reaches the lower pole, and the second extends a little below the equator. *d* and *d'* represent a typical fourth cleavage. *d*, view of the upper hemisphere; Arabic numerals represent the order of appearance of the fourth grooves. *d'*, view of the lower hemisphere of the same stage; the second cleavage grooves meet each other and are almost perpendicular to the first, and the third grooves extend a little below the equator. *e* and *e'* is the commonest type of the fifth cleavage. *e*, view of the upper hemisphere; Arabic numerals represent the order of appearance of the fifth cleavage grooves. The fifth cleavage is performed by two sets of grooves, one dividing the four central cells vertically or horizontally (8 in the figure), and the other appearing in the twelve marginal cells in the horizontal direction (1—7 and 9, 10 in the figure). *e'*, view of the lower hemisphere of the same stage; all of the third cleavage grooves join the first near the pole, while some of the fourth grooves reach the second. *f*, camera drawing from the section of the early blastula, showing the holoblastic type.

this margin it travels at a gradually decreasing speed until it reaches the vicinity of the equator.

The second groove appears at about this time. It is also meridional and perpendicular to the first, traversing it at the middle *(Text-figure 1, b)*. The wrinklings are also recognized on each side of the new groove *(Pl. III, Fig. 2)*. This groove proceeds at about the same rate as the first; but beyond the margin of the blastodisc, its progress is gradually retarded. By the time the second groove comes to the equator, the first groove reaches the lower pole, although this is rather difficult to make out in a living egg. In the preserved materials, however, the groove can readily be traced to the lower pole.

The four grooves which constitute the third cleavage are, in all the cases observed, vertical, instead of horizontal as in other frogs' eggs, conforming to the pattern of ganoids and teleosts *(Text-figure 1, c and c')*. The order of their appearance is irregular, beginning in this or that quadrant, as shown in *Plate V*. In some cases two or three minutes elapse from the time when any groove of the third cleavage is first visible to the time of the appearance of the last one. All of the grooves pass over the surface of the egg at the same rate as the first and second cleavage grooves, and terminate apparently also in the vicinity of the equator. At this stage the first cleavage line remains usually straight, but the second cleavage line, originally straight, often becomes drawn into a zigzag line on account of the shifting of the blastomeres *(Pl. III, Fig. 3)*. In preserved eggs I have found that the second cleavage grooves at this stage meet each other at the lower pole where they are perpendicular to the first with but few exceptions. The third cleavage grooves, which start from the second cleavage groove at nearly equal distances from its point of intersection with the first, usually join the first cleavage line near the lower pole, but in a few cases some of them reach the pole *(Text-figure 1, c')*.

The eight grooves of the fourth cleavage are formed in the manner shown in *Pl. III, Fig. 4*; therefore, this cleavage may also be termed vertical. In some eggs, however, one or two grooves, rarely even all of them, pass horizontally. These grooves do not appear simultaneously, but appear earlier in the four blastomeres on each side of the first cleavage line than in the others, and form the Chinese ideograph 田 as shown in *Text-figure 1, d*. Of the eight grooves, the four which start from the third cleavage lines meet the second cleavage line at points farther from the lower pole than the point of intersection of

the third groove with the first *(Text-figure 1, c')*. As the result of this fourth cleavage the egg is cut into four small central cells and twelve larger marginal cells. In living eggs these grooves are barely observable beyond the equator and cannot be traced to the second cleavage line. At this stage the second cleavage lines are sometimes recognized at the lower hemisphere even in living eggs.

The fifth cleavage may well be termed horizontal in the strict sense, but it is highly probable that many irreguralities appear from this cleavage on *(Text-figure 1, c)*. After this stage, cleavage goes on only in the upper hemisphere and the resulting cells become smaller and smaller, while the lower hemisphere remains almost unaltered, so that the egg in these stages might well be taken for meroblastic by a casual observer. However, a crucial examination reveals invariably that the egg is perfectly holoblastic, which fact may easily be made out in the preserved eggs or in sections. *Text-figure 1, f* is a camera drawing of a section of the early blastula. The lower hemisphere is cut into large blastomeres, which fact is hardly perceptible in the living materials. But in a more advanced blastula, the blastomeres are easily recognized on the lower hemisphere, while, on the other hand, they become rather indistinct on the upper hemisphere owing to their very small size.

b) Cleavage Rhythm

The successive cleavages were followed in living eggs, put in a watch-glass with water (at 15°C—17°C), and the exact time of appearance of each cleavage was taken and tabulated. (TABLE I)

As is clear from the table, the interval between the first and second cleavage was 56 minutes, and that between the second and third 63 minutes on the average, the result being thus largely in conformity with that obtained by JORDAN and EYCLESHYMER in various species of *Bufo* and *Rana* at about 18°C. The fourth cleavage furrows make their appearance also about one hour after the third cleavage. The fifth cleavage may be said to occur about one hour after the fourth, but after this stage the rhythm is gradually disturbed on account of the irregularities in the appearance of the cleavage furrows.

c) Blastulation and Gastrulation

In 20 to 24 hours after oviposition, the upper pole of the egg becomes translucent as already described by IKEDA in the case of

TABLE I

Egg No.	First cleavage	Second cleavage	Interval betw. 1st & 2nd clvg.	Third cleavage	Interval betw. 2nd & 3rd clvg.
1	8 : 20 a. m.	9 : 22 a. m.	62 min.	10 : 25 a. m.	63 min.
2	8 : 31 „	9 : 27 „	56 „	10 : 24 „	57 „
3	8 : 31 „	9 : 21 „	50 „	10 : 21—10 : 25 „	60—64 „
4	8 : 32 „	9 : 32 „	60 „	10 : 35—10 : 36 „	63—64 „
5	8 : 32 „	9 : 39 „	67 „	10 : 38—10 : 39 „	59—60 „
6	8 : 40 „	9 : 35 „	55 „	10 : 32—10 : 38 „	57—63 „
7	8 : 48 „	9 : 50 „	62 „	10 : 48—10 : 52 „	58—62 „
8	10 : 30 „	11 : 28 „	58 „	12 : 30 p. m.	62 „
9	10 : 30 „	11 : 40 „	70 „	12 : 50 „	70 „
10	10 : 40 „	11 : 50 „	70 „	12 : 55—12 : 58 „	65—68 „
11	10 : 40 „	11 : 25 „	45 „	12 : 30 „	65 „
12	10 : 40 „	11 : 46 „	66 „	12 : 54—12 : 56 „	68—70 „
13	10 : 42 „	11 : 36 „	54 „	12 : 30 „	54 „
14	10 : 48 „	12 : 04 p. m.	76 „	1 : 08 „	64 „
15	10 : 58 „	12 : 00 „	62 „	1 : 03 „	63 „
16	10 : 58 „	12 : 05 „	67 „	1 : 09 „	64 „
17	11 : 05 „	12 : 03 „	58 „	1 : 08—1 : 10 „	65—67 „
18	11 : 07 „	12 : 02 „	55 „	1 : 10—1 : 11 „	68—69 „
19	11 : 07 „	12 : 15 „	68 „	1 : 15 „	60 „
20	11 : 15 „	12 : 15 „	60 „	1 : 17 „	62 „
21	11 : 15 „	12 : 08 „	53 „	1 : 12 „	64 „
22	11 : 15 „	12 : 15 „	60 „	1 : 25 „	70 „
23	11 : 17 „	12 : 20 „	63 „	1 : 20 „	60 „
24	11 : 17 „	12 : 08 „	51 „	1 : 07 „	59 „
25	11 : 17 „	12 : 10 „	53 „	1 : 15—1 : 17 „	65—67 „
26	11 : 17 „	12 : 08 „	51 „	1 : 14 „	66 „
27	11 : 18 „	12 : 16 „	58 „	1 : 13—1 : 15 „	57—59 „
28	11 : 20 „	12 : 16 „	56 „	1 : 20 „	64 „
29	11 : 20 „	12 : 14 „	54 „	1 : 12—1 : 14 „	58—60 „
30	11 : 20 „	12 : 08 „	48 „	1 : 12 „	64 „
31	11 : 20 „	12 : 21 „	61 „	1 : 15—1 : 17 „	54—56 „
32	11 : 20 „	12 : 07 „	47 „	1 : 06 „	59 „
33	11 : 22 „	12 : 15 „	53 „	1 : 20—1 : 25 „	65—70 „
34	11 : 25 „	12 : 20 „	55 „	1 : 22 „	62 „
35	11 : 35 „	12 : 20 „	45 „	1 : 22—1 : 24 „	62—64 „
36	11 : 35 „	12 : 26 „	51 „	1 : 24—1 : 26 „	58—60 „
37	11 : 37 „	12 : 26 „	49 „	1 : 30 „	64 „
38	11 : 40 „	12 : 21 „	41 „	1 : 20 „	59 „
39	11 : 46 „	12 : 31 „	46 „	1 : 32 „	60 „
40	11 : 50 „	12 : 27 „	37 „	1 : 29—1 : 30 „	62—63 „

Rhacophorus schlegelii. This translucent area is the surface manifestation of an inner segmentation cavity, and extends naturally with the enlargment of the latter; it vanishes finally with the decrease and obliteration of that cavity. When the egg is put into water, the margin of this translucent area becomes depressed as shown in *Pl. III, Fig. 7*. When this translucent area grows to its maximum size, which is about 2.2 mm. in diameter, the dorsal lip of the blastopore, 'RUSCONI's groove,' makes its first appearance close below the equator of the egg. On the egg laid in the morning on the 27th of May, the translucent area appeared at 8 a. m. on the following day and the groove was observed first at 6 : 30 a. m. on the 29th. In the next five hours or more the ventral lip of the blastopore was formed and thus the blastoporic area or yolk-plug was completely encircled. This blastoporic area is very large, compared with that of other frogs' egg, owing partly to a quicker development of the lip and partly to the dorsal lip making its appearance at a point 10° to 20° below the equator in most cases, and sometimes even 5° below it, contrary to the case in the egg of *Bufo* or *Rana* where it appears 28° to 30° below the equator. The dorso-ventral diameter of the blastopore, at the time when the ventral lip is formed, is about 5/6 of the egg diameter as shown in *Pl. III, Fig. 11*. The rate of these developmental changes, however, varies greatly with the atmospheric temperature. But usually, in 20 to 27 hours after the appearance of the dorsal lip, the blastopore is completely closed.

Hand in hand with the diminution of the translucent area of the segmentation cavity, another translucent area makes its appearance and enlarges gradually—this is the superficial indication of the archenteron. When the diameter of the blastoporic area becomes about 1/3 of the egg diameter, the gastrula begins to rotate slowly, with the result that its resting point shifts gradually towards the ventral side of the future embryo. Thus at the end of gastrulation the now much reduced blastopore is detected from above on the posterior side of the future embryo, and at this stage the neural plate has already become recognizable faintly on the equatorial zone (region between the upper translucent area of the segmentation cavity and the yolk-plug). After this stage the neural groove and folds are to be found in two or three hours and the embryo becomes clearer and clearer. In some cases, however, the neural groove and folds are first discernible after the complete disappearance of the yolk-plug. As described above, in 20 to 27 hours after the encircling of the blastopore, the latter complete-

ly disappears and this marks the end of gastrulation. During the last stage of the closure of the blastopore, the lateral lips rapidly approach each other, and the pore becomes elliptical in shape, and later converted into a slit-like opening, as stated by MORGAN in other frogs.

d) Embryo and Larva

As described above, at the time when the gastrula rotation begins, the neural plate is recognizable faintly on the equatorial zone, as shown in *Pl. III, Fig. 13*. In the next two or three hours the neural groove (primitive groove) can be observed first as a rather broad groove *(Fig. 14)*; and one or two hours later, the neural fold becomes noticeable along each side of the groove *(Fig. 18)*. When the neural folds become distinct and meet each other at the anterior region (head fold), the sense plates make their appearance as the lateral extension of the neural folds *(Fig. 23)*. At this stage the entire egg is elliptical, instead of spherical as before. Meanwhile the sense plate is differentiated into an anterior sense plate and a posterior gill plate *(Fig. 24)*. The neural folds approach each other and fuse finally along the median line, converting the groove into a tube. This fusion of the neural folds begins at the neck region of the embryo and proceeds both forwards and backwards from this point. In 70 to 90 hours after oviposition, this fusion is completed along the whole length of the fold, and at the same time a few mesodermic somites appear in the pectoral region of the embryo *(Pl. IV, Fig. 31)*. A little later, a pair of longitudinal prominences are formed along the external sides of the region of the somites—these are the superficial indication of the pronephric ducts. As the development goes on, the longitudinal furrow limiting the somites from the pronephric prominence on both sides, is deepened and the body of the embryo is more elevated on the surface of the egg, as shown in *Figs. 31—36*. In *Fig. 32* the eye-bulb is seen making its appearance on either side as a lateral protrusion of the neural tube. In front of the somites and somewhat to the side of them, are found three pairs of gill plates. All these plates grow in the antero-lateral direction, so as to embrace the head and also the stomodeal depression, which has appeared in the middle part of the sense plate. In about four hours later the heart and anus are recognized. The embryo at this stage is quite different from that of other frogs, and reminds one very much of that of a ganoid. The head is depressed over the large elliptical yolk mass, while the tail is

somewhat elevated, so that the embryo does not take the shape of a bean like that of other frogs at this stage. In the next twelve hours or more the head is raised and the tail straightened gradually, but the prominence becomes more obscure than before. As the tail grows backwards, it becomes compressed and curved to the left, while attached closely to the yolk mass *(Fig. 39)*; both the dorsal and ventral edges become thin as in other frogs. At this stage the eye-bulb develops into a perfect eye, except for the lack of pigment; the rudiments of oral suckers appear on either side of the longitudinal groove of the sense plate below the stomodeal depression, as shown in *Fig. 40* and *42*. In about three hours later (viz. 100 to 120 hours after oviposition) the rudiments of the external gill make their appearance as a pair of papilliform processes of the skin. Somewhat later, the heart commences to beat, at first slowly, but soon about 60 times per minute. The number increases gradually to its maximum of about 170 per minute in the tadpole with well-developed vitelline veins, and thence decreases again with the development. At the time when the heart begins to beat, the embryo commences to twitch within the egg membrane. The space between the egg membrane and the yolk mass is now filled with yellow liquid, possibly a kind of secretion from the body. Just before hatching, namely about six days after oviposition, pigment appears first in the pectoral region of the larva. The latter soon begins to wriggle about in the frothy mass.

The newly-hatched tadpole is about 12 mm. in length. At this stage the auditory vesicles, nasal pits and vitelline veins are distinctly visible. The main trunks of the vitelline veins are two in number on each side of the yolk sac; and they run into each other close behind the heart.

In seven days after oviposition the three pairs of the external gills reach their full size; each of the anterior pair is composed of seven branches or more, the middle of about six branches, while the posterior pair, which appear after the first two, remain in the rudimentary state each with only two bud-like processes. After this stage the gills diminish rapidly in size and are covered over by the integumental fold or operculum which develops first in the region in front of the heart, and grows backward to fuse with the skin of the ventral side of the body, leaving a small opening or spiracle on the left side. It is on the ninth day after the oviposition that the development reaches this stage. The dorsal surface of the body and the sides of the tail are well pigmented, while the ventral surface is pigmentless and looks

yellow owing to the presence of yolk within. The vitelline veins are now obscure. The tail of this tadpole is very long, measuring about twice as long as the body, and moreover, the body is very slender, compared with that of other frogs, so that the tadpole looks more like a fish. On either side of the tail, myomeres are seen through the skin. Blood corpuscles flowing in the capillaries are also clearly visible in the intersegmental regions. At about the same time as the external gills are completely covered by the operculum, the coiled intestine begins to be formed, which increases rapidly in length. The lateral line system is easily seen in the larva of this stage preserved in KLEINENBERG's picro-sulphuric solution.

In about ten days after oviposition the first rudiments of the hind limbs are recognizable as small white papillæ on each side of the tail above and in front of the anus.

The metamorphosis takes place at the beginning of August, if the food supply is sufficient.

Several of the eggs were immersed in water to see whether the development is arrested or not under this abnormal condition. It was found that most of them were able to develop in water into perfect tadpoles, provided that the water was clean and sufficient. But the younger the stage was, the less the chance of unarrested development was found. Even the uncleaved eggs grew often to the tadpoles. The result is thus at variance with that obtained by IKEDA in *Rhacophorus schlegelii* and also with that reported by SIEDLECKI in *Polypedates reinwaldtii*. According to IKEDA, "Those that have not yet hatched can never thrive in water, and if placed artificially in it by way of experiment, they soon die," while SIEDLECKI reports "Wenn man die frisch aus den Eihüllen ausgeschlüpften Larven in Wasser eintaucht, so quellen dieselben stark auf und sterben in 1–4 Stunden ab." The author also states that the tadpole of this frog must remain at least one day after hatching in the frothy mass before going into the aquatic life.

Summary

1. *Rhacophorus schlegelii* var. *arborea* has a peculiar breeding habit in that one female is accompanied by four or five males and the whole group make a frothy mass out of the mucus discharged from the female and the eggs are laid in this mass. The mass is hung on twigs or leaves above a pond or marsh.

2. The egg is pigmentless and highly telolecithal. The first two cleavages are meridional and perpendicular to each other. The third cleavage is vertical instead of horizontal as in other frogs' eggs, and its four grooves start from the second cleavage line at nearly equal distances from the intersection of the second line with the first, and usually join the first near the lower pole.

3. The eight grooves forming the fourth cleavage are also vertical as a rule and start from the first and third cleavage grooves and join the second. The fifth cleavage may well be termed horizontal in the strict sense, but irreguralities are very common from this cleavage on.

4. In the living egg all of these cleavage lines are hardly recognizable on the lower hemisphere, so that the egg has the appearance of a meroblastic egg, though in reality it is perfectly holoblastic.

5. The cleavages occur at intervals of approximately one hour at about 17°C.

6. In 20 to 24 hours after oviposition a translucent area begins to appear as a surface manifestation of an inner segmentation cavity. When this area grows to its maximum size, the dorsal lip of the blastopore is formed just below the equator of the egg.

7. The dorso-ventral diameter of the yolk-plug is at first about 5/6 of the egg diameter. When the former diminishes to nearly 1/3 of the latter, the gastrula rotation begins. At this stage the neural plate becomes noticeable faintly.

8. In the next two or three hours, the neural groove can be observed, and one or two hour slater, the neural fold appears along each side of the groove. The fusion of the folds is completed in 70 to 90 hours after oviposition. The pronephric ducts are also easily observed from the surface at this stage.

9. The early embryo differs much from that of other frogs and reminds one somewhat of that of a ganoid.

10. Just before hatching, namely, about six days after oviposition, the pigment appears first at the pectoral region of the larva. In nine day after oviposition the coiled intestine begins to be formed. The metamorphosis takes place one to two months after oviposition, if the food supply is sufficient.

11. Even uncleaved eggs, immersed in water, continue to develop to perfect tadpoles. In this point this frog differs from others of the same genus, and also from *Polypedates reinwaldtii* in Java, whose embryo is reported to die one to four hours if transferred into water even after hatching.

II. THE LOCALIZATION OF THE MEDIAN PLANE OF THE EMBRYO AND THE MODE OF BLASTOPORE CLOSURE

Material and Methods

So far as I am aware, GOODALE ('11) was the first to discover the applicability of aniline dye—Nile blue sulphate—to the study of amphibian development. He succeeded in making out the mode of blastopore closure of the egg of *Spelerpes bilineatus* by making several spots about the equator of the egg in the late blastula stage and by tracing the change in the position of the spots in the later stages of development. This dye leaves a relatively permanent mark on the egg and is not so toxic as to interfere with the normal course of development. SMITH ('14 & '22), who investigated the mode of blastopore closure and the origin of the bilateral symmetry of the egg of *Cryptobranchus alleghcniensis*, obtained good results by applying a small drop of strong aqueous solution of Nile blue sulphate to the surface of an egg with a fine pipette. More recently, VOGT ('25) has devised a more elaborate method for producing a definite and permanent mark on the egg. Briefly stated his method is as follows: A piece of agar is soaked in one-percent solutions of Nile blue sulphate, neutral red and Bismark brown for a few days, and then dried on a glass plate. The dry agar is cut into small pieces of appropriate size and placed on the surface of the egg. To prevent the dye from spreading, the egg is kept in a hole of proper size made in a piece of wax, and the piece of agar is inserted between the surface of the egg and the wall of the hole with a very fine needle. The affinity between the dye and the egg is greater than that between the dye and agar, so that the egg is easily stained by diffusion. To stain the upper surface of the egg, the agar is put on the desired place and then pressed with a strip of tin-foil. By this method VOGT and GOERTTLER obtained excellent results in their study of the urodelan development. I worked with this method in my study of the development of the egg of *Rhacophorus schlegelii* var. *arborea* in its breeding season of 1929 and 1930 to make out the relation of the median plane of the embryo to the plane of first cleavage, and the mode of blastopore closure. The lack of pigment in the egg of this frog makes the material very suitable for this method. As just stated, I adopted VOGT's method of vital staining, with the only modification that a strip of cellophane was used instead of tin-foil, because its

transparency made the necessary manipulation easier. This idea I owe Prof. Yô K. OKADA. Magnified by a ZEISS binocular microscope, the eggs were accurately marked by applying the stained agar to them for 30–50 minutes, and then carefully placed in water in a shallow vessel, the bottom of which was covered with cotton. They were left thus very quietly for one or two hours until the stain of the vitelline membrane faded completely away. This is a necessary precaution to take, otherwise the stain of that membrane may leave another mark on the egg by the rotation of the egg in that membrane. The marks were observed and sketched at intervals. Views of the lower and the lateral surfaces of the eggs were easily obtained by means of a ZEISS prism rotator.

I wish here to acknowledge my indebtedness to Prof. Taku KOMAI for his valuable criticism and great kindness in looking through the manuscript. I am under deep obligation also to Mr. Iichi KITAYAMA, the Principal of Mizuno Primary school, who kindly let me stay in that school while I was pursuing my work.

Observation

The breeding season this year (1930) lasted from the beginning of June to the beginning of July. The village of Mizuno, where the present study was undertaken during that time, lies on the slope of Mt. Atago about six miles north-west of Kyoto and is as desolate as Bessho except that electric light is available there.

a) Localization of the median plane of the embryo

By the method described above, both of the opposite ends of the first cleavage groove were stained. Of about one hundred eggs thus stained, some perished shortly before the gastrulation, probably on account of overstaining, while in others the marks had faded away by the time of the first appearance of the dorsal lip. In all, 79 eggs survived to the gastrula stage and were available for observation. On the morning following the day of oviposition the dorsal lip appeared —much sooner than usual on account of the extraordinarily warm weather. The position of the first trace of the dorsal lip in relation to the mark is shown in Table II.

Of the 57 eggs of the "coincident" type, 42 showed the plane coinciding perfectly with the first trace of the dorsal lip, while in the remaining 15 eggs a deviation of within 10 degrees was found, which

TABLE II

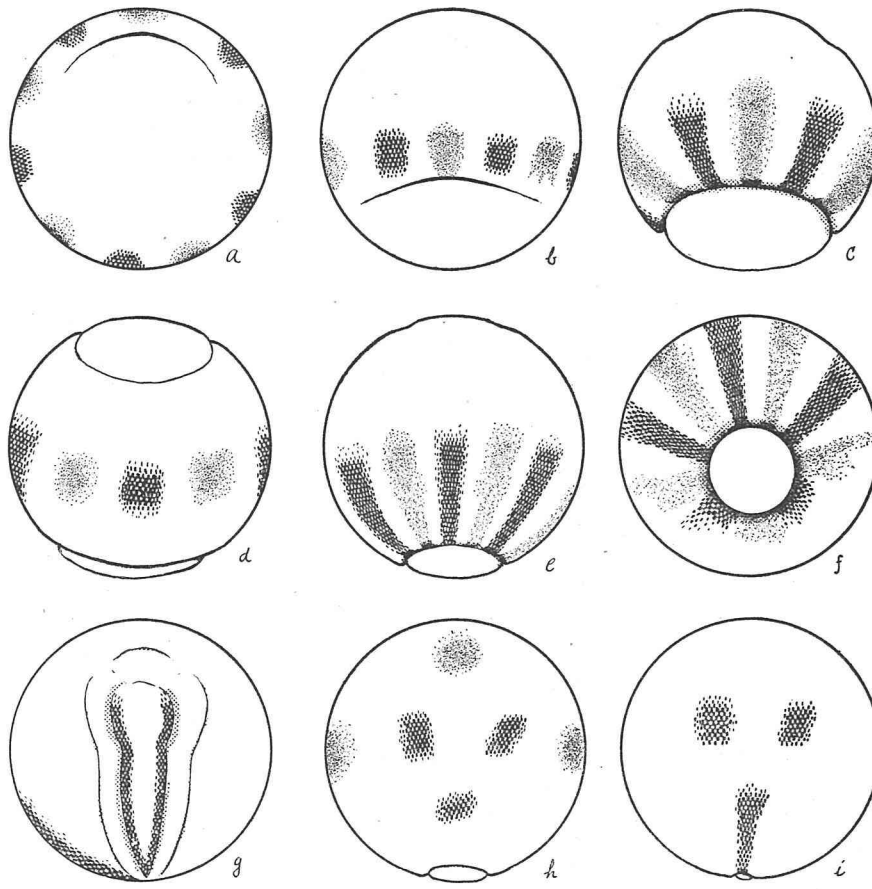
Group	Relation of the dorsal lip to the first plane of cleavage		
	Coincident	At right angles	Oblique
A	3	1	0
B	9	1	2
C	5	0	1
D	7	1	1
E	17	0	4
F	6	3	1
G	10	1	6
Total	57	7	15
Percentage	72%	9%	19%

I have grouped also under the "coincident" type. Among the eggs belonging to the third or "oblique" type there were departures of between 10 and 45 degrees in 9 and between 45 and 90 degrees in 6 eggs. This observation shows clearly that the plane of first cleavage coincides with the median plane of the embryo in the majority of cases, since it is already known that the plane passing through the middle of the dorsal lip coincides with the median plane of the embryo.

b) The mode of blastopore closure

Text-fig. 2, a–g show the mode of blastopore closure of an egg, which was taken from a batch deposited in the early morning, June 18th, and in which the dorsal lip of the blastopore had extended a great deal at 11 a. m. the next day, when ten marks were made around the equator—five with Nile blue sulphate and the other five with neutral red alternately as shown in the figure. *a* is a view from the lower hemisphere immediately after marking. *b* is a dorsal (frontal) view two hours later; all the spots on the dorsal region have extended more or less, especially the median red mark which has reached the margin of the blastopore. *c* and *d* are two different views at the same stage, sketched at 4 p. m. on the same day. These figures show clearly that the area covered by the dorsal spots becomes elongated towards the lip and partly inturned in it, while the part with the

ventral marks remains unchanged both in size and situation. *e* and *f* give the upper (dorsal) and posterior views of the same egg, sketched at 9 p. m. on the same day. By this stage the rotation of the egg as a whole has already begun and naturally the yolk-plug can be seen from above. By this time the marks made with neutral red have become paler and more difficult to trace, while those made with Nile blue sulphate have turned into sharp bands on the dorsal side. *f* shows that the ventral side has scarcely extended, but is inturned *in situ*. This fact offers evidence that the downgrowth of the dorsal lip is of such a great extent as to cover the original yolk pole completely, whereas the growth of the ventral lip is very slight, so that the blas-



Text-figure 2. *a–g*. showing the process of gastrulation and formation of neural plate. *h* and *i* showing the downgrowth of the material existing in front of the dorsal lip at the late blastula stage.

topore closes at the point midway between the original yolk pole and the primary ventral lip. *g* is an upper and somewhat posterior view of the egg at the stage after the neural folds become visible, sketched at 7 : 30 a. m. on the third day after oviposition (20 th). From the mode of extension of the colored band from the original position to the point of closure of the blastopore, the position of the prospective neural plate can be told approximately. In other words, the material out of which the neural plate is formed, is present at the equator of the egg before gastrulation and stretches towards the point of closure of the blastopore. To make out this extension of the material more precisely, the equatorial region of the egg was stained at the stage when the diameter of the yolk plug was about half the egg diameter. It was found that the material existing between the mark and the dorsal lip rolled in and was replaced by the stained cells, which are arranged in an elongated band. Thus it is more than probable that of the material lying in the original equatorial region, that which grows down in the early stage forms the chorda-mesoderm, while in the later stage of downgrowth it differentiates into the greater part of the neural plate. Text-fig. 2, *h* and *i* are examples affording evidence for the above view : *h* is an upper view immediately after making, sketched at 10 : 30 a. m., June 28th ; *i*, drawn four hours later, shows the elongation of the single spot in front of the dorsal lip, while the other spots remain almost unaltered. Three spots marked with neutral red had faded away and could not be sketched in the figure.

To make out the position of the anterior end of the neural plate, the animal pole of the late blastula was stained and the position of the head was carefully observed in relation to the spot, when the head became visible. From this observation it has become clear that the head develops at a point somewhat below the animal pole, although the accurate number of degrees from the pole could not be determined. It is neither so high above the equator as in *Triton* (VOGT) nor so close to it as in *Rana* (BRACHET).

All these observations lead to the belief that the body of the embryo is formed by the materials that lie in the semicircular area of the equator of the late blastula, except a part of the head which arises from the material lying above the equator. The neural folds are brought close together towards the median line by an actual shifting of the material.

Summary

1. Observations carried out with the staining method strongly corroborate the view that the plane of the first cleavage usually coincides with the median plane of the emleryo.

2. The results of observation of the blastopore closure were quite at variance with the view of 'convergence'; the blastopore is closed neither by the equal growth of the lip all around, nor by the process of 'concrescence.' The blastopore lip grows much faster on the dorsal side than on the ventral, and is closed finally midway between the original yolk pole and the point where the ventral lip first appears.

3. The material onginally localized in the equatorial region moves towards the blastopore and develops the neural plate. The chorda, the mesoderm and even a part of the endoderm are formed by the involved material which has been situated in the equatorial region before gastrulation.

4. The position of the anterior end of the neural plate is above the equator, but not so close to it as in *Rana* (BRACHET) nor is it so high above it as in *Triton* (VOGT). The neural folds are brought together towards the median line by an actual shifting of material.

Additional Remark

While the present paper was ready for press, I. MOTOMURA's paper "On the Presumptive Position of the Material of the Medullary Plate in the Frog's Egg, *Rhacophorus schlegelii* var. *arborea* (Okada et Kawano)" appeared in the "Science Reports of the Tôhoku Imperial University, Vol. V, No. 3." The results of his observation are largely in accord with mine, though there are some differences in detailed points. The main point of agreement between us lies in the fact that the anterior end of the prospective neural (medullary) plate is located below the animal pole and, the greater part of the material forming this plate exists in the equatorial region on the dorsal side of the egg, consequently the greater part of the neural plate is formed in the area occupied by this material. As for the mode of blastopore closure, however, he maintains that the blastopore lip converges equally from all sides towards the yolk pole, so that the pore is closed precisely at the yolk pole. Unfortunately I can not agree with him in this view. The downgrowth of the dorsal lip is much quicker than the converging development of all the other parts of the margin of the

blastopore and consequently the pore closes at the point midway between the original yolk pole and the spot where the ventral lip first appears. However, the dorsal lip never advances anteriorly beyond the lower pole of the egg, because the lower pole of the egg shifts gradually from the center of the yolk-plug towards the ventral side of the future embryo on account of the rotation of the egg as a whole.

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EXPLANATION OF PLATES III—V

LIST OF ABBREVIATIONS

a, anus; *av*, auditory vesicle; *cd*, circular depression; *dl*. dorsal lip; *eb*, eye-bulb; *ex*, external gill; *fb*, fore-brain; *gp*, gill plate; *h*, heart; *hb*, hind-brain; *hf*, head fold; *im*, inner membrane; *m*, stomodeal depression; *mb*, mid-brain; *nf*, neural fold; *ng*, neural groove; *np*, neural plate; *nt*, nasal pit; *om*, outer membrane; *op*, operculum; *pr*, pronephros; *s*, sucker; *sm*, somite; *sp*, sense plate; *ta*, translucent area of archenteron; *ts*, translucent area of segmentation cavity; *v*, vitelline vein; *y*, yolk-plug. Roman numerals represent the order of cleavage.

PLATE III

1. Eggs during the first cleavage. The wrinklings are seen on each side of the groove. The space between the outer and inner membranes is widened by absorption of water.
2. Eggs at the beginning of the second cleavage; wrinklings are seen on each side of the new groove.
3. Egg after the third cleavage. The first cleavage line is more obscure than before, but nearly straight, while the second cleavage line becomes a zigzag line by the rearrangement of the blastomeres.
4. Egg after the fourth cleavage.
5. Egg after the fifth cleavage, sketched from preserved material. The fifth cleavage may be considered as consisting of two sets; one is horizontal and the other vertical. Some of the vertical lines may be those of the sixth cleavage.
6. Early blastula, sketched from preserved material.
7. Later blastula. (about 35 hrs. after oviposition) The translucent area of the segmentation cavity is circumscribed by a furrow-like depression.
8. Appearance of the dorsal lip of the blastopore (about 50 hrs. after oviposition).
9. Side view of the gastrula showing the growth of the lateral lip of the blastopore.
10. Upper view of the same egg showing the translucent area of the inner segmentation cavity.
11. Lower view of gastrula just after the appearance of the ventral lip of the blastopore.
12. Gastrula sometime after the encircling of the blastopore.
13. The neural plate is faintly perceptible on the equatorial zone.
14. Dorsal view showing the faint indication of the neural groove.
15. Posterior view of the same egg.

16—22. Eggs showing the diminution of the blastopore and the appearance of the neural folds. In Fig. 22 the translucent area of the archenteron is clearly visible.

23. The stage when the neural folds are distinct and approaching each other in the pectoral region of the future embryo. Sense plate has appeared as a lateral extension of the neural fold. Head fold is distinct.
24. A stage a little later than in Fig. 23. The original sense plate is divided into the sense plate and gill plate.
25. Posterior view of the same egg.
26. Anterior view of the egg; the sense plates are united each other on the median line where they are faintly connected with the anterior end of the head fold.

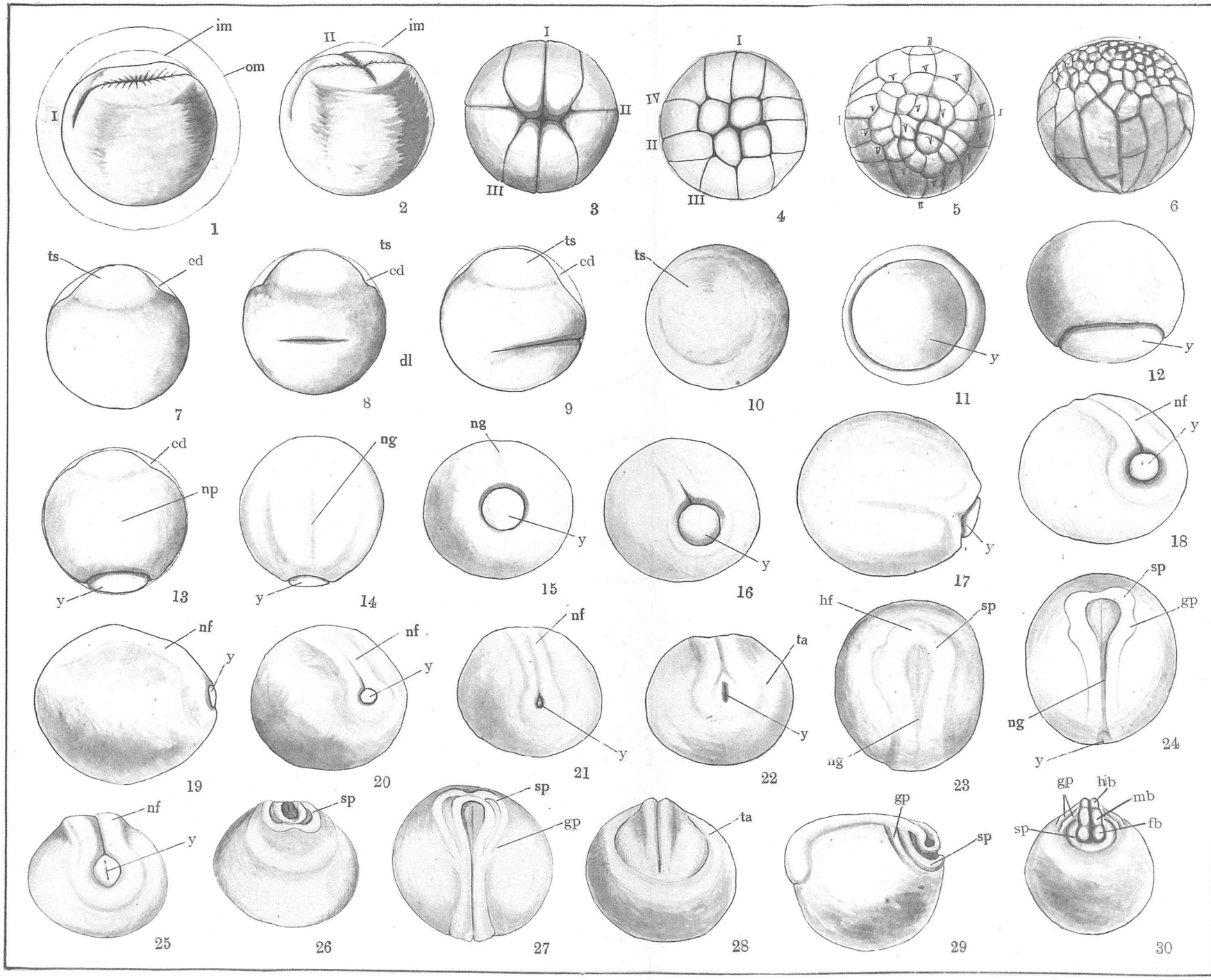
27. Dorsal view of the same egg.
28, 29. Posterior and antero-lateral views of the egg.
30. Anterior view of the egg later than the previous one. Differentiation of fore-, mid- and hind-brains is perceptible and gill plates are two pairs in number.

PLATE IV

31. A few somites and the pronephric duct are present on each side of the neural tube.
32. Side view of the egg; eye-bulb is distinct in this case, while the fusion of the neural folds is not completed. Gill plates are three pairs at this stage and the embryo is elevated somewhat; stomodeal depression is visible.
33. Dorsal view of the egg about three hours later than in Fig. 31.
34—37. Posterior, anterior, lateral and dorsal views of the egg about 20 hours later than in Fig. 25; heart and anus are formed (80 to 95 hrs. after oviposition).
38—39. Anterior and posterior views of the egg; the tail is elevated and elongated somewhat, while the head is less elevated.
40—42. The stage when the embryo begins to twitch. The oral suckers have been developed on each side of the longitudinal groove of the sense plate; the eye-bulb is now a perfect eye, except for the lack of pigment.
43. The stage at which the first rudiments of the external gills are formed and the heart begins to beat (100 to 120 hrs. after oviposition). 9 mm. in length.
44—46. The stage when pigment is seen first in the pectoral region of the embryo. Fig. 45 is the left gill of the same embryo (×35).
47. Newly-hatched tadpole; the nostril has been formed.
48. Fully developed right gills. The flowing of the blood corpuscles can easily be seen in these gills (134 to 145 hrs. after oviposition).
49—55. Young tadpoles with external gills atrophying. Fig. 51 shows the vitelline vein.

PLATE V

All figures show the cleavage pattern and rhythm as far as the third cleavage. The precise time at which each groove appeared, is noted in the figure. The dotted lines represent the first cleavage groove, the heavy lines the second, and the thin lines the third grooves.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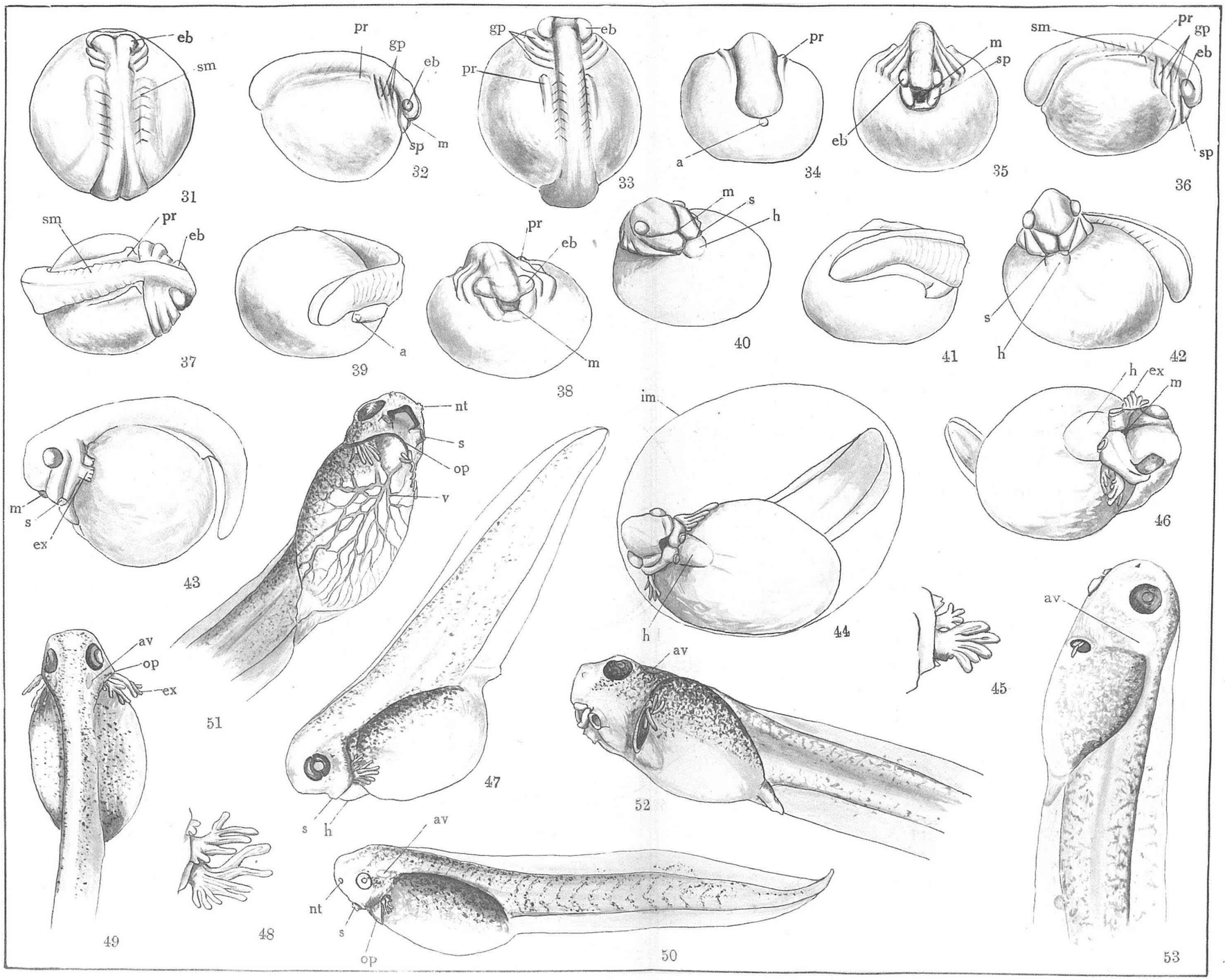
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