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EMBRYONIC AND LARVAL DEVELOPMENT OF *SCAPHIOPUS BOMBIFRONS* COPE

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INTRODUCTION

At the beginning of the last half-century, the field of descriptive embryology had been rather extensively covered, and the development of representatives of most animal groups had been described. Since that time, investigators, realizing that descriptive embryology alone is inadequate to provide an understanding of many of the processes of development, have for the most part turned toward experimental work, with the result that descriptive phases of study have received but little attention, and the embryology of a number of animals remains completely unknown. Since one can hope for an understanding of developmental processes only when knowledge of the morphological features of development provides an adequate foundation for experimental work, there still exists a need for work in descriptive embryology.

In spite of the unusual amount of interest attaching to toads of the genus Scaphiopus, the embryology of these forms has never been studied. The present work has as its aim a description of the normal development of Scaphiopus bombifrons Cope; it has been based mainly on consideration of external structure, although doubtful points have in many cases been investigated by reference to serial sections. Every phase of embryonic and larval development has been repeatedly followed in living specimens, and the study has been supplemented by consideration of an abundance of preserved material. It has been convenient for purposes of description to divide the developmental process into a number

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of stages based upon external morphology and the age at a given temperature (hours after fertilization at 23° to 25°C.). Each stage is considered as lasting until the beginning of the succeeding one, and description is based primarily upon the state of development which initiates a stage. A complete time record based upon study of living material is presented in connection with the description of stages.

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MATERIALS AND METHODS

Material for study was obtained during the spring season of each year from 1934 to 1938, inclusive. Adults (most often clasping pairs) were taken from their breeding congresses before eggs had been laid. In the laboratory pairs were isolated and put into culture dishes containing tap water to a depth of about two inches. The dishes were partially covered to prevent escape of the toads, and were placed in a dark room. Under these conditions amplexation takes place and egg laging, which begins after one or two hours, will continue even when the toads are taken into a lighted room. Eggs were removed as soon as possible to finger bowls, Syracuse watch glasses, or culture dishes labeled with the time of laying and with a number assigned to the pair of adults producing them. In some cases the time of laying was known only within one hour, but in those eggs chosen for time observations it was known exactly. Eggs and embryos were observed under a binocular dissecting microscope and, by means of notes, descriptions, and sketches, observations on development were recorded as completely as possible and for as many embryos as could successfully be studied. In a large number of embryos the exact time of appearance of the various sets of cleavage furrows was noted, and similar records were made for the appearance of all structures and reactions up to the time of hatching. Afterward, larvae were observed at intervals, and the rate of growth checked

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as fully as possible up to the time of metamorphosis. Time records have been based upon study of living material. Even with temperature regulated variations exist which make it quite difficult to prepare a reliable record of the ages at which various structures appear; this is especially true in case of the stages after hatching, since tadpoles of the same age and living in the same environment vary greatly in size at any one time, some being much larger and some much smaller than the average. The time record (that is, the ages at which each stage begins and ends) has been compiled after a careful study of all data gathered during the five years.

In 1934 tadpoles were kept until they had attained a length of about 9 mm., at which time the last ones were fixed. In 1935 and 1936, a number of tadpoles completed metamorphosis, and in the latter year about 40 young toads were kept until the end of September (Trowbridge and Trowbridge, 1937). In 1937, a small series of earlier stages was preserved by A. H. Trowbridge and turned over to the author for study. In 1938 tadpoles were again reared through metamorphosis.

Tadpoles were kept after hatching and up to the time of transformation in large culture dishes, each containing from 15 to 20 specimens. They were in all years except 1935 fed filamentous green algae, lettuce, the yolk of hard-boiled eggs, raw beef liver, bits of lean chopped beef, and fragmented insects. In 1935 several hundred specimens were allowed to develop in large cement tanks, and were fed mainly on a liver diet. Under these conditions development was not normal. At about the beginning of the third week the body became swollen and turgid, in the hind leg a large amount of fluid collected beneath the inte-

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gument, and in some specimens the tail became bent and distorted. Most tadpoles died before completing metamorphosis. None of these abnormalities developed when the more varied diet was given in other years. Klatt (1927) observed that special diets may lead to malformations of the tail or body in salamanders, and Noble (1931) has stated that "overfeeding with liver frequently leads to distended bodies and bent tail in both larvae and adult salamanders." As the latter pointed out, a one-sided diet should be avoided under laboratory conditions where healthy animals are required.

During the first years the developing embryos and tadpoles were kept at a temperature ranging usually from 23 to 25 degrees Centigrade, but occasionally varying between 23° and 27° C. In 1938 the temperature was as nearly as possible maintained at between 23° and 25° C, and the time schedules given are the ones observed to hold at that temperature.

In 1934 a series of embryos and tadpoles up to a length of 9 mm. was preserved, and in 1937 only a few (up to about Stage 8) were fixed, but in the other three years complete series from uncleaved egg up to fully transformed young toads were preserved. A number of fixatives were used, and abundant material for the study of every stage was obtained. In each of three years a complete series was fixed and preserved in 10% formalin. Bouin's fluid was used for a series from blastula to metamorphosing tadpoles; some of the larger tadpoles were cut open to permit better penetration. Goldsmith's fluid (Goldsmith, 1929) was used for a complete series. Another series was fixed in Smith's fluid, either according to the formula of B. G. Smith (1912, page 91) or to that of Galigher (1934, page 49). No attempt was made to remove egg

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membranes or jelly before fixation, since the presence of these was found not to hinder penetration to any great extent. Goldsmith's fluid dissolves the jelly layers, leaving only the chorion surrounding the egg or embryo. For removal of the envelopes before embedding, the specimens were exposed for a short time to the action of a one or two per cent solution of sodium hypochlorite, which should be agitated to hasten removal of the jelly. It is necessary to remove the embryos and wash them thoroughly in water as soon as the jelly is removed, but while the chorion is still intact. Care in observing this precaution precludes any possibility of injury through dissolution or disintegration of the surface of the embryo.

Material intended for study of external features was stored in 70% ethyl alcohol or in 5% formalin, depending upon the method of fixation. Material intended for sectioning was imbedded as soon as possible (in no case more than four weeks) after fixation. Tertiary butyl alcohol was used as the dehydrating and clearing agent for a number of embryos and larvae, but in the majority of cases ethyl alcohol, anilin oil, and toluol were used, in the author's modification of the method of Hamlett (1930) for yolky material. For embryos up to about the time of hatching, the steps from 70% alcohol are as follows:

2/3 70% alcohol plus 1/3 anilin	1 hour
1/3 95% alcohol plus 2/3 anilin	1 hour
Pure anilin (change once)	30 min.
2/3 anilin plus 1/3 toluol	1 hour
1/3 anilin plus 2/3 toluol	1 hour
Pure toluol (change several times)	1 hour

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Add shavings of paraffin, (53° to 55° M. P.), gradually increasing to saturation, over a period of from 6 to 12 hours. Keep at a temperature of 37°C., adding more paraffin if necessary, for several more hours. Place in the paraffin oven at a temperature of 56°C., and add melted paraffin at intervals of an hour or less until object is in pure paraffin. Change paraffin at least twice. Imbed after the embryo has been in pure paraffin about two hours.

Material prepared by either of the above methods and stored in tightly sealed jars in which a moist atmosphere is maintained yields excellent paraffin sections. Sections may be cut as thin as 6 or 7 micra, although most of those prepared were cut at either 8 or 10 micra. If slides are coated before staining and again before the final clearing with a 1% solution of celloidin there is no difficulty due to buckling or loss of sections.

Embryos may be successfully oriented under a dissecting microscope at the time of embedding. Such points of reference as the various cleavage furrows and the gray crescent can as a rule be distinguished without difficulty.

Material fixed in Smith's fluid may be embedded by the anilin method and sectioned successfully even after remaining three or four years in formalin, although staining is not satisfactory. Even those embryos fixed and stored for several years in formalin can be sectioned by this method, but during staining sections tend to fall off the slides before coating with celloidin can be accomplished. This was attempted only in those cases in which it was desired to section embryos showing points of especial interest.

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Smith's fluid and 10% formalin have been found the most satisfactory fixatives for study of the external features of cleavage. Formalin preserves the embryo in a form most closely approaching that of the living one in that the furrows remain deep and definite and the blastomeres slightly rounded, color is faithfully preserved, and the gray crescent is well marked. It has the fault, however, especially in the early cleavage stages, of occasionally producing a slight dent or depression near the animal pole or in the region of the gray crescent. It also causes slight swelling, and after several years may bring about destruction of pigment. With fixation in Smith's fluid, the surfaces of the blastomeres are more flattened, the furrows not quite as definite, and the gray crescent less well marked, although form and size of the embryo are well preserved. For later blastulae (Stages 10 and 11) Smith's fluid gives better fixation for external study than does formalin, since the cell outlines are a little plainer, pigment is better preserved, and there is no tendency toward collapsing. Embryos and larvae fixed in Goldsmith's fluid are so brittle that they can hardly be handled without breaking, and cleavage furrows are not as well outlined nor pigment as well preserved as after fixation in either formalin or Smith's fluid.

Smith's fluid is superior to any of the others used for external study of embryos from early formation of the neural structures to the time of hatching. Bouin's fluid gives good results on larvae from the time of hatching up to a length of about nine millimeters. Used for embryos before hatching, it contracts the chorion close to the body, flattens the early neural ridges, and sometimes causes a precipitation of flocculent material inside the chorion and on the surface of the embryo.

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Early neural folds are not well shown after fixation in either Goldsmith's fluid or formalin. The latter may cause a dent in the dorsal part of the body, and, in later stages up to the time of hatching, brings about a slight amount of swelling. Smith's and Bouin's fluids give better preservation of pigment in embryos from the time of appearance of neural structures up to the time of hatching than do either formalin or Goldsmith's fluid. Tadpoles from the time the integument becomes transparent up to the time of metamorphosis can be fixed in formalin, which preserves the body shape quite well.

Material fixed in Smith's, Goldsmith's, or Bouin's fluid sections well when prepared by either the tertiary butyl alcohol or the anilin oil method. Both Smith's and Goldsmith's fluids have been found to give satisfactory fixation for nuclear study. In most cases, however, the former has been used, as its results are much better for study of external aspects.

Although some eggs and embryos were stained <u>in toto</u> with Galigher's alum-hematoxylin, with picro-carmine, or with Grenacher's alcoholic borax-carmine, the majority were stained on the slide with Heidenhain's iron-hematoxylin or with Galigher's alum-hematoxylin, both without counterstain. Iron-hematoxylin gives a sharp nuclear stain, but the yolk granules stain so deeply that they may, especially in the thicker sections, interfere with observation of nuclei. Galigher's alum-hematoxylin stains the nuclei sharply, the yolk granules lightly, and marks out cell outlines plainly.

It is estimated that during the course of the study at least 30,000 eggs, embryos, and larvae have been procured.

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All sketches have been drawn from preserved material with the aid of a camera lucida.

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STATUS OF SCAPHIOPUS BOMBIFRONS COPE

As has been pointed out in a previous paper (Trowbridge and Trowbridge, 1937) the specific name bombifrons is used with some doubt. The status of bombifrons has long been in dispute. Some authorities (Stejneger and Barbour, 1923, Slevin, 1928, and Ortenburger and Freeman, 1930) consider bombifrons a synonym of hammondii. Others (Burnett, 1924, Taylor, 1929, and Gilmore, 1924) treat bombifrons and hammondii as separate subspecies of the species hammondii. Still others (as Smith, 1934) consider the two as distinct species. Our adults have been identified by Hobart M. Smith as bombifrons, but he agrees with us that the tadpoles are not typical of that species. The latter correspond with the description by Wright (1929) of S. hammondii Baird, save that the median conical horny tooth in the roof of the mouth is present. All tadpoles reared in the laboratory have been of the same type.

It has been indicated in the section on Food Habits of Tadpoles that differences in habitat and available food may cause slight differences among tadpoles of the same form as regards body shape and size, and degree of development of jaw muscles and cornified mouth parts. Laboratory-reared tadpoles have been compared with tadpoles apparently of the same form taken from their natural habitat in pools near Norman, as well as with specimens taken near Colorado Springs and sent to us by R. J. Gilmore. The latter are of two types so distinct as to be

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separated at first sight and without the aid of a microscope; further study shows that one group corresponds with the description given by E. H. Taylor (see Smith, 1934) for S. bombifrons, and the other, save for the presence of the median tooth in the roof of the mouth, with Wright's (1929) description of S. hammondii. It is concluded that environmental factors do not bring about structural differences great enough to confuse the identity of the form.

Since our adults have been identified as S. bombifrons Cope, and since that name has been used in the preliminary publication, it is retained in the present paper. To which species or subspecies the form may eventually be assigned must await a more thorough study of the entire genus than has yet been made, and one which will take into consideration both larval and adult characters.

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LIFE HISTORY

Notes from the Literature

Appearance and Habits:

Since the time of their discovery by Holbrook in 1842, spadefoot toads of the genus Scaphiopus have, on account of their peculiar habits and appearance as well as their rapidity of development, aroused more interest among naturalists than have most other amphibia. Hargitt (1888) referring to Scaphiopus holbrookii, wrote, "Altogether, they are certainly the most peculiar and erratic of any of the order; and, under the peculiar difficulties in the way of continuous study, it will be long ere its life-history can be said to be thoroughly known." The accuracy of this prediction is shown by the fact that even today, despite the interest attaching to spadefoot toads, their life history is but incompletely known, and only scattered notes have been written concerning their development.

As to general appearance, Holbrook (1842) described S. holbrookii as follows: "This is a strange animal-- an odd mixture of toad and frog, having the teeth of one, and the rudimental posttympanal glands of the other; it approaches, however, nearest the toad in its forms and habits .... The skin is very delicate, and though warty or granulated after exposure, when first taken from its hold the Scaphiopus presents the

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etiolated appearance of a real subterranean animal." Among the Anura of Oklahoma, spadefoot toads are distinguished from all others by the vertical pupil of the eye and the convex anterior interorbital region. S. bombifrons differs from toads of the genus Bufo in the absence of a distinct parotid gland. The "spade", a large cornified inner metatarsal tubercle with a free cutting edge, is a further characteristic and the one which gives the group its name. Complete descriptions of Scaphiopus bombifrons Cope have been given by Cope in 1889 (as Spea hammondii bombiffons Cope) and more recently by Smith (1934).

Much of the mystery attached by earlier authors to the life of spadefoot toads lay in their belief that, while the animals emerged to breed during the time of heavy rains in the spring or summer, the remainder of the year they spent in their subterranean burrows. Smith (1934) has pointed out that this belief is probably due to the fact that during the time of breeding the toads can be located in congress by their calls, and it is largely under such conditions that they have been collected. Even so, "if the observer does not happen to be on hand at the right time and in the right place, the animals will escape observation entirely." (Storer, 1925). After breeding, the toads apparently scatter widely, and since they do not sing when they emerge for food, they are seldom found.

Even now it is not definitely known how frequently, aside from their appearance at the time of breeding, spadefoot toads come out of their burrows. Most observers have seen them only at night. Smith (1934) has observed adults of S. bombifrons at night in the sand dunes near Medora, Kansas, hopping about in considerable numbers in light

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showers. Little and Keller (1937) took two specimens of S. hammondii on bare adobe loam soil on the Jornada Experimental Range in New Mexico one afternoon, but concluded that they were probably driven out of their burrows by the heat of a kerosene burner being used to eradicate poisonous plants. According to Wood (1935) adults of S. hammondii are relatively common after sundown in the cottonwood belt along Green River about fifteen miles southwest of Ouray, Utah. Apparently young or immature toads are taken more often on dry nights than are adults. Kellogg (1932) found the young toads (S. hammondii) coming out at night in the summer months independent of rains (along the Powder River near Powderville in Montana). Stone (1932) reported, for S. holbrookii in the New Jersey pine barrens, that on two successive nights he and others "found the open pine woods fairly well populated with the toads and a number of specimens were obtained all of which seemed to be about half grown (body length 1.50 inches)." Allen (1932) found S. holbrookii coming forth every night, the temperature permitting, during the latter half of March and the first part of April, 1932, in the bottom lands of the Tchoutacabouffa River near Biloxi, Mississippi; none of the specimens which were seen or taken exceeded 25 or 30 millimeters in length, excepting one, a full grown individual observed on a night attended by a light precipitation and on which immature specimens were noted to be more abundant. Linsdale (1938), in the Toyabe Mountains area in central Nevada, found emergence of breeding adults of S. hammondii Baird independent of rainfall, and extensive foraging of young ones in daylight hours. In August, 1932, he saw hundreds of recently transformed toads active early in the morning, and later retiring into

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shallow excavations, sometimes covered with earth. In 1933, from mid-May to mid-June, he found a large proportion of the 1932 brood had survived, and were foraging on the surface in daylight hours, being seen most often in early morning or late afternoon. By 8:00 a.m. most of those in sight were burrowing, but a few were seen at mid-day on clear hot days. "Evidently they take advantage of the opportunity, whatever time of year it comes, to feed. The long periods when the toads are underground must result from necessity induced by unfavorable conditions and not from any particular choice for living in burrows."

Noble (1931) has pointed out that structure of skin often restricts range of species, since thin and delicate skins, as those possessed by spadefoots, are more subject to desiccation than are thicker and more heavily cornified ones. However, the correlation between skin structures and environment is not always close. "Many Salientia.... burrow to avoid desiccation. The Spade-foot Toads are equipped with large metatarsal tubercles which are doubtless of great assistance in this operation." Storer (1925) has pointed out that the digging equipment with which toads of the genus Bufo are variously provided is developed to an extreme degree in the spadefoots, which are thus enabled to dig for themselves suitable individual shelters in which to spend the daytime when the amount of atmospheric moisture is dangerously low. Goldsmith (1926) found that digging reactions are induced in S. hammondii by evaporation, and that this species is sensitive to a humidity change of 10 per cent at a temperature of 27° C.

Scaphiopus bombifrons usually chooses sand or soft ground for its burrow. In burrowing, both newly-transformed and adult toads dig from

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the rear by means of the "spades", shuffling the hind limbs in quick movements. Adults are able to conceal themselves in a minute or less; young toads usually require from three to five minutes for the process. In adults, the end of the muzzle and the frontal convexity are in some cases covered with a black cornified layer, which is possibly of use to the animal in keeping the burrow open to the surface, or in packing the ground forming the walls of the burrow. This character is not constant, the horny material varying in amount and position; it may be found only on the tip of the snout, or on top of the head from snout to interorbital boss.

Breeding Places:

At the time of breeding spadefoots may gather by the thousands in their congresses. Dickerson (1913) wrote as follows of S. bombifrons: "In its range it is very abundant, and so when it does appear, every ditch and pool of water may show representatives." Ortenburger (1924) reported for S. couchii and S. bombifrons, 13 miles north of Tucson, Arizona, "In the series of roadside mud puddles where the observations were made there were well over 1500 individuals by actual calculation".

Apparently only temporary pools are chosen as breeding places. Gilmore (1924) has stated that in the low rolling hills east of Colorado Springs, at the time of heavy rains of early summer, the sandy soil is saturated, and temporary pools are formed in the valleys; to these pools the toads migrate in incredible numbers. "The spadefoot chooses temporary ponds in which to rear its young. In no cases have

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eggs or tadpoles been observed in permanent lakes, although such lakes are available. The ponds are roadside mud-holes and low areas in fields ranging from a few inches to a few feet in depth. The water is muddy and warm." Goldsmith (1926) agrees with these observations; during the breeding season he transferred to a small permanent pool fifty adults which failed to reappear, and which he concluded were probably destroyed by the abundance of snakes in the pool. Little and Keller (1937) observed hundreds of individuals and clasping pairs of S. hammondii at artificial ponds and temporary pools after summer rains. Moore (1937) found S. bombifrons abundant in a roadside pool and in terrace ditches at Stillwater, Oklahoma. Driver (1936) observed S. holbrookii breeding in temporary rain ponds in bottom-lands of the Connecticut River near Northampton, Mass., where they preferred the part of the pond over a recently plowed corn field to that over grass; the eggs were draped around submerged corn stalks.

Role of the Voice:

Concerning the role of the voice, Storer (1925) recorded the following observations: "Scaphiopus, in combination with its digging equipment, has other characteristics which would seem of decided benefit to an amphibian living amid arid surroundings. The voice of the male at spawning time is very loud, equaling or exceeding that of Hyla regilla and many times stronger than that of Bufo b. halophilus. Upon the advent of rain in amount sufficient to form pools we may expect that the first male Scaphiopus to enter a rain pool would begin calling; this would serve to attract females and other males so that a breeding

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colony would be established quickly. Rain pools do not necessarily always form in the same place in successive years. If, as we have reason to suppose, the adults are more or less scattered when in their burrows, the strong voice of the first male entering a pond suitable as a breeding environment would serve to concentrate the local population there." Ortenburger (1924) stated: "How the first ones find the puddles was not determined, but after a few calls of the first comers numbers could be seen coming directly towards the sound of the calls. There can be little doubt that they were guided by the calling of others in the water." Goldsmith (1926) has also indicated that voice may play a part in guiding toads to the pools.

The song of the male has been variously described. Ortenburger (1924) described that of S. hammondii (which he considers synonymous with S. bombifrons) as "sounding much like a loud purr of a cat but at the same time having the metallic mechanical sound of grinding gears! It is given by the male while the animal is in the water, each time he kicks his hind legs. The song lasts but 1-2 seconds." Gilmore (1924) observed: "After arriving at the ponds the male spadefoot indulges in very vigorous nuptial song, which continues without interruption until the mating has been completed. The effect has been described as 'weird, plaintive cries', 'hoarse and woeful'. The individual song somewhat resembles that of Rana palustris." Kellogg (1932) stated, for spadefoots taken in Montana: "The call of this toad is quite weird and unusual, and may be likened to the squawk of some animal when severely injured, or a resonant ye-ow. Once heard this distinctive call is not likely to be forgotten."

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Time of Breeding:

It is quite generally agreed that the time of breeding is dependent upon the rains of late spring or early summer. Some observers have found only one breeding congress each year, and others more than one. According to Smith (1934), Dr. E. H. Taylor has observed S. bombifrons to breed in Morton County, Kansas, as early as June 8, and as late as August 8: "Specimens were taken....after very heavy rainfalls. Large numbers congregated at breeding places. Three such groups were found in a radius of two miles and more than fifty specimens were taken on each of the two dates." Smith continued: "It is probable that they breed at any time coincident with the first heavy showers after the middle of spring, even though it be so late as the last of summer. -It has never been proved that they lay more than once a year." Gilmore (1924) has stated that near Colorado Springs, Colorado, the time of breeding is dependent on heavy rains of early summer. Goldsmith (1926) wrote of S. hammondii (probably the same form which Gilmore calls S. hammondii bombifrons) that, on the dry plains to the east of Colorado Springs, after May 1, the breeding period of the individuals in the various areas depends entirely upon a local rainfall sufficient to fill the temporary pools: "Rains insufficient to fill the pools will often bring out a portion of the toad population, which will breed and return to burrows in the soil. A later rain will again bring out a portion of the individuals inhabiting the territory and these will go through their breeding activities. This successional breeding has been observed to occur two and three times in one season in single pools. A heavy rain

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in May or June results in a general breeding period which completes this activity for the season." Little and Keller (1937) found in New Mexico mating of large numbers of S. hammondii after the three important summer rains on May 23, July 24 and August 26, 1934. In the spring of 1936 Moore (1937) observed three breeding congresses of S. bombifrons at Stillwater, Oklahoma. These were on April 28, May 8 and May 22, with the main one on May 8. In the same spring only one congress, that of May 8, occurred at Norman (Trowbridge and Trowbridge, 1937). As Moore pointed out, the difference is apparently due to differences in climatological data for the two regions. Driver (1936) reported for S. holbrookii near Northampton, Mass., one appearance in 1933 and three, with eggs laid at each, in 1934. Brandt (1936), in the case of S. holbrookii holbrookii (Harlan) in eastern North Carolina, found a congress occurring on March 20, 1933, after four days of warm heavy rains; similar conditions early in May of the same year failed to bring about another congress.

On September 6, at Continental, in Santa Cruz Valley, Arizona, Campbell (1933) found males of S. hammondii, Bufo cognatus, and B. woodhousii calling in temporary pools in a large open prairie, while the females of each species were out in the grass, accompanied by numerous subadults, apparently feeding. He concluded that probably the breeding season was over and the ovaries spent, but the males showed no diminution of ardor.

Mating, Egg-laying, Retreat from Congress, etc.:

Mating begins soon after the spadefoots have entered the water.

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Amplexation is normally inguinal. Wright (1932), speaking of S. holbrookii, remarked: "If heavy rainy weather is on they like other species of spadefoots will breed by day, even start first in the daytime but darkness is the preferred period for spadefoots in general (S. holbrookii, S. couchii, S. hammondii)." Gilmore (1924) has stated: "The process of mating and egg laying occupies from twenty-four to forty-eight hours. If rains continue the adults may remain in the water for several days; but more commonly they leave the pond immediately after the eggs have been laid." Ortenburger (1924) observed that the toads left the ponds during the day and came back after dark, none arriving until after it was quite dark. Goldsmith (1926) noted that "breeding activities at a particular pool last two or three days and then end as suddenly as they began, the large contingent of the population leaving during a single night." Storer (1925) wrote of California spadefoots. (S. hammondii),"once concentrated, spawning is evidently accomplished with speed as indicated by the large number of eggs in similar stages of development found in the ponds near Santa Maria following the first heavy late spring rains." Little and Keller (1937) saw in New Mexico pairs of S. hammondii Baird both day and night for several days after prolonged rains.

According to Gilmore (1924), "The egg masses vary in size. Large masses contain 200 to 250 eggs, smaller ones 10 to 50. The mass is attached to submerged vegetation, or to any object protruding from the bottom. The mass is elliptical in shape."

Goldsmith (1926) has observed the retreat of S. hammondii from their breeding congresses. "When breeding activities are over, the

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adults move off up the drainage lines to the warm dry soil of the hills, digging shallow burrows for protection during the day. The distances covered per night by the advance of this migration vary from 60 to 150 meters."

Notes on Development:

Although it has been recognized for some time that spadefoots develop with unusual rapidity, no author has described the process in any detail. Shortness of the developmental period is a character of marked benefit to an amphibian living in an arid environment. As Ruthven (1907) pointed out, "The transient nature of the water bodies on the plains makes it necessary that the immature stages of the amphibians of this habitat be brief." Even so, temporary pools often dry up before the tadpoles have had time to develop through metamorphosis. On August 1, Ruthven found six individuals (three males and three females) of S. couchii Baird breeding in a small pool on the flood-plain of the Santa Cruz River (mesquite association) near Tucson, Arizona. "The pool was small and shallow, owing its origin to a few showers that had occurred previous to this date, one the night before. Five days later the pool had become entirely dry and hundreds of tadpoles were dying in the mud." Driver (1936) near Northampton, Mass., found temporary rain ponds drying up and tadpoles (S. holbrookii) dying on the thirty-first day. At Stillwater, Oklahoma, Moore (1937) observed a remarkable resistance of the tadpoles of S. bombifrons to drying. Eggs were laid May 8. On June 3, no water was left in the pool, and on June 4 twenty-five per cent of the tadpoles were dead. "There was so little moisture that a

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farmer had driven a wagon through the place, and his tires made a depression less than 1 inch in depth." In the late afternoon and evening of June 4 a rain of 1.62 inches fell, and the next day the tadpoles were so numerous that their losses seemed insignificant. "How long they could withstand such unfavorable conditions is uncertain, but they were exposed to the direct sunlight of 1⅓ days and about 75% survived."

A number of observations are available on the hatching period of S. holbrookii. Nichols (1852) observed that five days after he found the spawn it had become tadpoles. Abbott (1884) stated: "During this interval (June 26-28) these animals spawned, the eggs being attached to blades of grass and slender twigs. The eggs hatched on the 2nd of July...." According to Sherwood (1898) the hatching period is "about a week". Overton (1915) recorded, on August 4 and 5, 1915, "a great congress-- enormous numbers of eggs of the spadefoot and Fowler's toads were readily identified in the pools. On the 7th, the eggs were hatched." In Florida, Wright (1932) noted more rapid development. "On August 18, 1928, we have the field note that two lots 'are hatched early morning of August 18. In fact, some in pond were almost hatched August 17 at noon or 1¼ days after laying. Certainly 1½-2 days after egg deposition these have hatched. Water must have been 75° or 80° when rain came and more when the sun came out.'" Driver (1934) found that the eggs hatched in two days at a water temperature between 50° and 60° F. Tadpoles kept in tanks and fed on earthworms and scraps of meat developed hind legs at the thirty-fifth to the thirty-eighth day, and the front legs emerged and the young toads left the tanks at forty-eight to sixty-three days. Tadpoles in natural ponds died when the water dried up on the

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thirty-first day. He believes the "reputed" rapidity of development may occur only with high mid-summer temperatures.

For S. couchii Baird, King (1932) has recorded that near Tucson, Arizona, hatching takes place in one or two days: "The little tadpoles grow rapidly and are sufficiently developed in a week to make their way into the mud at the bottom of the puddle and all have vanished by two weeks." Stecker (1908), for the same species at Waco, Texas, noted that the eggs hatch in eight to ten days, the limbs begin to appear on the twentieth to the twenty-third day, and on the twenty-seventh to the thirtieth day the young toads leave the water with their tails still in evidence. Strecker also reported that at Santa Maria hatching takes place at not more than five days, and that at seventeen days the tadpoles have attained a total length of eighteen and one-half to forty-three millimeters.

As concerns S. hammondii, Storer (1925) has written the following notes: "The embryonic developmental period is found to be short. The larval period is probably also short if we may judge by analogy from the known fact in the case of the other two widely distributed species of Scaphiopus in the United States. The newly transformed young spadefoot has at once the burrowing reflex of the species, which it must, in the case of prairie ponds lacking a border of aquatic vegetation as temporary shelter, put to immediate use to protect itself from desiccation." For S. hammondii in New Mexico, Little and Keller (1937) reported that thirty-six to forty-eight hours elapse between laying and the tadpole stage.

The most extensive account of the development of any spadefoot

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is the following one given by Gilmore (1924) for S. hammondii bombifrons Cope:

> The incubation period as observed in the field seems to be less than 48 hours. This is probably due to the very warm condition of the water. In 1924, long continued cloudy weather retarded the hatching.
>
> The newly hatched tadpole is a trifle less than one-fourth of an inch in length. Within less than five days it has doubled in size. Within another five days it has attained a length of one inch. The legs then begin to develop. Fifteen days later it has reached its maximum size. Two and one-half inches is the maximum length of the majority of adults in any tadpole community. A small minority attains three to three and three-quarters inches in length. At about the thirtieth day after egg-laying, arms begin to appear, and the process of transformation begins to be evident in all parts of the animal. The complete absorption of the tail and the completion of remodeling of all structures into adult form is accomplished by the fortieth day.
>
> In 1921, specimens were found to be completely transformed after thirty-six to forty days. In 1923, the shortest observed period was thirty-nine days....
>
> At no time during the season of growth are all tadpoles of even approximately the same size. From ten to twenty per cent of specimens known to have hatched at the same time are considerably larger than the average. Some may be two to three times the size of the average. These larger forms are frequently found with smaller ones half eaten.

Food of Tadpoles:

Gilmore (1924) recorded the following observations on the food of S. hammondii bombifrons.

> The spadefoot chooses temporary ponds in which to rear its young. In no cases have eggs or tadpoles been observed in permanent lakes, although such lakes are available. The ponds are roadside mud-holes and low areas in fields ranging from a few inches to a few feet in depth. The water is muddy and warm. The vegetation consists of such microscopic plants as have passed the winter in a resting condition in the dried mud at the bottom. A few plants of Marsilea, some of the coarser grasses, sedges and rushes may be present. To the casual observer the ponds seem unusually barren. The animal life is rich and varied.

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Protozoa are present in abundance. Rotifera are common. Various of the smaller worms are abundant. The larvae of aquatic beetles, bugs and diptera are present in small numbers. The dominant fauna consists of crustaceans. Of the phyllopods several species of shrimp are numerous (Streptocephalus, Thamnocephalus, Apus, Estheria). Of the Cladocera, Daphnia and other genera are represented. These attain to unusual size and abundance. Of the Copepods, Cyclops, Diaptomus, and other genera are abundant.

In this environment, poor in larger plants, rich in animal life, the spadefoot tadpole develops. This may account for the development of the carnivorous habit.

The structures about the mouth of the spade-foot tadpole are admirably adapted for a diet of living animals. The horny jaws are constructed for seizing and holding prey. They are capable of being opened to accommodate large prey. On the roof of the mouth is a median horny recurved tooth. This is not found in herbivorous tadpoles. The lips....are flat and thin and probably assist in the capture and holding of prey. Food is not swallowed whole as in the adult toad, but is held in the jaws and sucked or torn to bits.

The feeding apparatus is operated by an unusual development of mouth muscles, an adaptation probably associated with a carnivorous diet.

Gilmore also stated:

The tadpoles of most frogs and toads are herbivorous and therefore have very long intestines. As they transform to the adult condition they take only animal food, and the long intestine is replaced by a short one. In the spadefoot the change from a long to a short intestine seems to take place before the beginning of transformation. In fact, some specimens seem never to have had a long intestine. It seems probable that the spadefoot tadpole is departing from the traditions of its ancestors and relatives and adjusting itself to a new type of diet. This adjustment is approaching perfection in the jaws, lips, roof of the mouth, and jaw muscles. The long intestine character has not been eliminated, but is in process of elimination. It seems to persist during early tadpole life and is later supplanted by a short intestine. The short intestine character will be subject to a wide range of variation until it has firmly established itself on the race.

Cope (1889) recorded larvae (Spea hammondii bombifrons) from a lake in Idaho eating grasshoppers, and noticed several specimens with

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the entire insects in their mouths. "In some instances the grasshoppers' bodies were too large and projected from their mouths. These precocious larvae were evidently air-breathers, and hopped about presenting a curious appearance as they dragged their large tails after them."

Little and Keller (1937) reported that tadpoles of S. hammondii kept in the laboratory ate algae, lettuce and other vegetable matter, insects, and small crustaceans. "Cannibalistic tendencies were observed in the laboratory and at Frog Pond. Tadpoles attached themselves to other individuals which they slowly absorbed through their small mouths until only outer skins were left."

Linsdale (1938) has recorded several observations on feeding habits of the same form. At one time he saw many dead tadpoles scattered on the bottom of a pool, and live ones feeding on them. One tadpole was seen feeding at the surface film, supporting its body vertically with rapidly moving tail and working the mouth at the surface. Wood (1935) in Uintah County, Utah, found in a small pond used for watering cattle numerous large spadefoot larvae (S. hammondii), with hind limbs just developed, which "seemed to be feeding upon vegetable matter either contained in the cattle dung or growing upon it".

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Observations Made in the Vicinity of Norman

In the vicinity of Norman, field observations on the life history of S. bombifrons have been made in the spring seasons of 1934 to 1938, inclusive. In the first three years field studies were made jointly by the writer and Mr.A. H. Trowbridge (see Trowbridge and Trowbridge, 1937). In the spring of 1937 I was not present at the time of emergence of the spadefoots; Mr. Trowbridge and Dr. A. N. Bragg have allowed me to quote from their field notes for this year. The writer is responsible, except where it is otherwise indicated, for observations made in 1938.

Breeding Season:

The following quotation and table (Table I) are taken from the above-mentioned publication (Trowbridge and Trowbridge, 1937).

> Following a heavy rain on April 4, 1934, two specimens were taken from a deep ditch west of Norman, but none were found elsewhere. On the night of May 3, 1934, the spadefoots were out in large numbers. In 1935 the spadefoots did not appear until April 28, when they were abundant in the ditches and in the large pool south of the campus. They appeared on the night of May 8, 1936. In this area at least, and for as long as we have studied it, the spadefoots have had a breeding range not to exceed two weeks. It probably varies in other regions of the state, according to the spring rainy season.
>
> As in Colorado, the time of breeding appears to be directly correlated with the season, and also with the amount of rainfall. Gilmore does not give rainfall data for the years in which his observations were made, but we have compiled these data for this area from the official weather station situated in Norman. They are summarized in Table I.

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TABLE I

THE DAILY AMOUNT OF RAINFALL FOR THE MONTHS IMMEDIATELY PRECEDING THE FIRST BREEDING CONGRESSES FOR A THREE-YEAR PERIOD. THE RAINFALL RECORD FOR EACH YEAR IS TABULATED ONLY UP TO THE DATE OF THE BREEDING CONGRESS OF THAT YEAR

Month	1934	1935	1936
April 4	1.55 inches		No rains
April 15	.23 "		in April
April 17	.35 "		
April 18	.02 "	1.05 inches	
April 19	.00 "	1.55 "	
April 25	.60 "	.20 "	
April 27	.19 "	.00 "	
April 28	.00 "	1.20 "	
May 2	.00 "		1.07 inches
May 3	2.00 "		.00 "
May 8			2.59 "
Total rainfall	4.94 inches	4.00 inches	3.66 inches

An inspection of Table I reveals a number of interesting facts. First, Scaphiopus bombifrons did not breed following the first heavy spring rains of any of the three years. Rather breeding was delayed until at least a total precipitation of 3.66 inches for the preceding five or six weeks had fallen. Second, in all cases a heavy rain was required to bring the toads out in numbers. Although the data are not included in the table, rains averaging from .75 to 1.5 inches fell after the breeding dates, some of them occurring as late as the latter part of May for all three years. Spadefoots have never appeared after the first breeding congress, except in 1936, when a gravid female was picked up in a pool formed in the Norman streets following a .23 inch rain, May 23. Neither have they appeared after heavy rains in June or subsequent months.

It seems to be certain that S. bombifrons in Oklahoma has a well-marked breeding season, and that this season is definitely correlated with climatic factors and time of year.... The evidence indicates a hereditary breeding pattern, more or less influenced by climatic conditions at the time the eggs are ready

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for laying.

In 1937 and 1938 distribution of rainfall resulted in a late congress in the former and an early one in the latter year. The data used in Table II were obtained at the University weather station in Norman.

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TABLE II

Daily Amount of Rainfall in Inches During the Spring Months of 1937 and 1938 *

Day	May '37	June '37	March '38	April '38	May '38
1	.01				
2		.35			
3	.02	.48			.82
4			.005	C	
6					1.17
7	.32	.36		.48	
8		.15	.110		
9		.10			
10	.57				
12	.15				
14				.44	
15		.46			.86
16		.20			
17		.62 C			
18					.84
19				.68	C
20				.04	
21	.15		.140	.98 C	2.61
22			.020	.37 C	1.10
25			.640		
26			1.820		
27		.56	.820		
28		.20	1.580 C		
29	.96		C		
30	.01				
TOTAL	2.19	3.48	5.135	2.99	7.40

*The total precipitation recorded for each day is that amount falling between 7:30 A.M. of the day in question and 7:30 A.M. of the following day.

C indicates the occurrence of a breeding congress.

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During March and the first part of April, 1937, but little rain fell. In the latter part of April rains of .50 and .94 inches were recorded on April 21 and 22, respectively. During May, precipitation totaled 2.19 inches, the greatest amount in any 24-hour period being .96 inches on May 29. During June, there were no heavy rains, and during July (not included in Table II) the total precipitation was only .97 inches. In this year only one congress was held. On the night of June 17 one was present in a muddy temporary pool in a corn field one mile directly south of the campus. Dr. Bragg and Mr. Trowbridge collected seven pairs of toads from this congress. On the same night, according to Dr. Bragg, eggs were laid in buffalo wallows in a pasture at the end of Jenkins Street. It is doubtful whether in this year any tadpoles lived to complete metamorphosis. In the buffalo wallows just mentioned, Dr. Bragg, while watching the development of tadpoles of Bufo cognatus, made observations on tadpoles of Scaphiopus which were also present. Clutches of B. cognatus eggs were seen in both pools June 18, and tadpoles June 21. On June 22 none remained in one pool, and only one in the other. The next day none were found. Since Scaphiopus tadpoles were present in both pools, Dr. Bragg concluded the Bufo tadpoles had been eaten by them, and possibly also preyed upon by Dytiscid beetles present as the pools dried to a low level. He followed the progressive drying of the pools until on June 27 both were dry and the mud was cracked, and there were no signs of tadpoles of either species. A heavy shower fell on the morning of June 28, and in the afternoon both pools contained considerable water, but there was no evidence of tadpoles. Dr. Bragg has written in his field notes,

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"Scaphiopus apparently could not withstand the drying of the pools. Perhaps they were not far enough advanced to do so as Moore's were at Stillwater." From June 19 to June 25 his readings of water temperature in the pools, taken in the afternoon, vary between 30° C. and 37° C.

In 1938, the unusually large amount of rain which fell during March resulted in the occurrence of a breeding congress earlier than any other recorded for this region. After rains totaling a little more than five inches for the six preceding days, a congress was held the nights of March 28 and 29. No spadefoots were heard during the afternoon of March 28, but at 9:00 o'clock males were heard calling in the pond one-half mile south of the campus (the same pond from which toads were taken in 1934 and 1935), and in bodies of water on pasture land and in a wheat field between this pond and the end of Jenkins Street. One pair was taken from the former pond at 9:15 P.M. Between this time and midnight males were heard calling from various buffalo wallows and roadside ditches; they seemed to be quite wide-spread in their distribution. At 1:00 A.M. two pairs were taken from the pool directly south of the campus. No other pairs were seen at this time, and the males seemed fewer in number than earlier in the night. When the pool was visited again between 3:00 and 4:00 A.M. there were markedly fewer males, and no females or pairs were seen. The number of Bufo cognatus males in the pool was also smaller than earlier in the night. At 1:00 A.M. March 29 the air temperature at the pond was 13.75° C.

During the afternoon of March 29 spadefoots were calling in various roadside ditches and shallow pools between Norman and Purcell. Between midnight andll:30 A.M. four pairs, one female, and a number of

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males were taken from the pool one-half mile south of the campus. All pairs were taken in shallow water near the margins of the pool, rather than in the deeper portions as in previous years. Throughout the pool the water was more shallow than on the preceding night. At 12:15 A.M. the air temperature was 19° C., and the water temperature near the middle of the pond was 17° C. The next night(at 11:30 p.m., March 30), according to Dr. Bragg and Robert Taylor, no spadefoots were calling in this pond.

Five pairs of toads were isolated in culture dishes in the laboratory and all produced eggs, beginning at 2:30 A.M. and continuing until early afternoon of the same day. Cleavage was slightly more irregular than in previous years, and many eggs did not develop at all. As in other years, almost none of the eggs laid during the late forenoon and early afternoon developed.

During the afternoon of April 4 the pool from which specimens had been taken was again visited. It was found to be completely dried up except for accumulations of water about two inches deep in hoof prints in the mud. No tadpoles were present in these depressions, nor were there any traces of them on the pond bed. No spadefoot tadpoles were obtained by seining in pools in the wheatfield and in the roadside ditches near Jenkins Street. Consequently, it is uncertain whether the early congress of March 28-29 was an entirely successful one. In all other years, congresses have lasted only one night. Moreover, the fact that on each of the two nights the numbers of toads became smaller as the night progressed seems an indication that at least during the first night some of the couples did not remain until egg laying had been accomplished. No ex-

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planation can be attempted for the fact that only a minority of the eggs laid in the laboratory developed. One would hardly expect breeding congresses to occur at a time when eggs and sperm were not ripe. On the other hand, on April 3 Dr. Bragg found in a pool one mile north of Norman spadefoot embryos ready to hatch; it seems most likely the eggs from which these developed were laid during the congress of March 28-29, and that their early development was retarded by low temperatures prevailing immediately after this congress. (At noon April 1 the temperature of water in an unheated room was 11°C. and the air temperature 9°C. At 1:30 P.M. the air temperature outside was 6° C.)

Occurrence of several other congresses in 1938 is a further indication that egg-laying was not general during that of March 28-29. On April 4, Dr. Bragg reported hearing spadefoots calling in the pool between railroad and interurban tracks. Calls were heard south of the campus the nights of April 21 and 22. (according to John Harms and Robert Taylor), and again on the night of May 19, at which time Dr. Bragg reported finding spadefoots present in small numbers at several other places. Probably the situation in this year was somewhat similar to that successional breeding described by Goldsmith (1926) as occurring in the dry hills to the east of Colorado Springs. That the first congress described (March 28-29) did not complete the breeding activity for the season cannot be attributed to insufficiency of rain, but possibly to its unusually early date or more probably to the low temperatures prevailing.

Breeding Places and Breeding Habits:

Congresses have been found to take place only in temporary bodies

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of water. The largest congresses observed have been those in a pond which forms after heavy rains in a cultivated field one-half mile south of the campus. In this pond the water is always muddy, and its depth, depending on the amount of rainfall, has varied between one and two feet. Congresses were held here in 1934, 1935 and again in 1938. Muddy roadside ditches and various pools in cultivated fields have been found to be used as breeding places, as have also buffalo wallows and temporary pools in pasture lands in which the water is clear. In 1938, a few tadpoles were taken from a pool 52 feet wide and 172 feet long (measurements obtained by Dr. Bragg);the water was clear, and its greatest depth about two feet. Tadpoles of Bufo cognatus were present by the thousands in the same pool.

Daylight appearance of spadefoots during the breeding season has been recorded on two occasions. "On the afternoon of May 4, 1934, following a two-inch rain, spadefoots were heard calling about four o'clock in the afternoon. By midnight they were present by the hundreds in the pool south of the campus and mating was well under way." (Trowbridge and Trowbridge, 1937). Again, during the afternoon of March 29, 1938, after a congress the preceding night, they were calling in ditches and pools in the vicinity of Norman and between Norman and Purcell. It seems likely that, as Goldsmith (1926) and others have pointed out, the first arrivals at a pool attract by their voices others from some distance away. Males are responsive to or excited by the calls of other males; this is shown by the fact that captured males as a rule remain quiet when they are at some distance from a congress, but begin to call vigorously when carried closer.

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Males have been found to arrive at breeding pools earlier than females and to remain after all the latter have disappeared. Congresses have, as a rule, lasted only one night, and clasping pairs have been taken on a second night on only one occasion (March 29, 1938).

The following quotation (Trowbridge and Trowbridge, 1937) sums up observations on mating, egg laying, etc.

> In the Norman area mating apparently does not begin for several hours after the males arrive at the pools. The males lie outstretched and give their call and, we believe, wait for the females to come to them.
>
> Before mating, individual toads are easily captured, but after clasping has taken place the toads become more wary. The greater part of a single swimming individual is easily discernible, but only the heads and eyes of mated pairs show when a light is flashed upon them. These pairs are quick to take alarm and may submerge almost instantly, but reappear on the surface within a minute or two at no great distance from where they disappeared. Amplexation is inguinal, and the eggs must normally be laid while the toads are swimming. We have found egg masses attached to submerged sticks, clumps of grass, etc., but never on the bottom of pools.

Egg Masses, Rate of Development, etc.:

Only a few observations have been made on egg masses in the field. At 5:00 P. M. on May 10, 1936, Scaphiopus embryos were found in a buffalo wallow in the high prairie in Johnson's pasture (about ten miles southwest of Norman). The water was clear, not over four to six inches in depth, and its temperature was 28° C. These embryos were at the point of hatching (when brought into the laboratory three hours later all had hatched) and were attached in clusters of four to twelve to submerged vegetation. The embryos (eggs had almost certainly been laid during the congress of May 8-9) were slightly more advanced in state of development than those in the laboratory. Dr. Bragg found on April 3, 1938,

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some embryos in the same stage, also in small clutches; six were counted in one mass, and none contained more than twenty. These also were attached to grass stems and other plants about one to two inches below the surface of the water. The embryos found in 1936 indicate that early development is at least as rapid, and probably more rapid, in the field than in the laboratory. Readings of water temperature taken at various times show that the afternoon temperatures, save during such times as the cold weather following the first congress of 1938, are higher in the pools than those at which material was kept in the laboratory; at night one would expect them to be somewhat lower.

During the spring of 1938 I made a number of visits with Dr. Bragg to two pools in which he had been watching the development of tadpoles of Bufo cognatus, and in which he had noticed also spadefoot tadpoles. One of these pools is a buffalo wallow 20½ by 23 feet with water six to seven inches deep, located in a pasture just north of the Norman cemetery. The water is clear, and the pool at the time of our visit contained large floating masses of filamentous green algae. The pool on April 25 contained thousands of Bufo tadpoles, and some large spadefoot tadpoles. Many of the latter were congregated under a mass of floating algae, maintaining themselves in a vertical position as they fed. Dr. Bragg, without making any effort of catch all in the group, scooped up thirty-one of these at one dip with a net. They were taken into the laboratory and metamorphosed there at a slightly earlier date than did those remaining in the pool. By May 4 eight of those in the laboratory had both front legs free and the tails partially resorbed; by May 9 all except one, which appeared abnormal, had transformed. On April 30 there

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were no more floating masses of algae in the buffalo wallow; the temperature two feet from the margin was 30°C. On May 7 Dr. Bragg noticed in the wallow some tadpoles in which both front legs had emerged. On May 12 three specimens were taken there in which metamorphosis was completed save for the retention of a tail stub 3 to 5 mm. in length. The young toads were sitting at the margin of the pool, with their heads out of the water. At the same time specimens were taken which showed no resorption of tails and in which the arms had not emerged. On May 16 completely transformed young toads were out on land near the margins of the pool, and several tadpoles remained untransformed in the pool. So far as is known egg laying occurred in this pool during the congress of March 28-29. The rate of development was probably retarded by the low temperatures which followed this congress. It is believed that eggs were laid in the pool at only one time; if this is true, these specimens, like those observed by Gilmore (1924), show the same differences in developmental rate as do those reared in the laboratory.

Gudernatsch (1914) found by feeding mammalian glands to four species of amphibian larvae that, next to the thyroid group, liver-fed tadpoles showed the most rapid progress in differentiation. Possibly liver fed to tadpoles brought into the laboratory caused them to metamosphose a few days earlier than did those of the same group left in the pool.

Food Habits of Tadpoles:

Tadpoles reared in the laboratory have been observed to take both animal and vegetable food as soon as the mouth parts are well

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enough developed to permit feeding. Cannibalistic tendencies have been observed as early as thirty-three hours after hatching, and at the same age both algae and liver are taken eagerly. From this time up to the onset of metamorphosis tadpoles consume, in comparison with their size, an enormous amount of algae. In addition, raw beef-liver, lean beef, hard-boiled egg yolk, lettuce, and fragmented insects have been taken. Algae and liver seem to be the preferred foods.

It has been found that in their natural habitat tadpoles of the same species also eat quantities of plant food. As has been noted, large tadpoles in the buffalo wallow near the Norman cemetery were on April 25 observed feeding on algae. These tadpoles were somewhat larger and heavier than those kept in the laboratory, the belly especially being much larger, rounder, and softer. They were found when dissected to have the stomach and gut crammed with algae; particles of soil and sand were also in evidence, and in some cases a few Copepods were present. Since Bufo tadpoles were living in the same pool, one would expect that they also were eaten; if so, their remains were not recognizable. In all cases the intestine was very long and much coiled. Tadpoles of the same species and probably of the same age were taken, also on April 25, from a large clear pond one mile north of Norman, between the railroad and the interurban tracks. This pond contained but little algae, and the tadpoles taken from it were slightly darker in color than those from the buffalo wallow, while their bellies were firmer and less rounded and the jaw muscles much better developed. Tadpoles from both these habitats had larger and softer bodies, better developed jaw muscles, and lips a little more heavily cornified than had those reared in the laboratory.

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A group of tadpoles collected near Colorado Springs and sent to the writer by R. J. Gilmore differed from laboratory-reared specimens in the same respects.

Tadpoles developing in the type of pond described by Gilmore (1924) must necessarily live almost entirely on animal food. The same is doubtless true of tadpoles which in the region around Norman develop in muddy pools and roadside ditches. One is led to wonder to what extent, under these conditions, their food is made up of tadpoles of their own as well as of other species. Tadpoles in the laboratory have been found to eat dead tadpoles of their own species, and, in at least one case, at an early age living tadpoles were attacked and their tails eaten off. In 1934, "we placed about 200 spadefoot tadpoles in a tank which contained approximately 1,000 Bufo cognatus tadpoles. Before many days none of the smaller Bufo tadpoles remained." It seems certain the latter were attacked and killed by the spadefoot tadpoles.

A large tadpole of Scaphiopus bombifrons which was taken from a pool and brought into the laboratory on May 12, 1938, provided us our first view of a spadefoot tadpole attacking and killing another tadpole. It seized a Bufo tadpole about ten millimeters in length near the middle of the body and held on for a minute or two, working its jaws and apparently making an effort to eat the victim. The dead and broken tadpole was then abandoned, and was shortly taken up by two other spadefoots, one seizing its head and the other its tail. As they neared the middle of the body each jerked at the dead tadpole until it was finally broken apart, after which each spadefoot finished eating the portion held in its mouth.

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Tadpoles taken from pools have been found to have the stomach and gut crammed with long filaments of algae. In the laboratory, tadpoles seize upon masses of algal filaments and swallow them as fast as they are able, without making any effort to break up the mass. Pieces of liver or fragments of soft-bodied insects which are sufficiently small are taken into the mouth entire, although they are chewed before being swallowed. When the yolk of a hard-boiled egg is crumbled into the dish and settles to the bottom, tadpoles often move about with head down and mouth close to the bottom of the dish as they feed. When large pieces of liver are put into the culture dish or tank groups of tadpoles soon collect around them, each individual nibbling and jerking in an apparent effort to remove small pieces. Occassionally a tadpole may seize and swim away with a piece of liver larger than its own body.

It seems that the tadpoles, while predominantly carnivorous, are not too strictly limited in their habitat and food requirements, and that their body shape and size as well as their degree of development of jaw muscles and cornified mouth parts depends to a certain extent on the character of food available.

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OBSERVATIONS

Uncleaved Egg

The eggs of Scaphiopus bombifrons when laid in the laboratory cohere in masses usually consisting of ten to fifty but occasionally of as many as eighty to one hundred eggs. Before the jelly layers swell the individual eggs of a mass are easily separated; later, they are held together quite firmly through cohesion of the gelatinous envelopes to one another. Occasionally the envelope of an egg may be united to that of an adjoining one by a short connecting cord which is quite elastic and which shows no spiral structure. In most cases numerous long fine strands of gelatinous material adhere to the envelopes and apparently aid in holding the eggs of a mass together.

The egg complement, as computed from the number of eggs laid by individual females and from dissection of females taken at the beginning of breeding congresses, is roughly from 400 to 700. Strecker (1908) has stated that the complement for Scaphiopus couchii at Waco, Texas, is from 342 to 528, and Storer (1926) has judged that for Scaphiopus hammondii in California it is about the same.

In connection with the description and naming of egg membranes, it should be pointed out that some confusion exists as to the use of the terms "vitelline membrane" and chorion. This difficulty was

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pointed out by van Bambeke as long ago as 1880. He noticed that certain of the enveloping layers had not been mentioned at all by some authors, and that in different works the same name had served for totally different structures. Smith (1926) has remarked:

> In the frog the thin membrane closely investing the egg, but separable from it, is usually called the vitelline membrane--a term which by some is limited to primary egg membranes, while others apply it to any thin membrane closely surrounding the egg.

The "vitelline membrane" of Triton and of the Axolotl, which corresponds to the membrane of the egg-cell (Eizellemembran of Remak) in the frog, van Bambeke found to be involved in cleavage, and the chorion, the transparent homogeneous layer next outside it, not so involved. Smith (1912) found in the female Cryptobranchus of 30 to 35 cm. body length the rapid development of two non-cellular membranes closely investing the egg within the follicle. The inner of these, the zona radiata, becomes at the time of maturation a simple cell wall to the egg, while the outer one, the zona pellucida, persists as the 'vitelline membrane' (quotation marks Smith's). He found the zona radiata arising from the peripheral cytoplasm of the ovocyte, and considers it a primary egg membrane; the zona pellucida is formed, he concluded, as a product of cellular activity of the follicle, and is therefore a secondary egg membrane. In a more recent paper (Smith, 1926) he has termed the latter a chorion. The chorion in Cryptobranchus takes no part in cleavage, but remains as a spherical sac enclosing the embryo until in post-gastrular stages it is ruptured and cast off. The inner membrane, on the other hand, is found to be involved in the process of cleavage.

Since in Scaphiopus there has been no study of oogenesis or forma-

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tion of membranes, no statement can be made as to the manner of formation of the investments of the egg. However, study of membrane formation in various anuran eggs has shown that the process is usually much as that described by Smith for Cryptobranchus. Moreover, authors for the most part agree that the thin primary inner membrane secreted by the egg itself should be called the vitelline membrane, and the term chorion applied to the thin but tough secondary membrane secreted by the follicle and separated after fertilization from the surface of the egg by the perivitelline space. Since in Scaphiopus the thin inner membrane serves as the cell-wall of the vitellus or egg proper, and since it is involved in cleavage and may by analogy be presumed to be a primary membrane, it will be referred to as the vitelline membrane. The term chorion will be applied to that thicker membrane which surrounds the egg and which persists as a sac (Figs. 154 to 157) enclosing the embryo up to the time of hatching.

The vitelline membrane is, of course, discernible as such only in sections. After swelling of the jelly envelopes has occurred, the chorion appears as a spherical sac surrounding the egg and separated from it by a short distance. Outside the chorion and closely adherent to it is a rather dense and highly refractive jelly layer averaging.05 mm. in thickness. The second and outermost jelly layer is much softer, quite clear and transparent, and averages .4 mm. in thickness; its outer surface takes the form of a very thin and slightly opaque film. Both jelly layers have attained their full thickness by the time the first cleavage furrows appear.

Eggs of Scaphiopus bombifrons have an average diameter of 1.5 mm., with no appreciable difference between vertical and horizontal

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diameters. Size variations are considerable even among eggs produced by the same female; in one such lot of eggs, diameters were found to vary from 1.01 mm. to 1.61 mm. Eggs 1.01 mm. and 1.02 mm. in diameter, although rather rare, have been observed to continue development and to form perfect but small tadpoles. In this connection, Chambers (1908) has found that in Rana temporaria and R. esculenta the size of cells of a tadpole or young frog is in direct relation to the size of the individuals and that therefore the size of the young animal is determined by the initial size of the egg.

Pigmentation is light in the egg of Scaphiopus bombifrons, the animal hemisphere being light brown in color and the vegetal creamy-white. The gray crescent can in most cases be distinguished, although not as easily as in later stages, when the various cleavage furrows furnish landmarks of use in determining its position. Since there is no black pigment, the crescent does not appear gray, but is marked rather by a lighter brown pigmentation and a higher extension of the non-pigmented region than that characterizing the opposite portion of the egg. The fosette germinative of van Bambeke or fovea germinative of Max Schultze is plainly evident in most uncleaved eggs; it marks the position which is or has been occupied by the nucleus. Pits marking the point of entrance or attempted entrance of spermatozoa are present at various points in the animal hemisphere, and have been observed at times to persist throughout the second cleavage.

The outer gelatinous layer of eggs, whether laid in the laboratory or collected from submerged vegetation in pools, is usually coated with particles of sand. It seemed likely that this coating

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had been acquired in the cloaca of the female, and dissection of a number of females taken from breeding congresses has supported the truth of this assumption; in most cases the cloaca has been found to contain a large quantity of sand. Coatings on the envelopes are sometimes so heavy as to interfere with observation of the developing embryos, and in any case necessitate removal of at least the outer layer of jelly before embedding and sectioning can be undertaken.

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Stage I

First Cleavage

The first cleavage furrow appears from forty to fifty (occasionally only thirty) minutes after deposition of the egg. At its first appearance the groove is shallow and smooth. As it deepens, small wrinkles appear on each side of it and soon coalesce to form deep and well-defined Faltenkranzen similar to those described by Wilson (1896) as occuring in Chorophilus (= Pseudacris) triseriatus. In the animal hemisphere the groove progresses rapidly and, when completed superficially, leaves the blastomeres quite widely separated. There is never any tendency toward fusion and disappearance, and subsequent reappearance, of the furrow after each cleavage, as has been described in some Salientia (Wilson, 1896). On the other hand, first as well as later furrows in the animal hemisphere remain quite widely open for a short time after completion of the groove with the blastomeres rounded and diverging strongly from the groove. The groove then gradually narrows and the blastomeres become more closely appressed, although still somewhat rounded and fairly well separated. At no time in development are the furrows ever fused or indistinguishable; rather (Figs. 146, 147, and 151), they remain remarkably distinct. The greater depth and openness characteristic of newly formed furrows is not long retained.

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First and second furrows can be distinguished by their appearance for only a short time after the second is completed, and by the time the third set of furrows is well established the first two are as a rule indistinguishable. Similarly, by the time either the fourth or the fifth furrows are completed the preceding sets are alike in appearance. With decreasing size of the cells, the distinction in appearance between recent and older furrows becomes less marked.

Progress of the first furrow is slower in the vegetal than in the animal hemisphere; the advancing furrow, save for its wider ends, is narrow, and the blastomeres separated by it lie rather closely appressed. It is completed externally in from nine to fourteen minutes.

The studies of Morgan and Tsuda (1894), Morgan and Boring (1903), Brachet (1906) and Jenkinson (1906) show that, in the Anura studied by them, in the majority of cases the first cleavage plane coincides with the median plane of the gray crescent, in some cases it may lie at right angles to the plane of symmetry of the crescent, and in fewer cases there are varying degrees of departure from coincidence. In Scaphiopus, study of later cleavage stages, particularly the second and the fourth, supports this general conclusion. When the first furrow passes through or near the middle of the gray crescent, the egg is usually divided into two blastomeres of equal or nearly equal size; when the plane of the furrow is at right angles to the plane of symmetry of the crescent, the two blastomeres are as a rule unequal in size, the dorsal one being the smaller. Of 97 embryos measured with this point in mind, 72 were divided into blastomeres of approximately equal size, and 25 into blastomeres of definitely unequal size.

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STAGE 2

Second Cleavage

Second cleavage furrows appear from ten to fifteen minutes after the beginning of the first furrow. At this time the first furrow has usually been completed externally. The second furrow is, like the first, vertical, advances rapidly in the animal and more slowly in the vegetal hemisphere, and is marked in the former by the occurrence of Faltenkranzen, which are not, however, quite as wide and deep as those accompanying formation of the first furrow. As is the case in most Anura, second furrows appear first at a point or points along the first furrow, and progress downward. Second furrows departing from a single point on the first furrow usually form at both their upper and lower ends a straight line at right angles to the first furrow (Fig. 1). When in the two blastomeres they depart from the upper and reach the lower pole at different points along the first plane of division, a cross-line or polar furrow is formed at each pole. Almost invariably, when a cross-line occurs at the upper pole, one is formed also at the lower pole, the two lying at right angles to each other. Figures 2 and 6 illustrate the type of cleavage which leads to the establishment of a polar furrow. The latter consists of that portion of the first furrow which lies between the points of departure of the second furrows;

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it is at first straight, but later (Figs. 7 and 8) becomes oblique through shifting of blastomeres. Living material has presented no evidence that the polar furrow, as Morgan (1897) stated, "may be brought about even subsequent to division by a shifting or readjustment of the blastomeres on one another." Of 113 embryos measured in the four-cell stage, in thirty-one cases the second furrows form a single straight line at right angles to the first. In 82, a segmental furrow is present, varying in length from .029 mm. (the shortest line considered to constitute a segmental furrow) to .29 mm., with an average of .12 mm.

Figures 10 to 14 inclusive illustrate examples of those aberrant cleavages which signify abnormality and approaching death of embryos. In those shown in Figures 10 to 13 inclusive, there is extreme inequality in size of the four blastomeres. Figure 14 represents an embryo in which the first furrow is vertical and the second horizontal and lying at the approximate boundary between pigmented and non-pigmented areas.

Variation in size is shown by two embryos sketched in Figures 15 and 16; their respective diameters are 1.01 mm. and 1.61 mm.

Study of more than 200 embryos, fixed at a time when the second furrow was either incomplete or very recently completed, has shown that there exist between the plane of the first furrow and the plane of symmetry of the crescent, and between position of the gray crescent and size of blastomeres, the same relationships that have been described as occurring in a number of other Anura. The plane of the first furrow usually (Figs. 1 to 3, 7, 8, etc.) coincides with the plane of symmetry of the crescent; in some cases (Figs. 4 and 5) it lies at right angles

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to the plane of the latter, and in a few cases (Figs. 17, 83, 95 and 105) there are varying degrees of departure from coincidence of either the first or the second furrow with the plane of the crescent. When the first furrow bisects the gray crescent and divides the embryo into two blastomeres of equal size, the second furrow as a rule (Figs. 3,20, and 22) separates two smaller dorsal from two larger ventral blastomeres. A first furrow the plane of which lies at right angles to the median plane of the crescent tends to separate the embryo into a smaller dorsal and a larger ventral blastomere (Fig. 19). When the plane of neither the first nor the second furrow coincides with the median plane of the crescent (Fig. 17) that blastomere which lies nearest the middle of the crescent tends to be the smallest, while the one opposite it is largest, and the other two are intermediate in size. Figures 17 to 22 inclusive illustrate embryos of the more common types.

In those embryos in which either the first or the second furrow separates two smaller dorsal from two larger ventral blastomeres, it is difficult to determine whether the furrow in question lies to the dorsal side of the upper pole, or to the lower pole, or of both. Except when polar furrows are present, the first and the second furrow, as seen in surface view, each usually forms a circle, since both their upper and their lower ends meet as straight lines. Second furrows sometimes curve toward the dorsal part of the embryo from their point or points of origin at the first furrow (Figs 19 and 20). Occasionally (Figs.18, 21 and 22) the first two furrows intersect in such a way as to form acute and obtuse angles at both upper and lower poles. Newport (1851) observed the eccentric origin (at a greater or less distance from the

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pole) of the second furrows, and concluded that they always form segments larger on one side than on the other. In Ambystoma, Eycleshymer (1895) found the second set arising at the pole or at a greater or less distance from it, and also described acute and obtuse angles formed by the first and second furrows in such a way as to give rise to an X.

It is particularly interesting to note that in Scaphiopus bombifrons there can be distinguished as early as the beginning of the second cleavage a dorsal eccentric area of accelerated cell division indicative of the bilaterality of the embryo. When the first furrow has passed through or near the middle of the gray crescent, the second furrows form in both blastomeres at the same time (Fig. 3). When, however, the plane of the first furrow lies at right angles to the plane of the crescent, the second furrow forms earlier in the dorsal than in the ventral blastomere. The number of embryos which illustrate this latter point is rather small, for the embryo must be one in which (1) the first furrow lies transverse to the plane of the gray crescent or varies only a few degrees from this position, (2) the position of the crescent can be established with certainty, and (3) observation is made or fixation accomplished at the time when the second furrow is just appearing. Out of a large number of embryos only seven were found which met all these conditions. In one of these, the beginning second furrows are equally well advanced in the two blastomeres. In the remaining six, the furrow in the dorsal blastomere is more advanced than that in the ventral blastomere. In four cases (Figs. 4 and 5) the second furrow, while present and well defined over a distance of about 30° in the dorsal blastomere, has not appeared at all in the

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ventral blastomere. In one case, early second furrows are present in both blastomeres, but that in the dorsal cell is deeper and better defined. In the remaining embryo (Fig. 6) there is a striking and probably not truly representative difference in degree of development of second furrows in the two blastomeres. It is concluded that the condition described in these embryos represents the first appearance of a dorsal eccentric area of accelerated cell division which is expressive of the bilaterality of the embryo and which, as is shown in discussion of succeeding stages, persists throughout development as far as the various cleavages have been followed. This early appearance of a dorsal region of accelerated cell division foretelling the position of the median plane may be considered a sign of differentiation which, as such, gives a basis for understanding of the later stages in development.

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Explanation of Figures

Stage 2 Second Cleavage

In figures illustrating cleavage stages, the view is, unless otherwise indicated, that of the upper hemisphere.

In figures illustrating either the upper or the lower hemisphere, the gray crescent lies, unless otherwise indicated, at the left.

Incomplete furrows, regardless of their state of development, are represented by broken lines.

All sketches X40.

1. Second furrows meeting to form a straight line at the upper pole.

2. Type of cleavage leading to establishment of a polar furrow.

3. Early second furrows in an embryo in which the plane of the first cleavage coincides with the plane of the gray crescent.

4. Earlier formation of second furrow in the dorsal blastomere.

5. As Fig. 4.

6. Abnormal acceleration in formation of second furrow in the dorsal blastomere.

7. Embryos with polar furrows of varying lengths.

8. Embryos with polar furrows of varying lengths.

9. Embryos with polar furrows of varying lengths.

10 to 14. Aberrant cleavages indicating abnormality and approaching death of embryos. Fig. 13 represents the gray crescent side of the embryo shown in Fig. 12. In the embryo shown in Fig. 14 the first furrow is vertical and the second horizontal.

15 and 16. Embryos illustrating size variations.

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17 to 22. Embryos showing the more common types of cell arrangement after completion of the second furrows.

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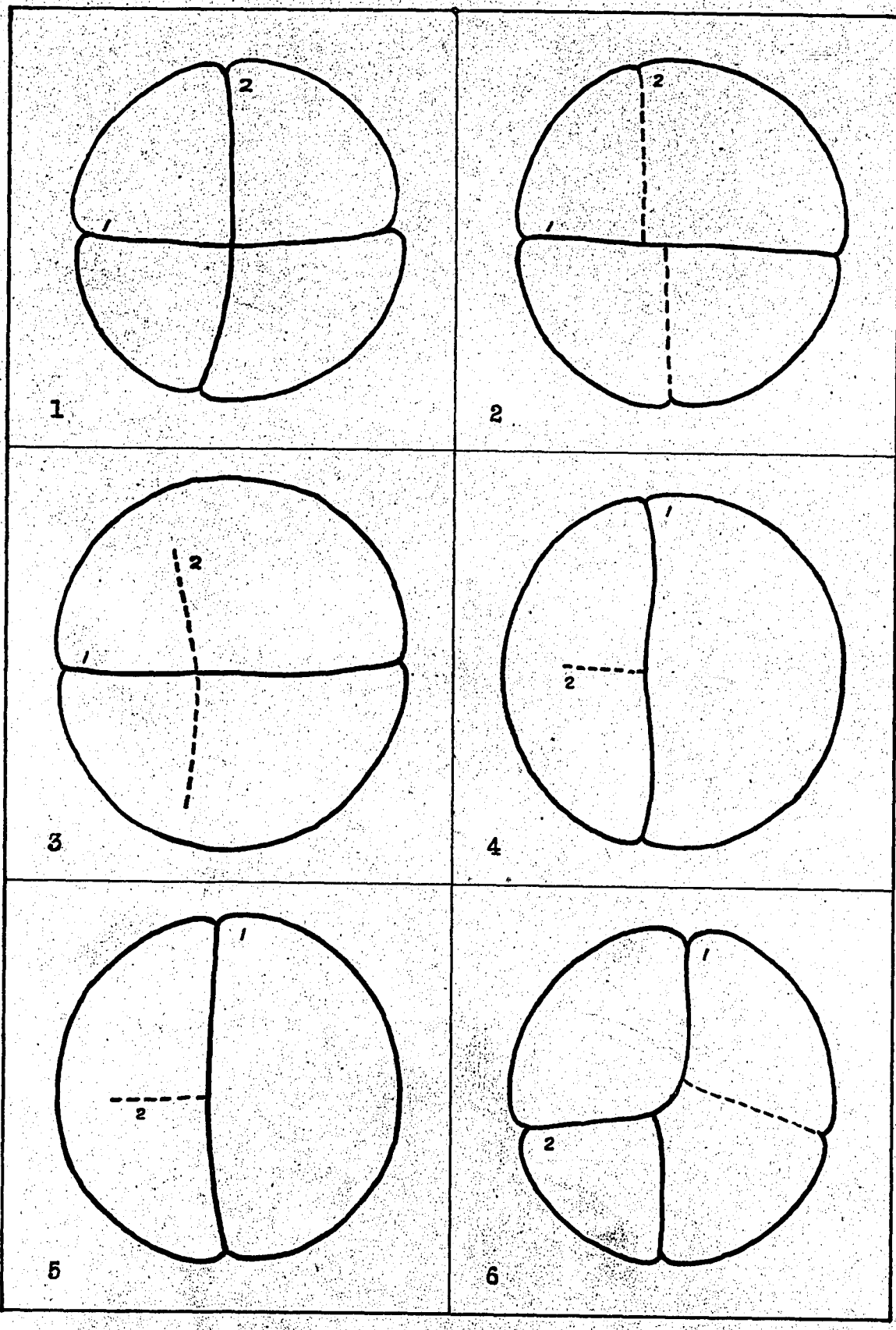

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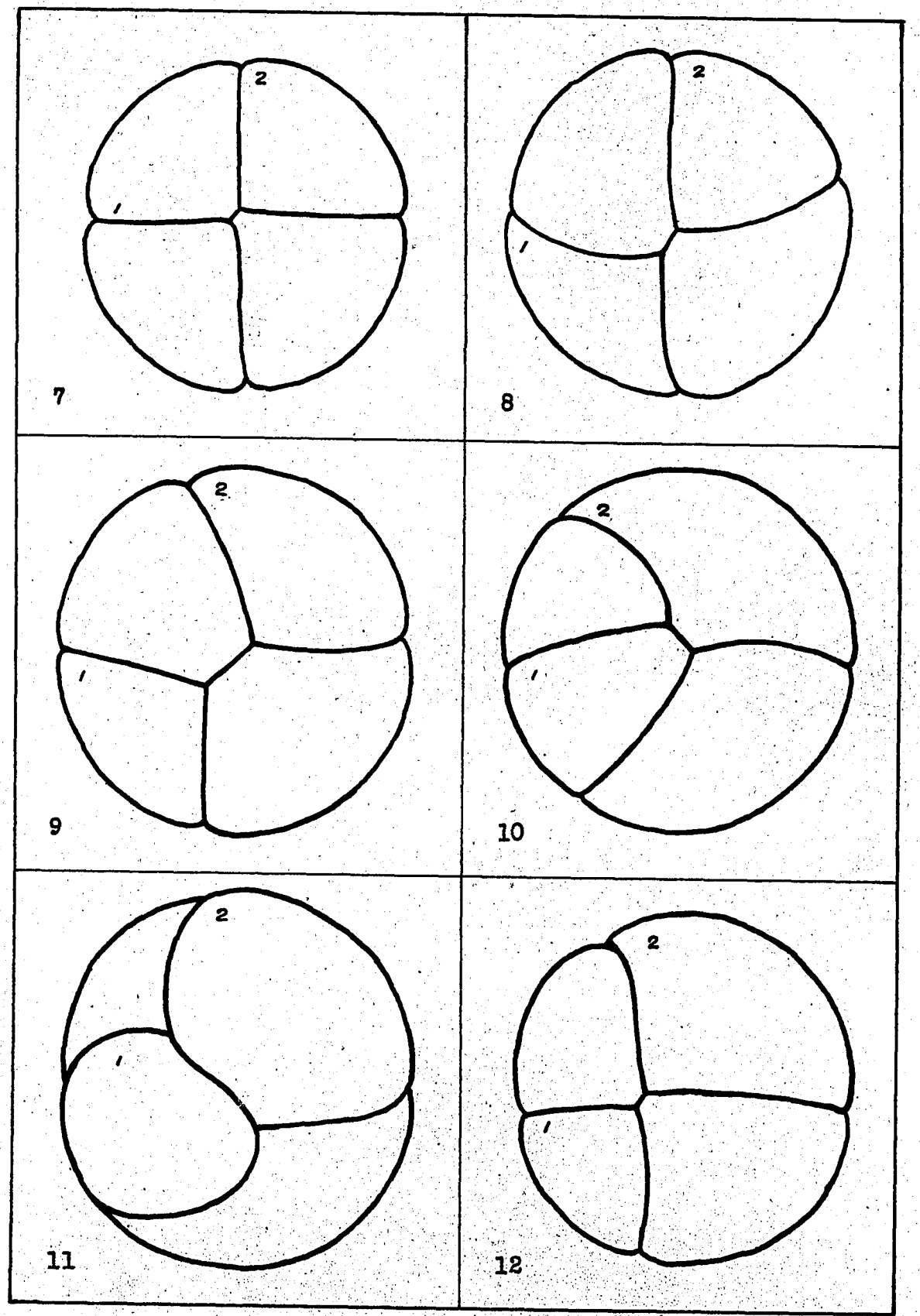

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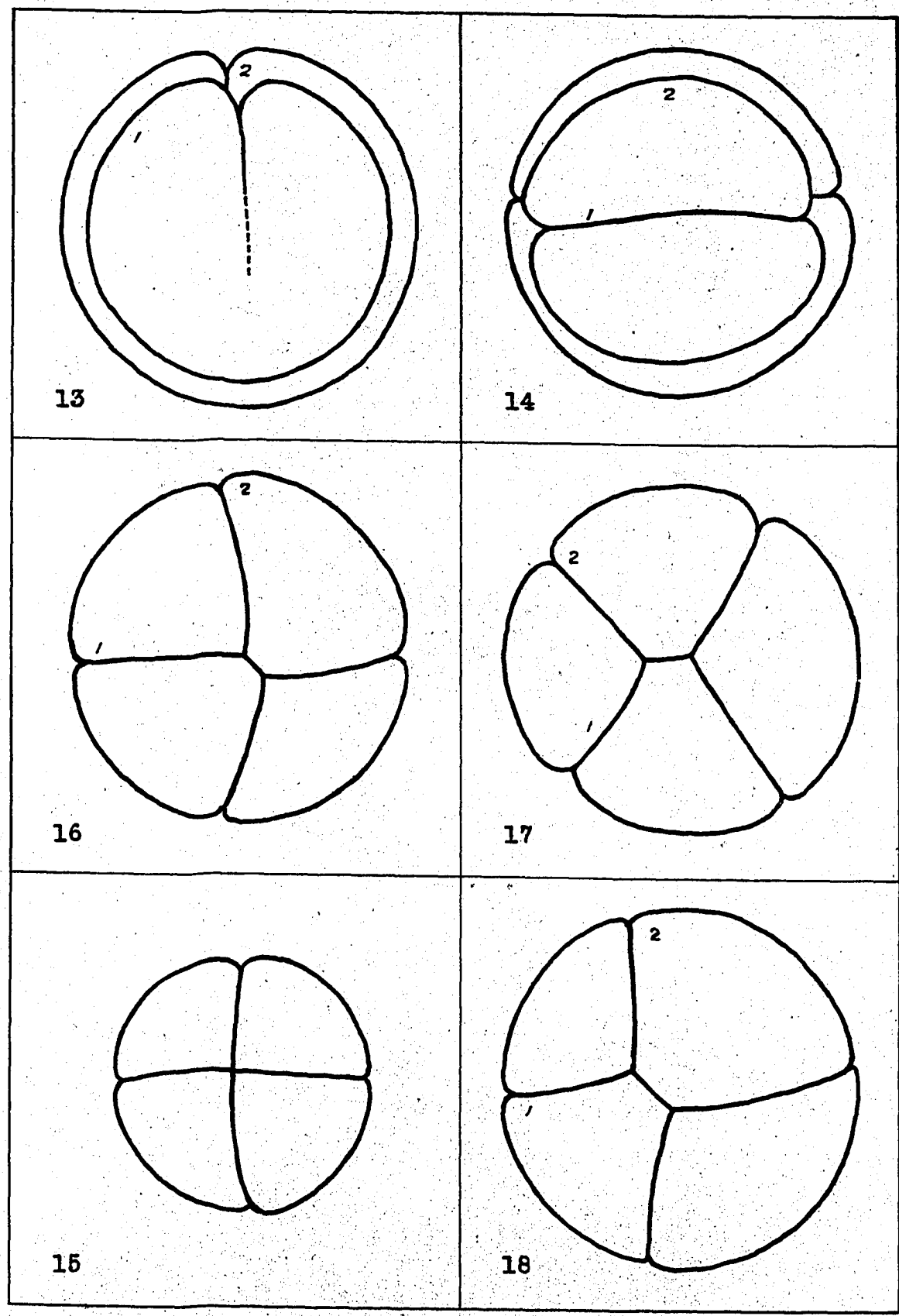

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STAGE 3

Third Cleavage

Third cleavage furrows appear from ten to fifteen minutes after the beginning of the second furrows, which by this time are as a rule externally complete. The third furrow is superficially completed in any one blastomere two or three minutes after its first appearance. Faltenkranzen are not so well marked as those accompanying the formation of the first and second furrows. At the upper border of the furrow they are fairly well marked, and at the lower border a few very shallow and transitory ones appear.

At the third cleavage the first external indication of furrow formation is the appearance of a faint line clearer or lighter in color than the surrounding area; such a clear band (lame de fractionnement) preceding appearance of the peripheral furrow has been described by Goette (1875) and also by van Bambeke (1880). In non-pigmented portions of the embryo this line appears only as a narrow clear band. In pigmented regions it appears as a clear and sharply defined pigment-free band. The surface of the embryo along the center of the clear band soon becomes slightly sunken, so that the early furrow appears as a long V-shaped depression. Through extension and deepening of this depression the furrow is finally completed superficially. The lame de fractionnement is not, as a rule, evident during formation

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of the first and second furrows, although in a few cases it has been seen to be faintly indicated in the lower hemisphere. In all succeeding cleavages, so far as they have been followed, formation of furrows takes place in the same manner as that described for the third set.

Since it becomes necessary in a consideration of cleavage to refer to the various states of development of individual furrows, some system of terminology must be devised in order to facilitate reference and description. Consequently, while it is recognized that a continuous process such as formation of furrows can be only roughly divided and only inadequately described by these terms, cleavage furrows in their stages of formation and superficial completion are referred to as very early, early, medium, almost complete, and complete. A furrow which, although no groove is actually present, is indicated by the presence of a clear band, is termed very early. One present in the form of a slight depression over part or all of the distance which it will traverse is referred to as early. A furrow is considered complete only when it has extended across the entire surface of the cell which it will divide, when in all parts of its length the bottom of the groove is no longer visible, and when, in the upper hemisphere, the blastomeres separated by it are rounded and strongly divergent from the groove. A furrow which is complete throughout all but a very small part of the distance which it is traversing is termed almost complete, while one which for any reason is intermediate between early and almost complete is referred to as medium.

It is necessary also to have some system of designation of cells for the purpose of defining their position with reference to the gray

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crescent. In embryos in which one of the first two cleavage planes coincides with the median plane of the crescent, those blastomeres on the side on which the crescent lies may be considered dorsal, and those on the opposite side ventral. In cases with discordance up to 45°, cells lying nearest the center of the crescent may be termed dorsal median. This sytem of designation has been used by recent authors, as for example Hall (1931) and Votquenne (1934), as well as by earlier investigators, and its use is well established.

During the third cleavage there is a well-marked tendency, observed both in living and in fixed embryos, for the furrows both to appear and to be completed earlier in the dorsal than in the ventral blastomeres. Fifty embryos fixed in the early part of Stage 3 were studied with this point in mind. In six of the embryos, an early third furrow is present in each of the four blastomeres. The remaining 44 embryos, as is shown in the fullowing summary, all give evidences of accelerated cleavage in the cell or cells lying in the region of the gray crescent; number of cases is indicated by the number at the left and stages of development of furrows in the statements at the right.

2------- Very early in the two dorsal cells, not visible in the two ventral cells.

7------- Early in the two dorsal cells, very early in the two ventral cells.

1------- Almost complete in the dorsal median cell, early in the two adjacent cells, very early in the ventral median cell.

2------- Complete in one dorsal cell, early in the other dorsal cell and in the two ventral cells.

2------- Complete in one dorsal cell, medium in the other dorsal cell and in the two ventral cells.

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7------ Complete in one dorsal cell, medium in the other dorsal cell, early in the two ventral cells.

14----- Complete in the two dorsal cells, early in the two ventral cells.

9------ Complete in the two dorsal cells and in one ventral cell, medium in one ventral cell.

Figures 23 to 27 illustrate embroys showing acceleration of cleavage in the dorsal blastomeres.

The third cleavage furrow is particularly interesting as considered from a comparative standpoint. As Smith (1912) has pointed out, a vertical third cleavage is characteristic of heavily yolk-laden and highly telolecithal eggs, as those of the fishes generally. In urodeles the condition is intermediate.

> "In Cryptobranchus the vertical type prevails; in Desmognathus, Necturus, and Diemyctylus there is increasing irregularity; in Amblystoma the third cleavage is latitudinal." (Smith, 1912).

In Salientia, with eggs less heavily yolk-laden, the plane of cleavage is latitudinal. However, there are in all cases deviations from the type, and the rule is far from absolute. Thus in Cryptobranchus (Smith, 1912) the third furrows, which as a rule depart from the second at a short distance from the upper pole, usually extend obliquely in the lower hemisphere to the first furrow at some distance from the pole; Smith therefore considered that the third furrows are in general

> "intermediate between a true meridional and a true latitudinal cleavage but approach more nearly to the former type."

In some cases he found one or more truly latitudinal third furrows. Hilton (1904, 1909) found regular and vertical third furrows in only

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two or three of a number of Desmognathus eggs, cleavage being irregular in the others. According to Eycleshymer (1904) and Eycleshymer and Wilson (1910) it appears that a type cannot be recognized for the third cleavage of the egg of Necturus; the irregularity is greater than in Cryptobranchus and there is a more marked tendency for the third furrows to come in latitudinally. In Diemyctylus (Jordan, 1893) there is even greater irregularity, and "with the completion of the second furrow all consistent regularity is at an end." Jordan and Eycleshymer (1894), who studied variations in cleavage in the eggs of Ambystoma punctatum, Diemyctylus viridescens, Rana palustris, and Bufo variabilis, found that a surprising proportion of eggs swerved from the amphibian "type". In Ambystoma some of the third cleavages were vertical. In a batch of frog eggs from one mother, the third furrow was found to be truly horizontal in only 29 out of 69 eggs, the other 40 showing considerable variation; in some embryos equatorial furrows appeared in three quadrants, and a true vertical in the other, while in others three cleavage planes were vertical and one horizontal, and in still others the whole third set of furrows was vertical. They concluded that in general about one-half of the eggs fail to form a true first equatorial plane in all four quadrants, and stated "All of our observations tend to emphasize the fact that great variation is a frequent occurrence in the early cleavage stages of the Amphibian egg. We have found irregularity to be the rule, regularity the exception."

In Scaphiopus the third furrow departs less from the "typical" condition than does that of many Amphibia. The furrow is in the majority of cases latitudinal, departing from either the first or the second

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furrows. It lies on the ventral side of the embryo approximately at the boundary between pigmented and non-pigmented areas, and on the dorsal side slightly below this boundary. Variation exists mainly in the fact that some furrows tend toward obliquity. It has been noted that in Rana palustris Jordan and Eycleshymer found a considerable proportion of truly vertical furrows, that is, furrows with the upper end located at or near the upper pole, in a position normally occupied by the upper ends of the fourth furrows. In Scaphiopus no truly vertical furrows have been observed; even those few which take a direction intermediate between horizontal and vertical have their upper ends located in or near the position normal for a latitudinal third furrow. In 80 of a series of 122 fixed embryos, the third furrow is considered horizontal in all four quadrants. These embryos are of the types shown in Figures 28, 29, and 30, that is, the furrows in all four blastomeres are at approximately the same level, so that all four micromeres lie in one plane; in them, the furrows in adjacent quadrants may meet exactly (Fig. 29), but more commonly there is a slight distance separating the points at which the third furrows join the first or second furrows, and subsequent shifting of blastomeres has produced a furrow (Fig. 30) similar in appearance and method of formation to the polar furrow of the four-cell stage. In 32 of the 122 embryos, a condition is found similar to that shown in Figs. 31 and 32; that is, one or more of the third furrows is slightly oblique, with the result that it meets the vertical first or second furrow at some distance from the adjacent third furrow, and the lower portion of the micromere separated by it lies nearer the equator than

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do those of the other three. In ten embryos of the series the tendency toward obliquity is carried still further; in each embryo one or more of the furrows takes a direction intermediate between horizontal and vertical, and joins the meridional furrow below the equator (Figs. 33 and 34) or not far from the lower pole (Figs. 35 to 40). The two embryos shown in Figures 41 to 46 illustrate the closest approach found to a vertical third furrow.

A relationship is found to exist between presence or absence of a polar furrow and obliquity of third cleavage furrows. Embryos with no polar furrow or with a very short one have all been found to fall into the first of the three above-mentioned types. On the other hand, all of those embryos which can be classed under the second and third types possess a polar furrow of some length, and the micromere with the oblique third furrow is located at one end of the polar furrow, rather than being one of the two micromeres the upper boundaries of which constitute the polar furrow. Viewed in this light, the formation of an oblique third furrow, or of one not markedly oblique but located nearer the equator than are the other third furrows, is not surprising. Since the upper boundary of the micromere in question is at some distance from the upper pole, formation of an oblique or low third furrow merely tends to prevent extreme inequality in size of the four micromeres. On the other hand, an oblique third furrow may at times, particularly when it is located on the ventral side of the embryo, result in formation of a micromere much larger than the other three (Fig. 33).

In an earlier publication (Trowbridge and Trowbridge, 1937) it

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was noted that as the time consumed in cleavage decreases, the time spent in rest increases. Evidently this is due merely to the fact that with decreasing size of blastomeres, furrows are completed more quickly in the later than in the earlier cleavages. A careful summary of the time of appearance of data on sets of cleavage furrows from the first to the eighth (as far as they have been followed in living embryos) shows that they can most accurately be described as occurring at ten to fifteen minute intervals.

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Explanation of Figures

Stage 3 Third Cleavage

23-27. Accelerated cell division in the dorsal cells.

23. Furrow complete in one dorsal cell, early in the other, very early in the two ventral cells.

24. Furrows complete in the dorsal cells, early in the ventral cell at lower right and medium in the one at upper right.

25. Furrows complete in dorsal cells, medium in the ventral cell at lower right, and early in that at upper right.

26. Furrows completed in all cells except one of the ventral two.

27. Lateral aspect of the embryo shown in Fig. 26.

28. to 30. Views of respectively, upper hemisphere, left side, and right side of an embryo in which all of the third furrows lie at approximately the same level.

31. and 32. Embryos with one or more oblique third furrows.

33. Embryo with a markedly oblique third furrow in blastomere a.

34. Same embryo tilted slightly to show the oblique furrow.

35. to 37. Views of upper hemisphere, side, and lower hemisphere, respectively, of an embryo with a markedly oblique third furrow.

38. to 40. Views as above of a similar embryo.

40. to 43. Views from the same respective aspects of an embryo with one third furrow approaching a vertical position.

44. to 46. Same respective views of another embryo illustrating the same point.

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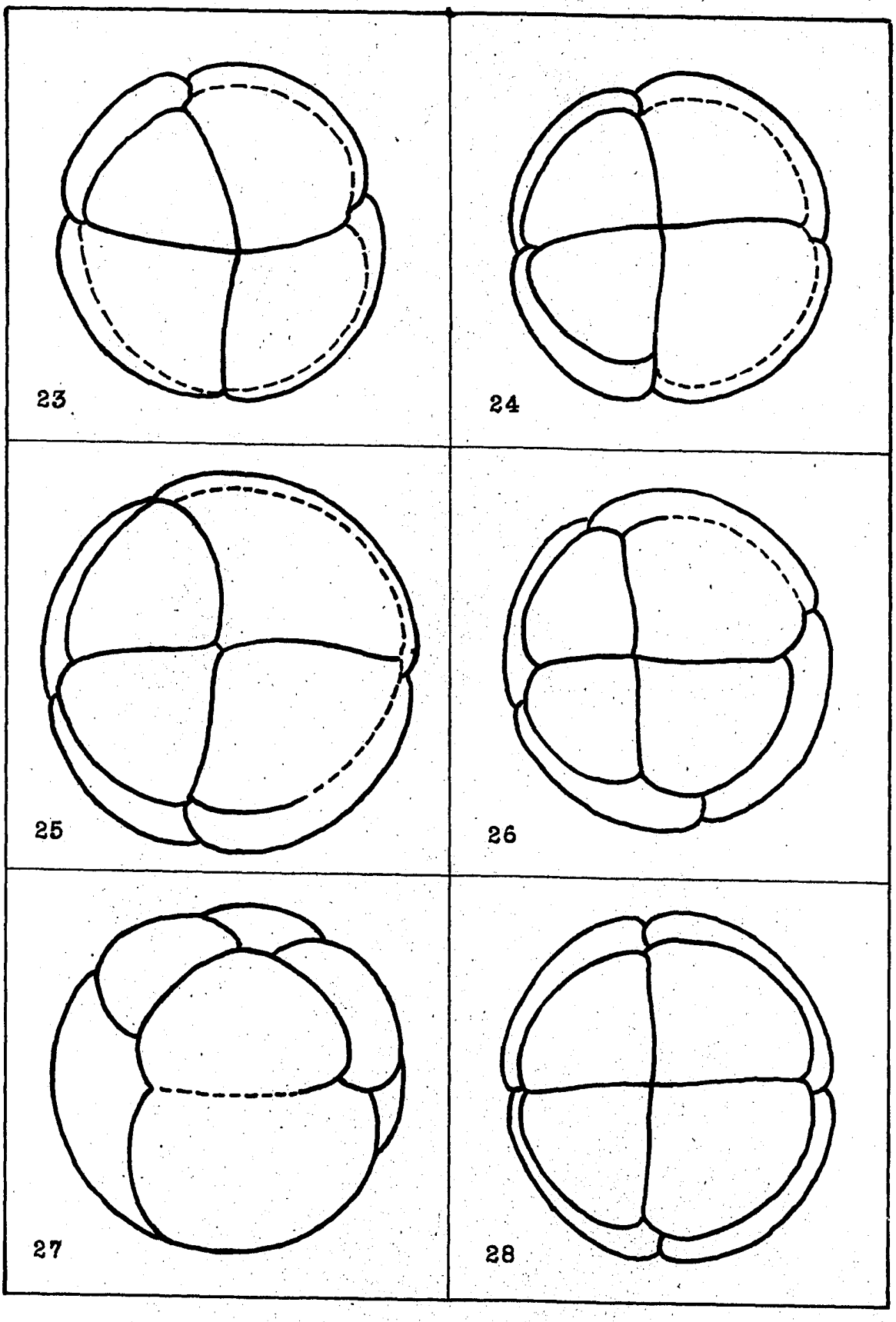

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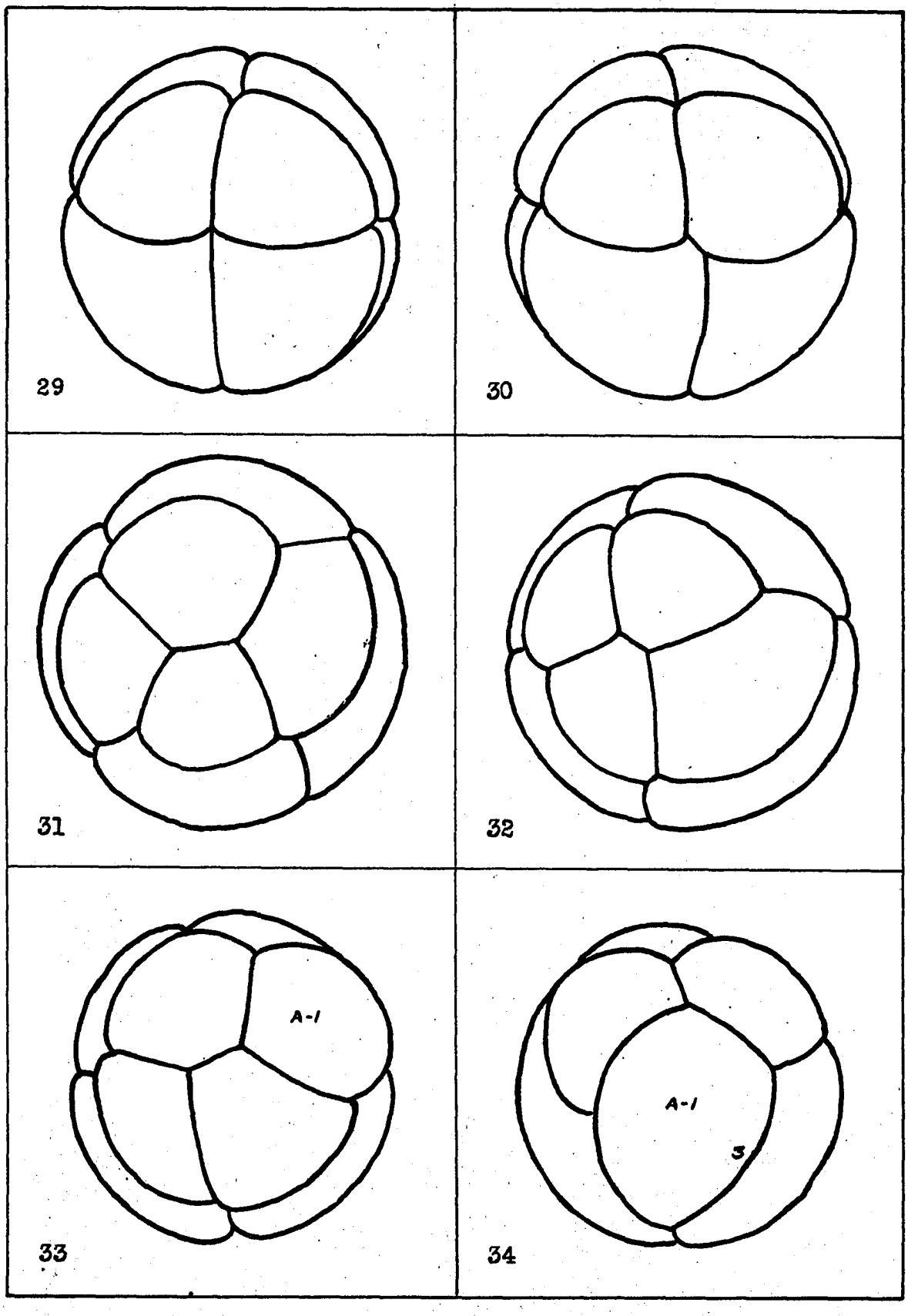

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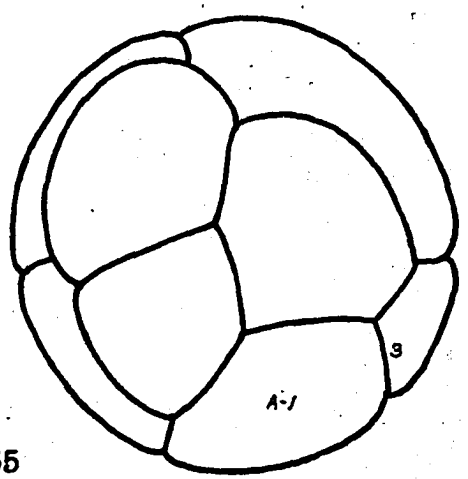

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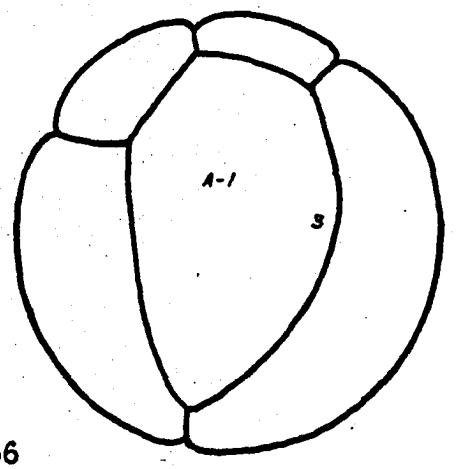

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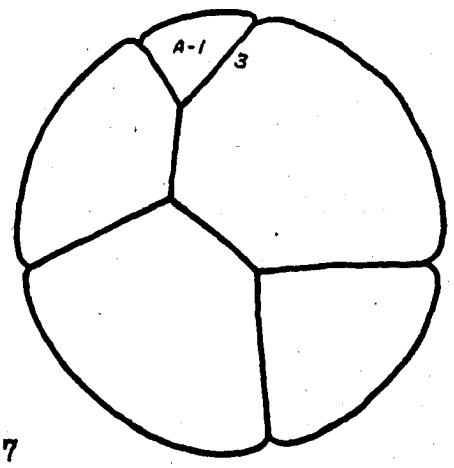

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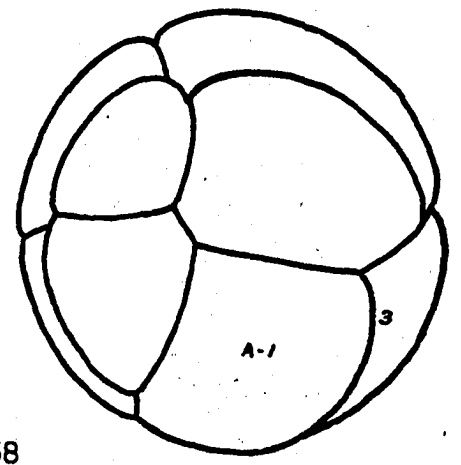

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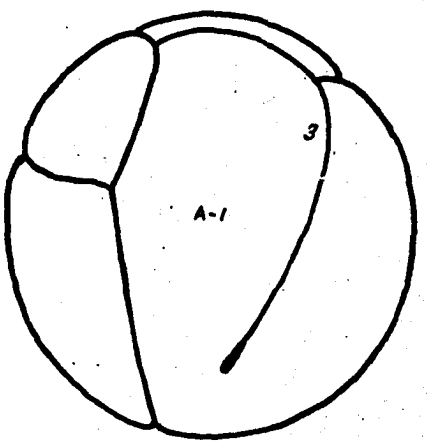

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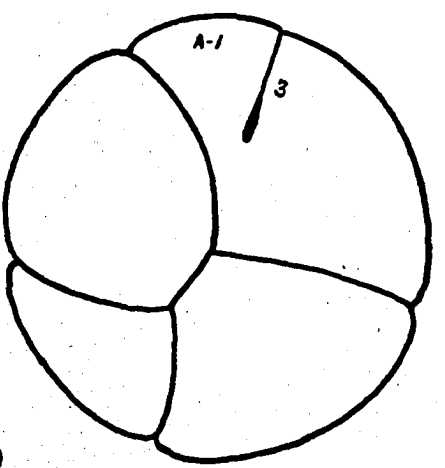

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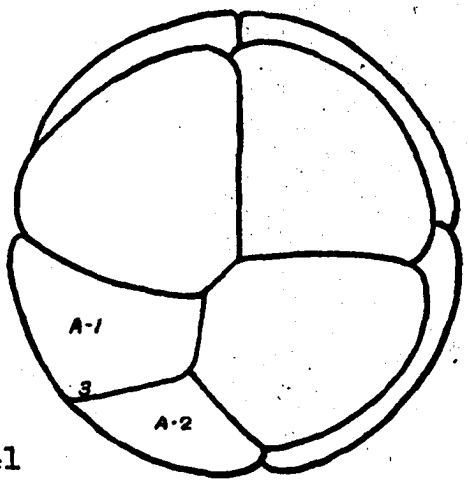

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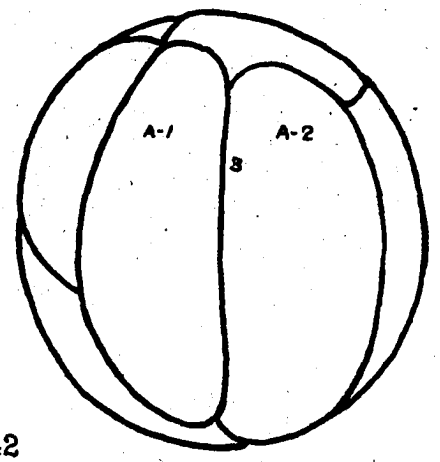

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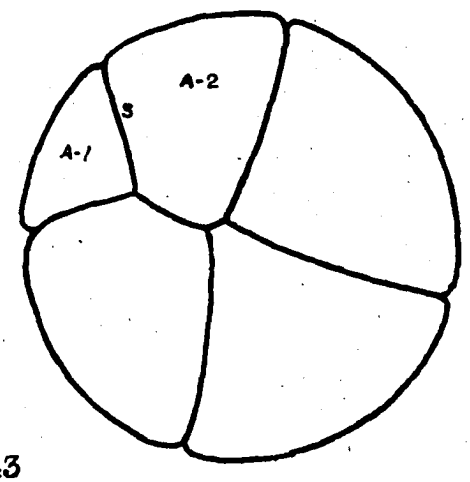

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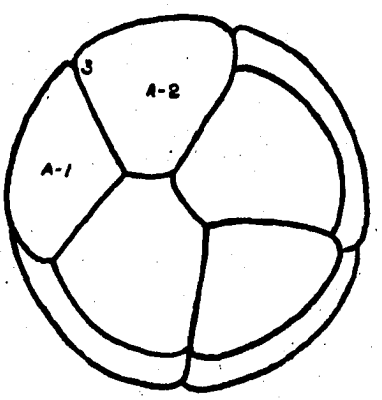

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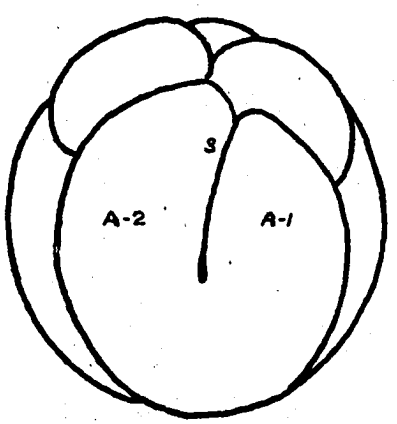

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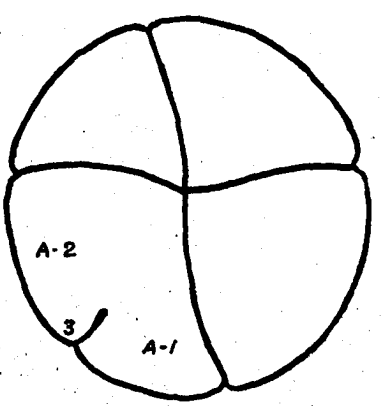

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STAGE 4

Fourth Cleavage

Fourth cleavage furrows are initiated from ten to fifteen minutes after the beginning of the third furrows, which are by this time always completed. They nearly always are first seen in the micromeres, and are completed in these cells in from one to two minutes. The furrow appears first about midway between the upper and the lower margin of the cell which it will divide, and, as it extends, may be completed first in some cases at the upper and in the other cases at the lower end. Formation of the furrow in any one macromere follows closely that in the micromere above, and occasionally the two appear simultaneously. In any case, the furrow is always present in a macromere before it is completed in the micromere above. Fourth furrows in the macromeres depart from the third cleavage plane and progress downward. According to data from the study of living embryos, they are completed in the macromeres about three minutes later than in the micromeres, and thus from five to six minutes after they first are to be seen in the latter cells. Small and transitory Faltenkranzen have been noted at the fourth and subsequent cleavages, but have not been studied in any detail. Schultze (1863) has described Faltenkranzen as occurring at the fourth and fifth cleavages, and Wilson (1896)

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has observed that in Chorophilus triseriatus they form at least up to the 128 cell stage.

As concerns location and direction, the fourth furrows are typically vertical, although they usually do not reach the upper and rarely the lower pole. Embryos with furrows incomplete in the micromeres give a better picture of location and direction than do those with the cleavage completed, since in the latter the first two furrows, coincident with shifting and readjustment of blastomeres, are often drawn away from their original positions. In some embryos (Figs. 47, 48, and 49) all four furrows in the micromeres are vertical. In others (Fig. 50) one or more may extend downward at right angles from one of the first two furrows. Again, one or more of them may be oblique or may approach a horizontal direction (Figs. 51 to 54). Figure 55 represents an embryo with fourth furrows so recently completed that shifting of micromeres has not as yet occurred.

During the fourth cleavage acceleration of cell division in the dorsal blastomeres is not as a rule manifest with the first appearance of the furrows; as is shown in Figures 47 to 54, early cleavage lines appear at about the same time in all four micromeres. In living embryos, however, as well as in those fixed at a slightly later stage, it is evident that cleavage is completed first in the dorsal cells (Figs. 58 to 62, and 147). In these embryos and in similar ones fourth furrows in the macromeres also tend to be further advanced in the dorsal than in the ventral region.

The majority of embryos in the sixteen-cell stage show a bilateral arrangement of micromeres; such a situation has been noted by many authors,

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among them Schultze (1863) and Roux (1883). The most common type of bilateral arrangement is that characterized by the occurrence of the eight micromeres in two more or less definite parallel rows of four cells each, the arrangement being such that the cells lie in an elongated configuration. This type of arrangement results when all of the fourth furrows meet one of the first two furrows, and is accentuated by shifting and readjustment of micromeres after completion of the cleavage. It is best marked in those embryos in which no polar furrow is present and in which the two fourth furrows in adjacent micromeres reach approximately the same point on that furrow (first or second) which forms the dividing line between the two rows of cells (Fig. 63). In case either one or both of these conditions does not exist, the resulting configurations are similar to those shown in Figures 64, 65, 67, 69, 71, 73 and 74. In these embryos, that furrow which forms the dividing line between the two groups of four cells does not remain straight, but is drawn into a zig-zag line, and the bilaterality of micromere arrangement is not so plainly evident. Since at this time first and second furrows are indistinguishable, it cannot be stated definitely in any case whether it is the first or the second which is joined by the fourth furrows and which thus comes to form the dividing line between the two rows of cells. However, since the gray crescent usually lies at one end of the micromere configuration (although sometimes at one side and occasionally in an intermediate position), and since the crescent has been found in most cases to be bisected by the first cleavage plane, it would seem that the furrow in question is usually (and possibly always) the first.

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Embryos exhibiting a slightly different type of bilaterality in micromere arrangement result when the fourth furrows in two adjacent micromeres join one of the two first furrows, and those in the other two micromeres join the other (Figs. 75 and 76) or reach the intersection between the two (Fig. 77). In embryos of this type, location of the gray crescent is most often as that indicated in Figure 76, and occasionally as that shown in Figure 75; in a few cases its median plane lies at an angle of 45° or less from either of the two positions indicated.

Configurations more or less intermediate in type between the two above-mentioned are formed when one fourth furrow joins one of the first two furrows and the remaining three join the other (Figs. 78, 79, 81, and 82).

In some embryos bilaterality of micromere arrangement is not present, nor do the micromeres lie in an elongated configuration. Four of them may extend to or near the upper pole, alternating with four which are separated from it by a greater distance (Figs. 83, 84, and 148). In these embryos, arrangements of micromeres is roughly radial. Other variations in the configuration of cells are illustrated in Figures 86 and 89.

Although some sixteen-cell embryos are rather difficult to interpret, recognition of the fourth furrows is nearly always possible. Recently completed fourth furrows in the upper hemisphere can be recognized by that depth and openness characteristic of newly formed furrows. A little later, when this distinction no longer holds, fourth furrows can nearly always be distinguished in the lower hemisphere; in most of the embryos studied they are not quite completed, and, when

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they have been completed, their points of intersection with the vertical first or second cleavage planes are usually located at some distance from the lower pole. Since the fourth furrow in a micromere and that in the macromere below it usually depart from about the same point on the third furrow, the identity of the plane in a micromere is, in all but the most irregular and unusual cases, established by the position of that in the macromere. Moreover, even in unusual types of cleavage (as those shown in Figs. 82 and 86) if one or more of the fourth furrows can be identified (as in Fig. 68) one has a guide to the location of the others, as they alternate with the first two furrows.

Fourth furrows in the macromeres usually fall short of the lower pole. The most common type of cleavage is that shown in Figures 66 and 70; it is the type found as a rule in embryos with micromere arrangements as those shown in Figures 63, 65, 67, 69, 71, 73, 74, 75, and 76. In these embryos the fourth furrow in a macromere joins at some distance from the lower pole that vertical first or second furrow other than the one joined by the fourth furrow in the micromere above; if the furrow separating the micromeres into two rows or four cells each be the first, then the fourth furrows join the second in the lower hemisphere, and vice versa. The macromeres below those four micromeres lying nearest the upper pole do not reach the lower pole, while those below the four micromeres lying somewhat removed from the upper pole (at the two ends of the elongated configuration of micromeres) do border on the lower pole. Thus four of the macromeres lie relatively high, and four low, and the result is a nearer approach to

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equality in size than would otherwise be attained. At the same time, there is formed a macromere configuration of four cells lying on each side of a vertical furrow, with the long axis of the configuration at right angles to that formed by the micromeres.

Occasionally (Fig. 72) in one or more of the macromeres the fourth furrow may join the same vertical furrow as that joined by the fourth furrow in the micromere above. In rare cases (Fig. 85) all of the fourth furrows reach the lower pole. Other cleavage patters in the lower hemisphere are shown in Figures 80 and 88. In the embryo represented in the latter figure an oblique fourth furrow has divided a macromere into two cells of highly unequal size.

In connection with the fact that cleavage furrows, as for example the fourth ones, do not generally pass through a common point at the upper pole, Rauber (1883) postulated the existence of a Polflucht and assumed that the furrows "alle suchen den Pol zu vermeiden". However, furrows do occasionally reach the pole, and as Jordan and Eycleshymer (1894) have pointed out, rather than their "avoiding" the pole, mechanical cell-stresses are rarely so adjusted that they intersect at the pole.

In an attempt to determine the percentage of embryos in Stage 4 which can be classified under the various types described, a series of 278 embryos was studied, in addition to those represented in the sketches; the results of the study are shown in Table III. The first and second columns of figures give, respectively, the number of the figure representing an individual embryo, and the number of specimens (of the series of 278) which resemble in the matter of micromere arrangement the embryo in question.

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TABLE III

Types of Micromere Arrangement after the Fourth Cleavage in Embryos of *Scaphippus bombifrons* Cope

	Figure Number	Number of cases	Total No. of cases	Percentage of Entire Series
Bilateral Micromere Arrangement of First Type	63	25		
	64	5		
	65	23		
	67	34		
	69	20		
	71	24		
	73	38		
	74	3		
			172	61.87
Bilateral Micromere Arrangement of Second Type	75	9		
	76	19		
	77	9		
			37	13.31
Arrangement Intermediate Between First and Second Types	78 or 79	12		
	81	18		
	one fourth furrow reaches intersection of 1st and 2nd	3	33	11.87
Radial Arrangement	83	5		
	84	9		
			14	5.036
Irregular Arrangement	60			
	61			
	86	22		
	89			
			22	7.914
Total Number of Cases		278	278	
Total Percentage				100.000

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As is shown in Table III, 61.87% of the embryos constituting the series have the micromeres arranged in two more or less definite rows of four cells each, 13.31% exhibit a slightly different type of cell arrangement (as illustrated by Figures 75, 76, and 77), and 11.87% have the micromeres lying in a bilateral configuration intermediate between the two types above mentioned. Thus 87.05% of the total number of embryos exhibit a bilateral configuration of micromeres. In 5.036%, the arrangement of cells are arranged in an irregular manner. Although the series of specimens is not large enough to serve as a basis for computation of reliable percentages, it is evident that certain types of embryos occur much more often than do others.

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Explanation of Figures

Stage 4 Fourth Cleavage

47. to 54. Embryos showing location and direction of early fourth furrows.

55. to 57. Embryo in which fourth furrows are so recently completed that shifting of cells has not occurred; views of, respectively, upper hemisphere, left side, and lower hemisphere.

58. to 62. Acceleration of cleavage in dorsal blastomeres.

58. Furrows complete in dorsal micromeres, and in dorsal macromeres complete over three-fourths of distance to lower ends. Furrows in ventral micromeres medium, in ventral macromeres very early over the upper half of the distance they will traverse.

59. Furrows complete in dorsal micromeres and early in dorsal macromeres, early in ventral micromeres, very early in one ventral macromere and not yet evident in the other.

60. Furrows complete in the dorsal micromeres, medium in the ventral micromeres. Furrow early in the dorsal macromere at lower left and almost completed in that at upper left, very early in each of the two ventral macromeres.

61. Furrow completed in one dorsal micromere, medium in the other three micromeres, almost complete in one dorsal macromere, medium in the other, and not yet evident in the ventral macromeres.

62. Side view of the embryo illustrated in Fig. 61. The furrow in micromere a1-a2 approaches a horizontal direction and that in macromere a3-a4 is oblique.

63-89. Various types of cell arrangement after completion of the fourth furrows. Fig. 66, 68, 70, 72, 80, 85, and 88 represent the lower hemisphere of the embryo shown in each case in the preceding figure. Figure 87 represents a side view of the embryo which is shown in Figures 86 and 88, and in which an oblique fourth furrow has separated two macromeres highly unequal in surface areas.

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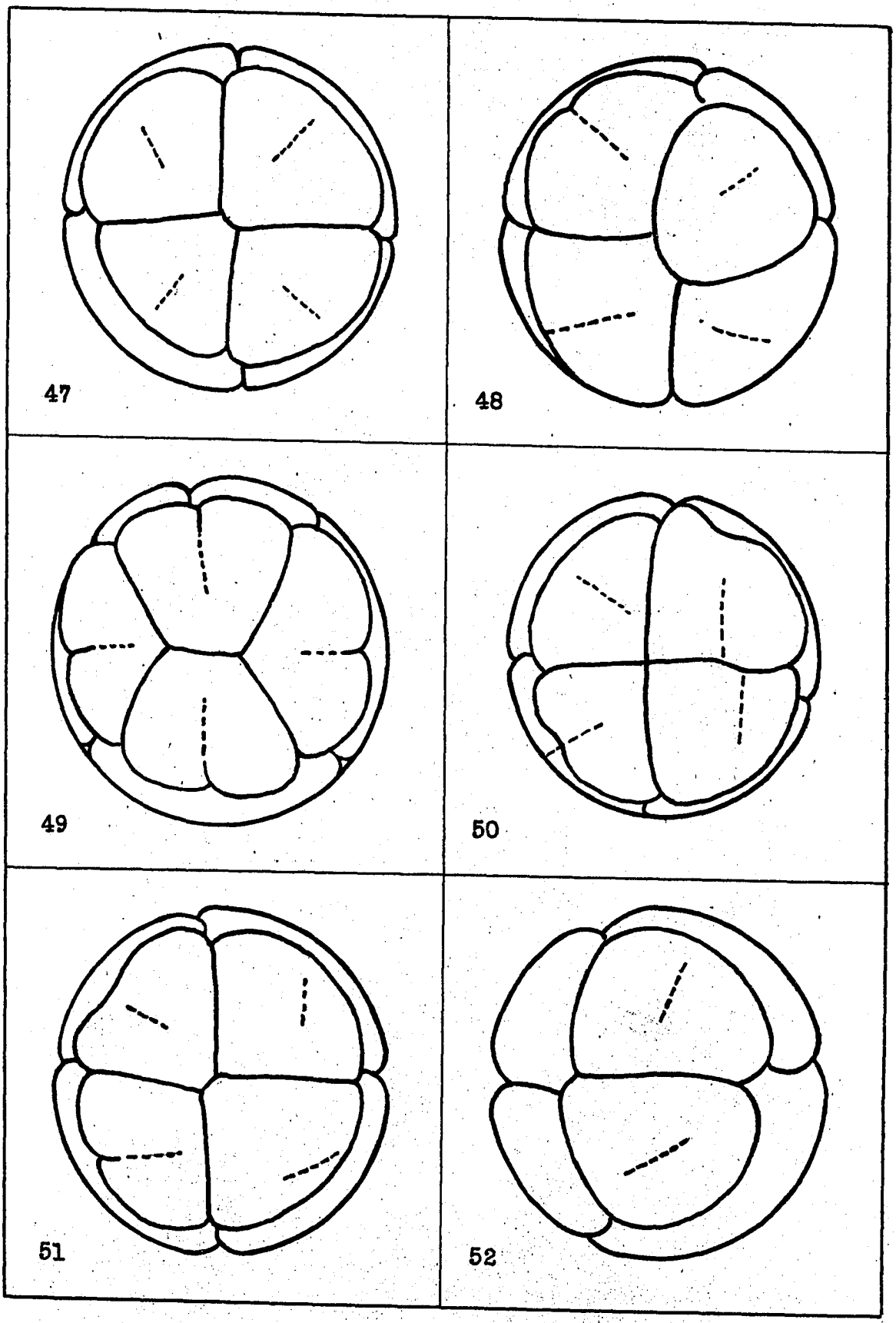

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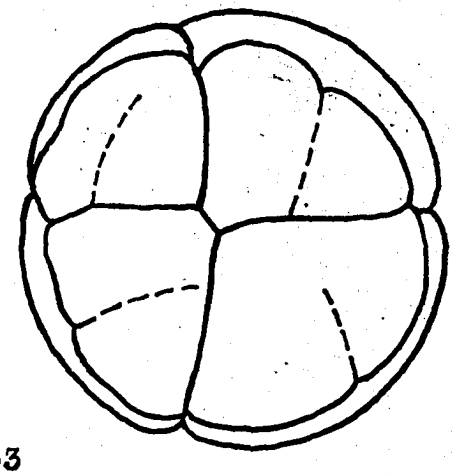

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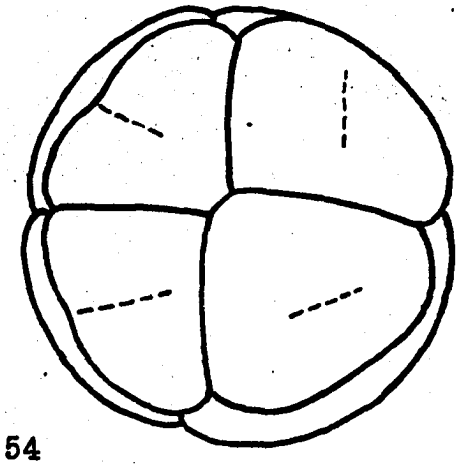

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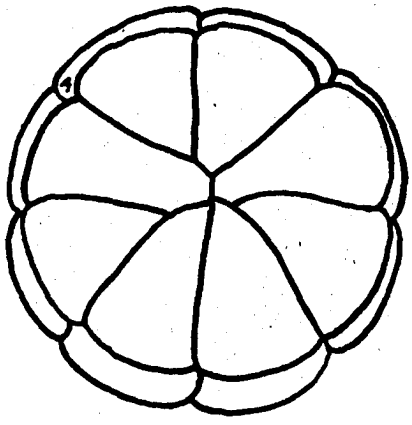

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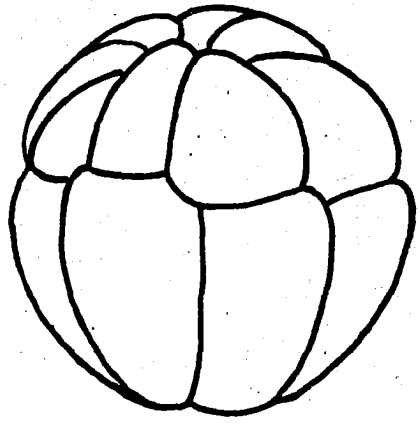

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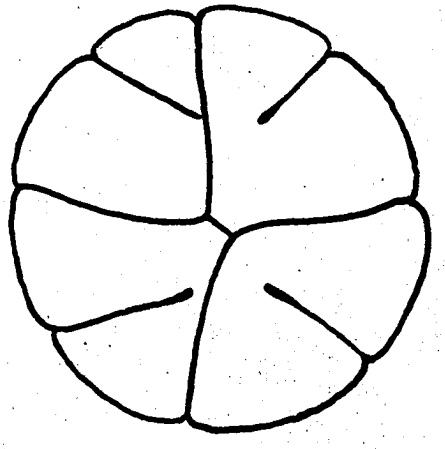

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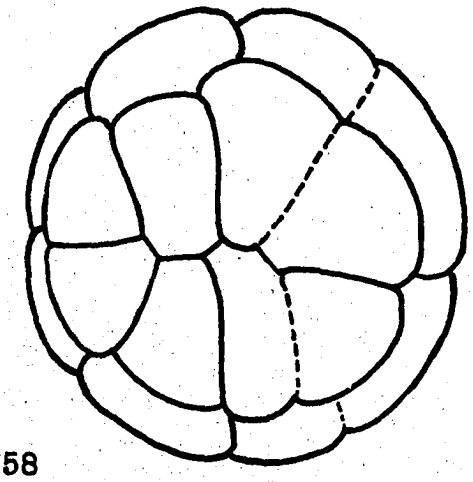

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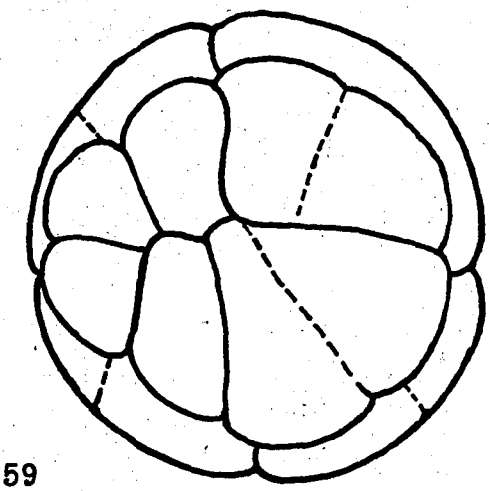

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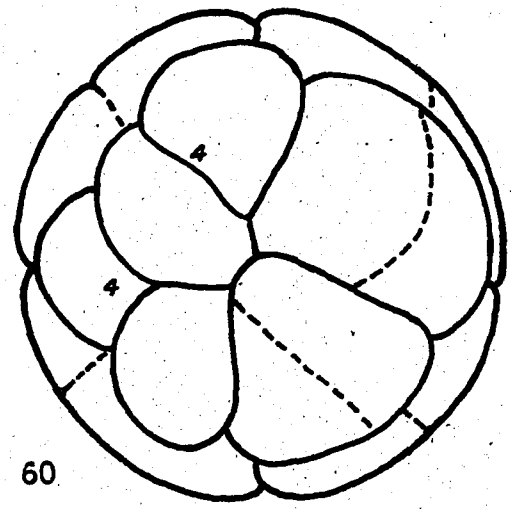

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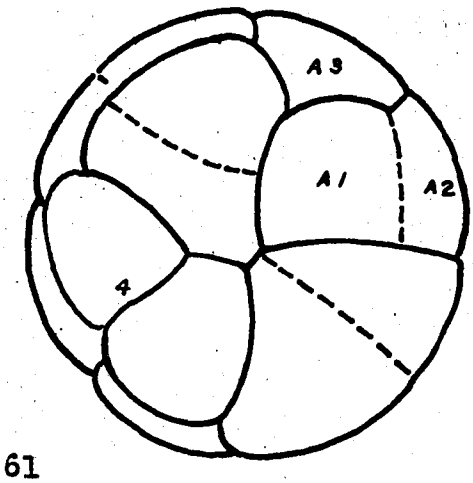

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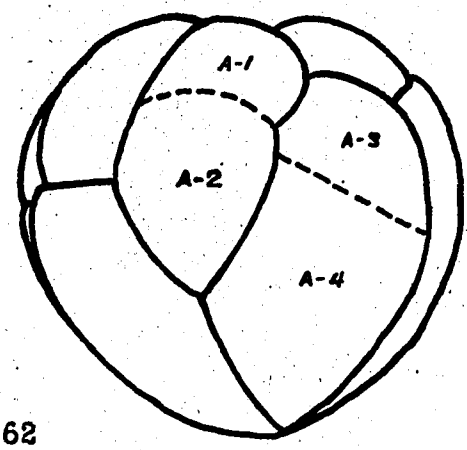

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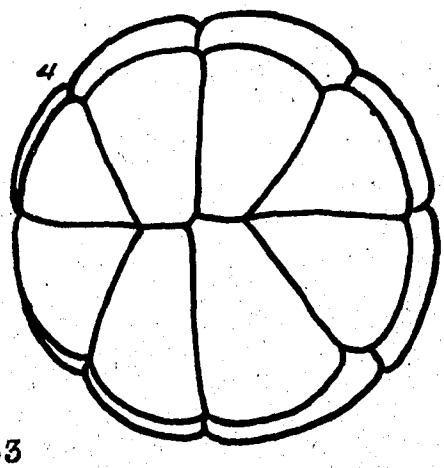

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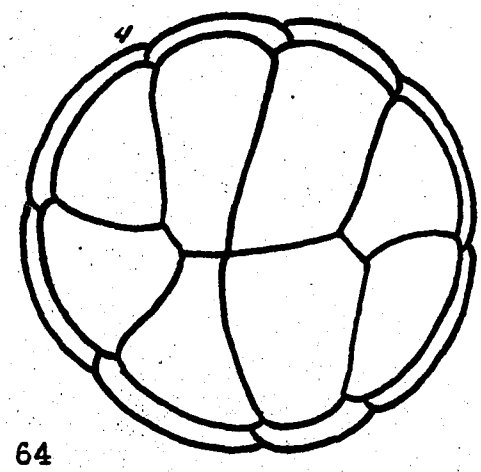

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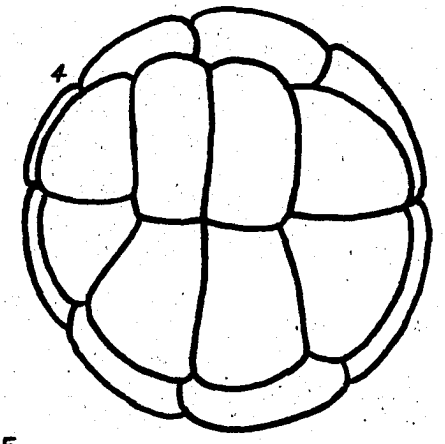

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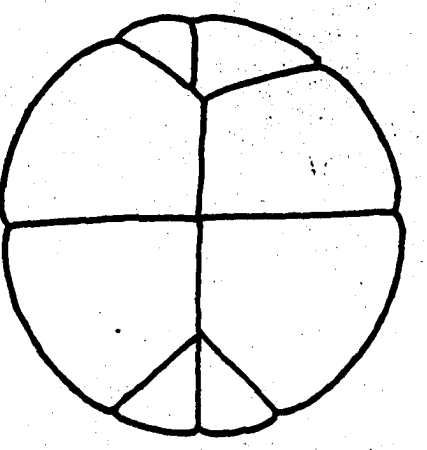

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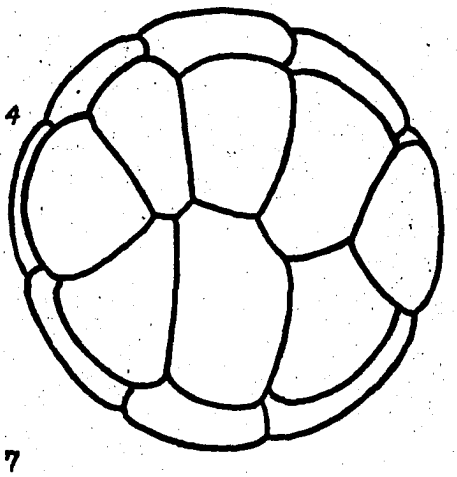

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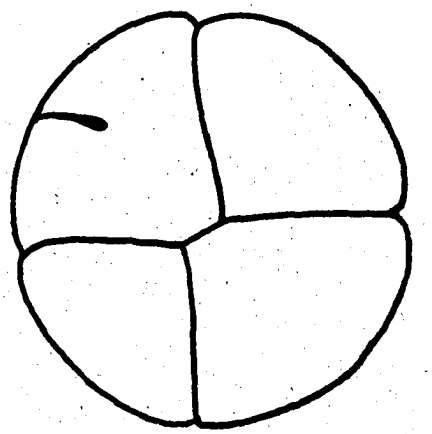

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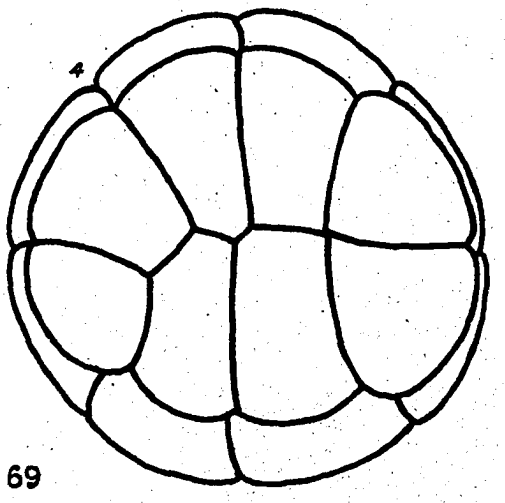

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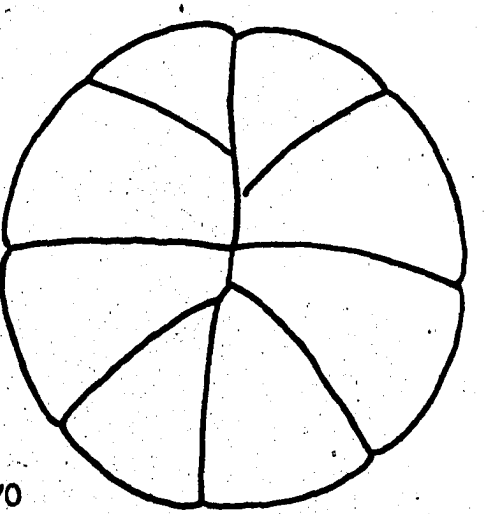

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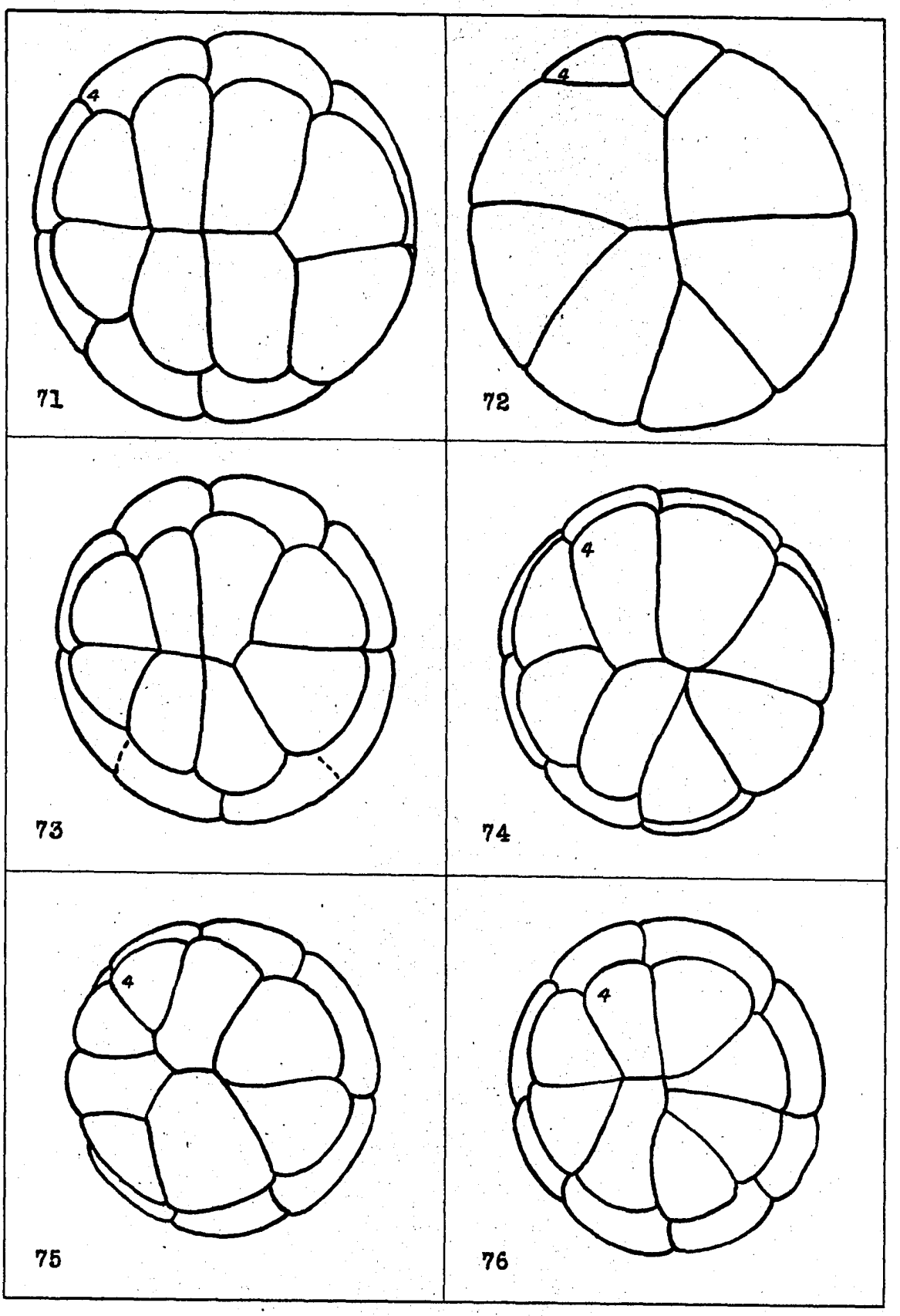

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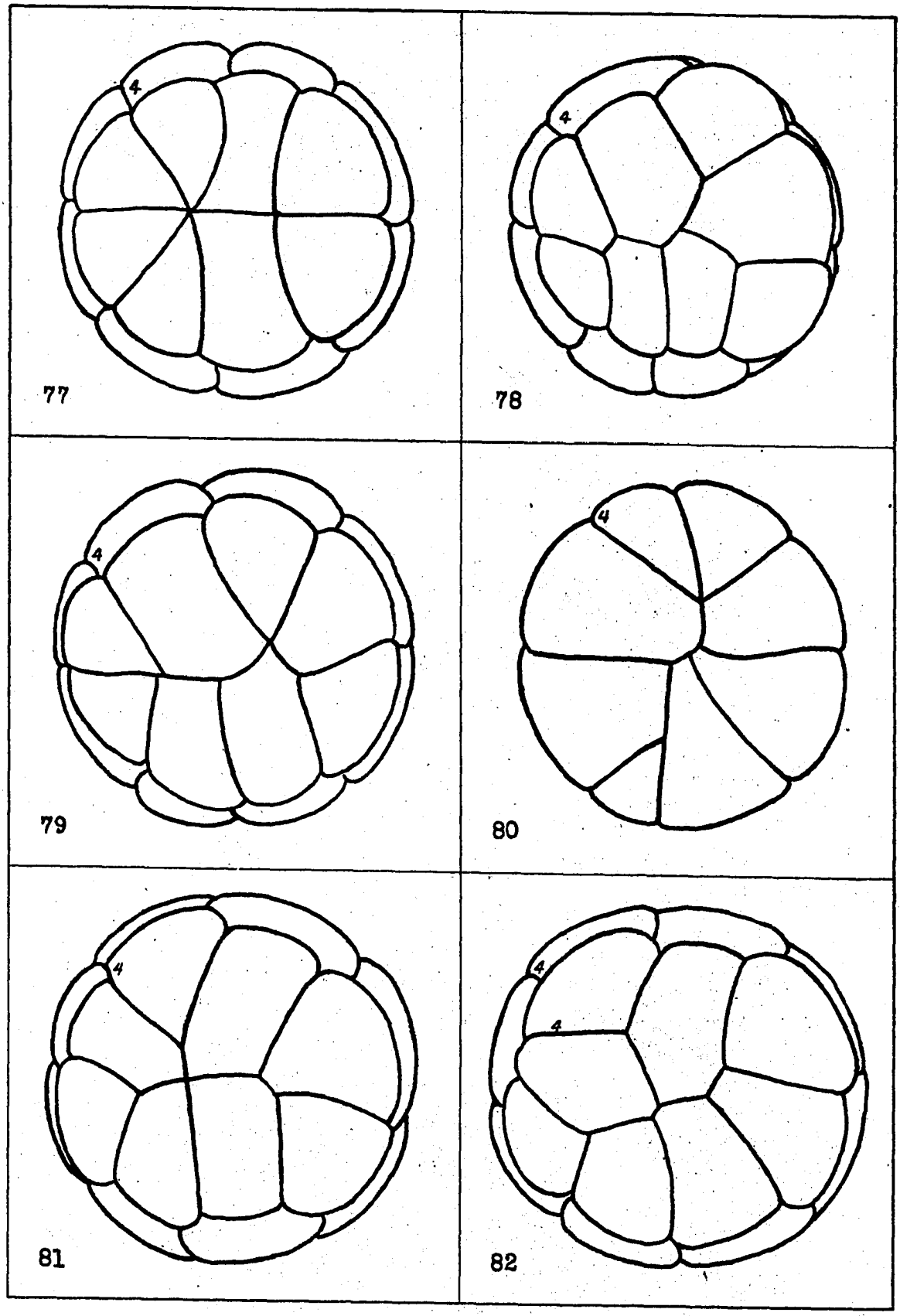

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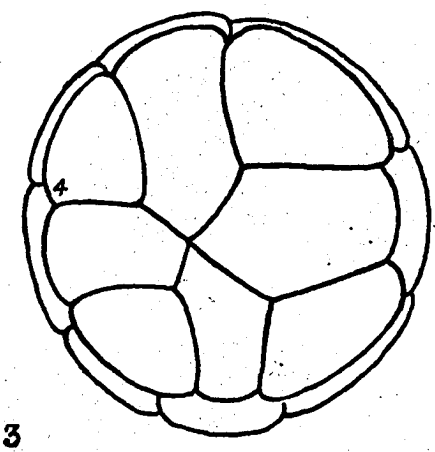

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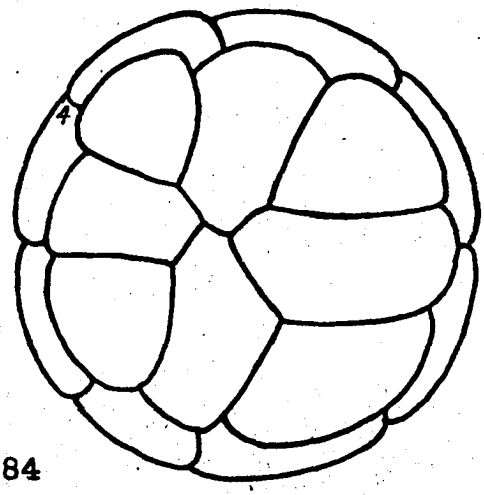

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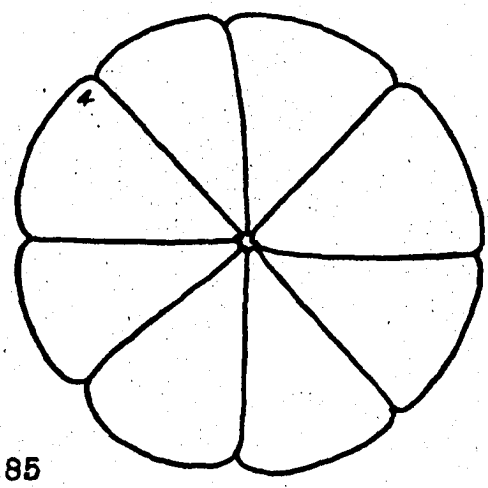

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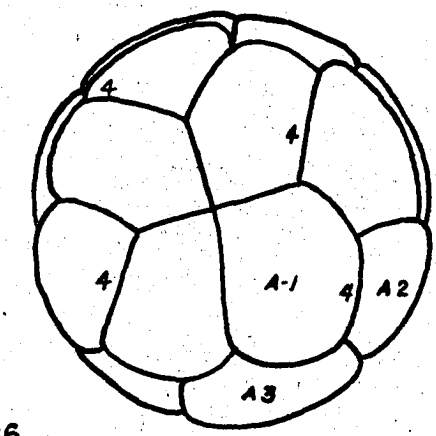

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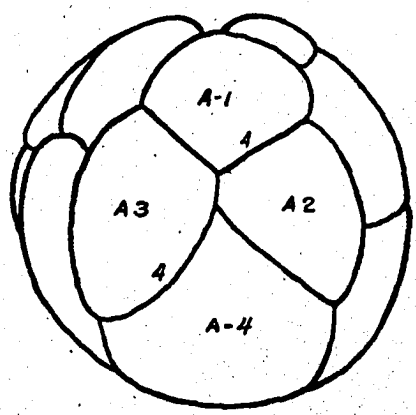

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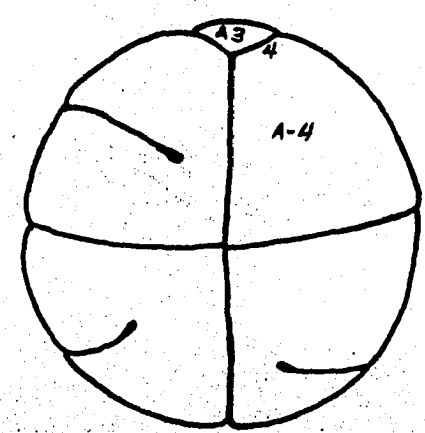

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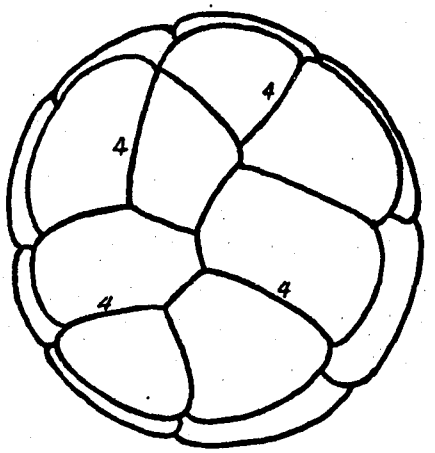

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STAGE 5

Fifth Cleavage

Fifth cleavage furrows appear from ten to fifteen minutes after the beginning of the fourth furrows. Like the latter, they usually are seen first in the micromeres, and are completed superficially in any one cell in from one to two minutes. In the macromeres they form slightly later, and are completed from one to three minutes later than in the micromeres. However, this relationship may be disturbed by earlier formation of furrows in the dorsal blastomeres, so that occasionally the fifth furrow may be almost completed in one or more of the macromeres before it has appeared in some of the micromeres (Figs. 90 and 93). Acceleration of cleavage in the dorsal blastomeres, which is well marked during this cleavage, is shown in the embryos illustrated in Figures 90 to 97, 149 and 150. These embryos have been chosen as representative of more than forty illustrating the same point. Only one embryo (Fig. 98) has been found in which fifth furrows are not better developed in the dorsal than in the ventral cells.

As concerns direction of the fifth cleavage planes, cleavage is seen to be more regular in Scaphiopus than in many other Anura. The figures of Jordan and Eycleshymer (1894) show that in the four genera of Amphibia studied by them the fifth furrows are quite irre-

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gular. They stated, "The appearance of the fourth set of furrows almost invariable marks the end of any constancy whatever in the relative position of the blastomeres." According to Morgan (1897); "During the fifth cleavage-period the irregularities in the division of the cells is generally so great that we cannot speak definitely of any special direction of the new planes. Nevertheless there is a tendency for some of the new furrows to come in at right angles to the last planes of division. Therefore, many and occasionally all of the fifth cleavage-planes are horizontal." In Scaphiopus the fifth furrows are nearly always latitudinal and approximately at right angles to the fourth division planes; in most embryos, they are so in all sixteen blastomeres. Occasionally one or more of the furrows may be oblique or vertical. Figure 96 illustrates an embryo in which one of the micromeres is being divided by a vertical furrow. In the embryo represented in Figure 97 the situation is somewhat difficult of interpretation, but it seems likely that one of the four micromeres of the eight-cell stage was divided by an almost horizontal fourth furrow, so that the two resulting micromeres were elongated in the latitudinal direction; in each the early fifth furrow is vertical. In one embryo three of the early fifth furrows are vertical. Apparently the direction of the fifth cleavage planes, as well as that of others, depends somewhat upon the shape of the cells divided off by earlier cleavages, as the furrows tend to come in at right angles to the longer axis of the cell as seen in surface view. In so far as the external aspect of a blastomere is indicative of the shape of the entire cell, this phenomenon is in agreement with the rule of Errara (Lewis, 1926) that the

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"incipient partition-wall of a dividing cell tends to be such that its area is the least possible by which the space content can be enclosed."

At the completion of the fifth cleavage thirty-two blastomeres are present, lying in four more or less definite horizontal rows or circlets. In most cases, as would be expected from the bilateral arrangement of the micromeres in the sixteen-cell embryo, the eight micromeres of the first or uppermost circlet assume an elongated formation consisting of two rows of four cells each. In other embryos they may be either roughly radial in plan, or may have no apparent regularity. These eight cells tend to be a little smaller than those of the circlet below. They may be triangular, quadrangular, pentagonal, or hexagonal in surface view, while those of the second and third circlets are nearly always quadrangular, and usually rectangular. In almost every embryo, and especially in those of the bilateral type, about two of the eight cells of the first circlet extend into the zone of light pigmentation constituting the gray crescent; they may be only slightly lighter in color than are the opposite micromeres, their lower portions may be only very lightly pigmented, or the entire cells may be light in color. The second circlet of cells always extends into the dorsal zone of light pigmentation, so that two or rarely three cells are lightly pigmented or almost non-pigmented. There is in most cases a sharp demarkation in degree of pigmentation between the cells of the second and those of the third circlet; this follows of course from the fact that the third furrow approximately divides the pigmented from the non-pigmented portion of the embryo. Cells of the third circlet may be only lightly pigmented on the side of the embryo opposite the crescent, and are always without pigment, that is, as light in color as the

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vegetal portion of the embryo, in the region of the crescent. Cells of the second and third circlets are of approximately the same size, although individual differences exist. In the three upper circlets dorsal cells are as a rule smaller than ventral cells. The eight macromeres nearest the lower pole retain the same type of arrangement as in the preceding stage. They may show, depending on degree of pigmentation of individual embryos, no trace of pigmentation, or the upper parts of those cells on the more darkly pigmented side of the embryo may be faintly tinged with brown. Figures 99 to 104 represent views of a "typical" embryo at the thirty-two cell stage.

Up to and including Stage 5 cleavage has been comparatively regular in that, in every stage, embryos show from surface view the expected number and a more or less orderly arrangement of blastomeres. However, with the beginning of the fifth cleavage, in some embryos a factor enters in which tends to modify this condition. This is the formation in some blastomeres of a fifth cleavage plane oblique to the surface. As a result one of the daughter cells has a large and the other only a small surface area. Cells of the latter type migrate or are pushed inward soon after the furrow is completed. This process is the first step in transition of the wall of the blastula from a single cell-layer to that of several cell-layers in thickness.

Inward movement of cells of this type has been observed in living embryos, and the various stages of the process are shown also in fixed material. Figures 105 to 115 illustrate embryos in which one or more of the fifth furrows is separating or has separated blastomeres highly unequal in surface area; Figure 116 shows some of the cells in surface

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view. In embryos in which the cells with small surface area lie at the same level as the other blastomeres, it is evident that only a short time has elapsed since completion of the fifth furrows, since in some (Figs. 105 to 111) the furrow is incomplete in one or more of the blastomeres, and in others (Figs. 112 and 113) there is as yet no indication of formation of sixth furrows. In fifteen other embryos of this type, with the cell in question lying at the same level as the other blastomeres, there is no indication of formation of the sixth furrows, except that in one case, in the dorsal cells of the second circlet, the nuclei are in anaphase or in early telophase. In a few embryos with no evidence of sixth furrows, the cell has been found to be slightly depressed (Fig. 114) or to lie definitely below the level of the other blastomeres (Figs. 115 and 151). In each of two other embryos with the cell in question lying at a much lower level, early sixth furrows are present in three dorsal cells of the second circlet; and in another of the same type, seven cells, located in both dorsal and ventral regions of the second and third circlets, exhibit very early or early furrows. In the later part of Stage 6 the immigrating cells are seen to be almost completely hidden from view, and study of sections has shown that in them the sixth cleavage planes are paratangential or oblique to the surface.

Since the cells in question have been found to possess an internal portion large enough to make their size approximately equal to that of the neighboring cells, their inward movement can probably be explained on a purely mechanical basis; with shifting and readjustment of blastomeres after completion of the cleavage, cells shaped as they are would

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tend to be pushed inward by pressure of the surrounding blastomeres with greater surface area.

Blastomeres with markedly small surface area are usually triangular or wedge-shaped in surface outline. They lie as a rule in the uppermost circlet of micromeres. In one embryo (Fig. 109), which was of an unusual type even before the fifth furrows appeared, the cell belongs in the second circlet. In the elongated bilateral type of micromere configuration the blastomere with small surface area has always been found to lie at one end of the configuration, with no apparent relationship to location of the gray crescent. In embryos of the type shown in Figures 114 and 115, it also lies in the first circlet, and is the upper daughter cell of one of those four micromeres of the sixteen-cell stage which lay somewhat removed from the upper pole. It seems evident that the location of these cells is not determinative, but that they occur in various positions.

Inward migration of cells during the fifth cleavage takes place in only a minority of the embryos.

Reed (1904) made a study of the embryo of frog (species not stated) to determine how the interior cells arise. Her sections showed that as late as the end of the 32-cell stage all the cells are divided by cleavage planes which appear on the surface. Beginning with the fifth cleavage, however, some of the micromeres are divided obliquely, giving rise to some cells that are partly submerged. These cells have the appearance of being pushed into the interior, since the portion visible in external view is small as compared with the portion lying mainly within the embryo. At the next cleavage, she stated, a

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cell of this kind would give rise to one cell extending to the surface and one entirely internal.

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Explanation of Figures

Stage 5 Fifth Cleavage

90. to 97. Acceleration of cleavage in dorsal blastomeres.

90. Furrow complete between a1 and a2, medium in the other dorsal micromeres, almost complete in macromere a3-a4, not evident in any of the ventral blastomeres.

91. Furrows complete in the dorsal micromeres, early in the ventral micromeres, not evident in any of the macromeres.

92. Furrow complete between a1 and a2, and between b1 and b2. Furrows medium in c1-a2, d1-d2, and h1-h2, very early in e1-e2, f1-f2, and g1-g2. Early furrows in a3-a4 and in b3-b4.

93. Furrows complete between a1 and a2, b1 and b2, and c1 and c2. Furrow medium in d1-d2, early in e1-e2 and in h1-h2, and very early in f1-f2 and g1-g2. a3 is almost completely separated from a4. Very early furrow in h3-h4.

94. Furrows medium in b1-b2, c1-c2, and d1-d2, early in a1-a2, very early in the four ventral micromeres. Furrows very early in macromeres a3-a4 and h3-h4.

95. Furrows early in a1-a2, b1-b2, and d1-c2, very early in d1-d2, very early in e1-e2 and f1-f2. No furrows evident in macromeres.

96. Furrows early in a1-a2, b1-b2, and d1-d2, very early in c1-c2 and in the four ventral micromeres. No furrows evident in macromeres.

97. Furrows complete between a1 and a2, b1 and b2, and c1 and c2, medium in e1-e2, and early in d1-d2, f1-f2, g1-g2, and h1-h2. Furrows very early in macromeres b3-b4 and c3-c4. The shape and position of cells d1-d2 and e1-e2, together with the vertical fifth furrows, indicates that the two cells in question were separated by a horizontal fourth furrows.

98. Cleavage not accelerated in the dorsal blastomeres. Furrows medium in b1-b2, a1-a2, h1-h2, and g1-g2, early in f1-f2, and very early in c1-c2, d1-d2, and e1-e2. No furrows evident in macromeres.

99.-104. Views of upper hemisphere, dorsal side, ventral side, right side, left side, and lower hemisphere, respectively, of one of the more 'typical' 32-cell embryos.

105-115. Embryos in which one or more of the fifth furrows is separating or has separated blastomeres markedly unequal in surface area. In those shown in Figs. 105 to 113, the cell with small surface area lies at approximately the same level as do the other blastomeres; in those

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shown in Figs.114 and 115, it is partially submerged.

116. Surface views of

1. Cell a, Fig. 105
2. cell b, fig. 105
3. Cell a, Fig. 106
4. Cell a, Fig. 107
5. Cell b, Fig. 107
6. Cell a, Fig. 108
7. Cell a, Fig. 109
8. Cell b1 and surrounding cells, Fig. 114

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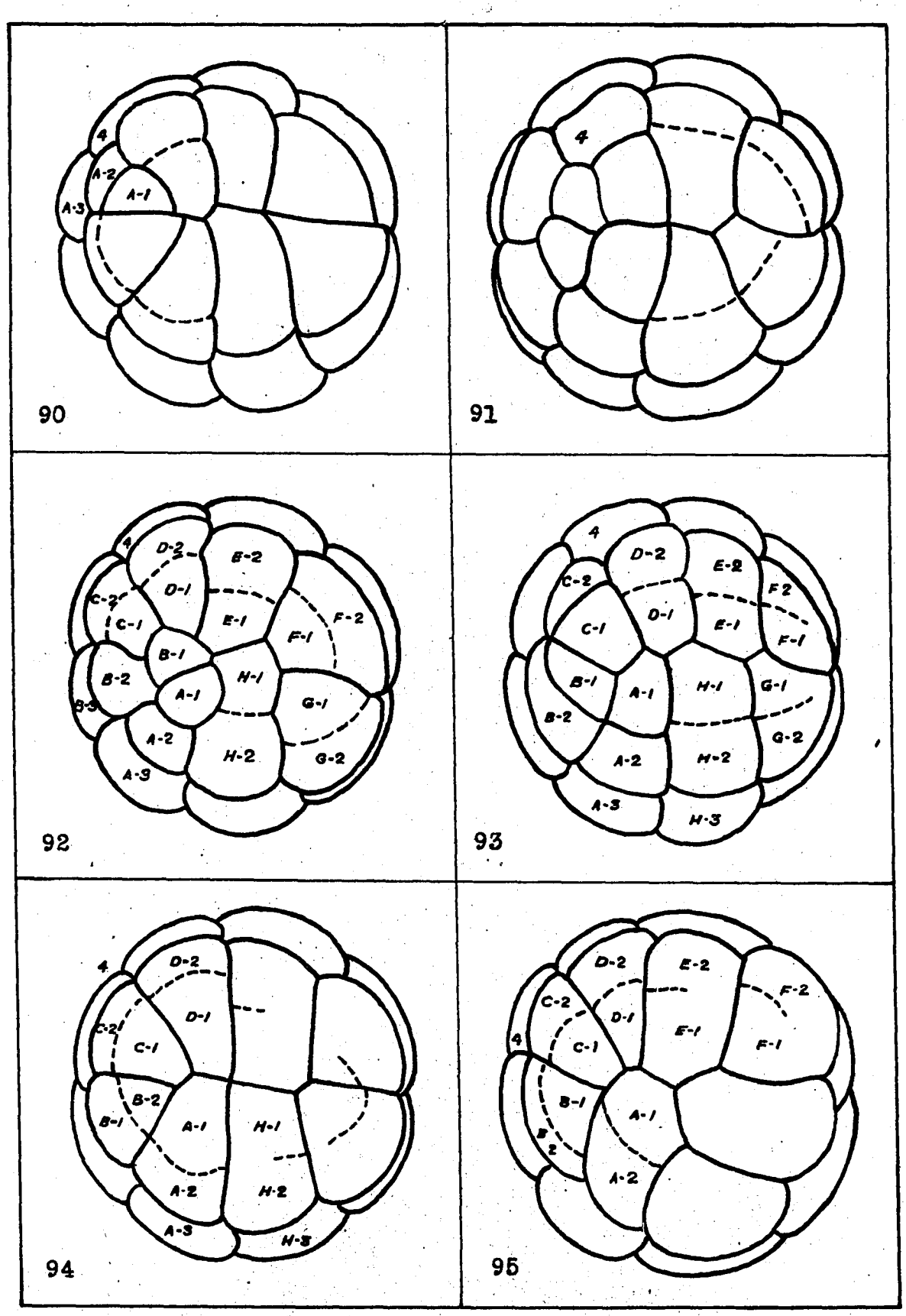

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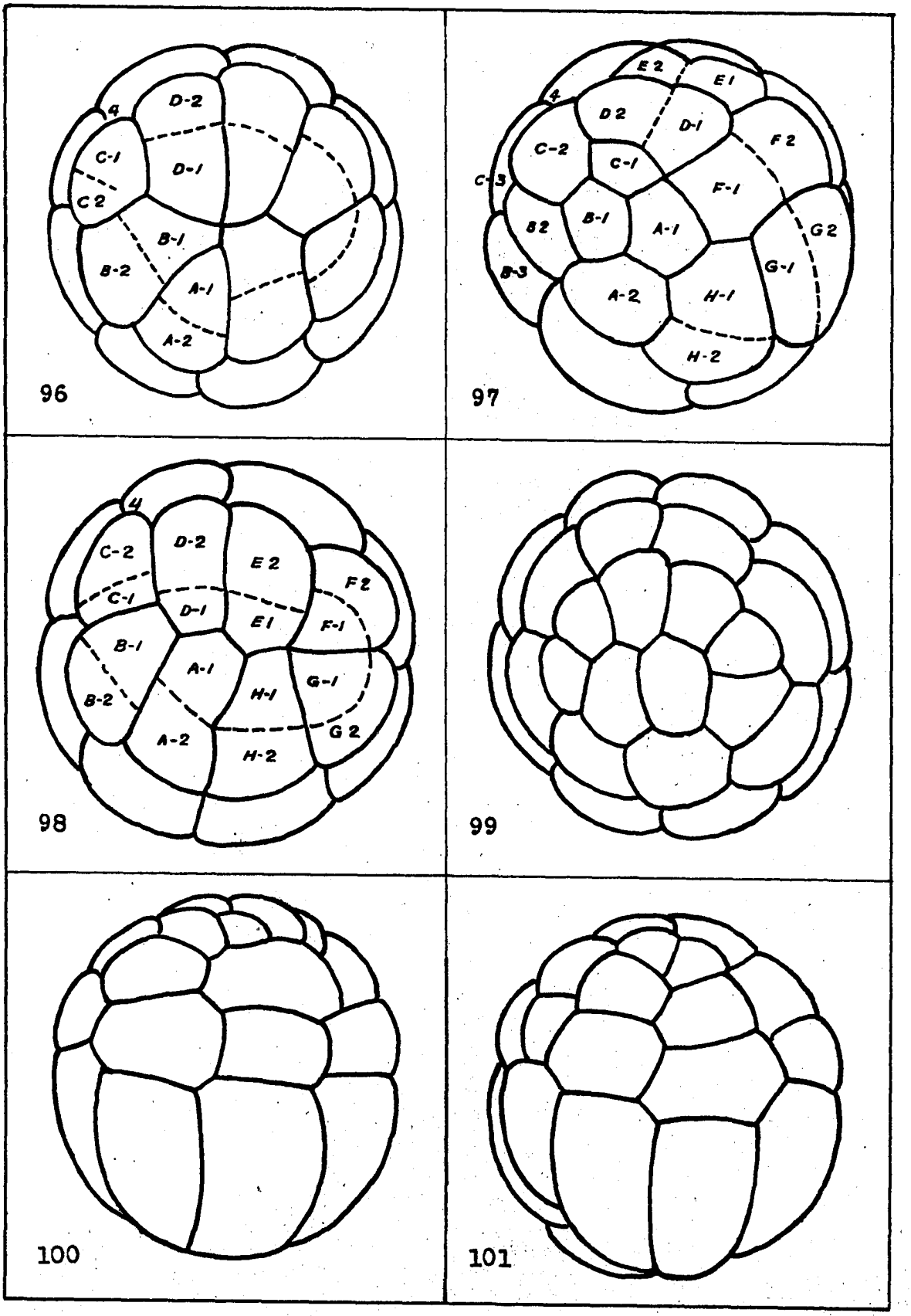

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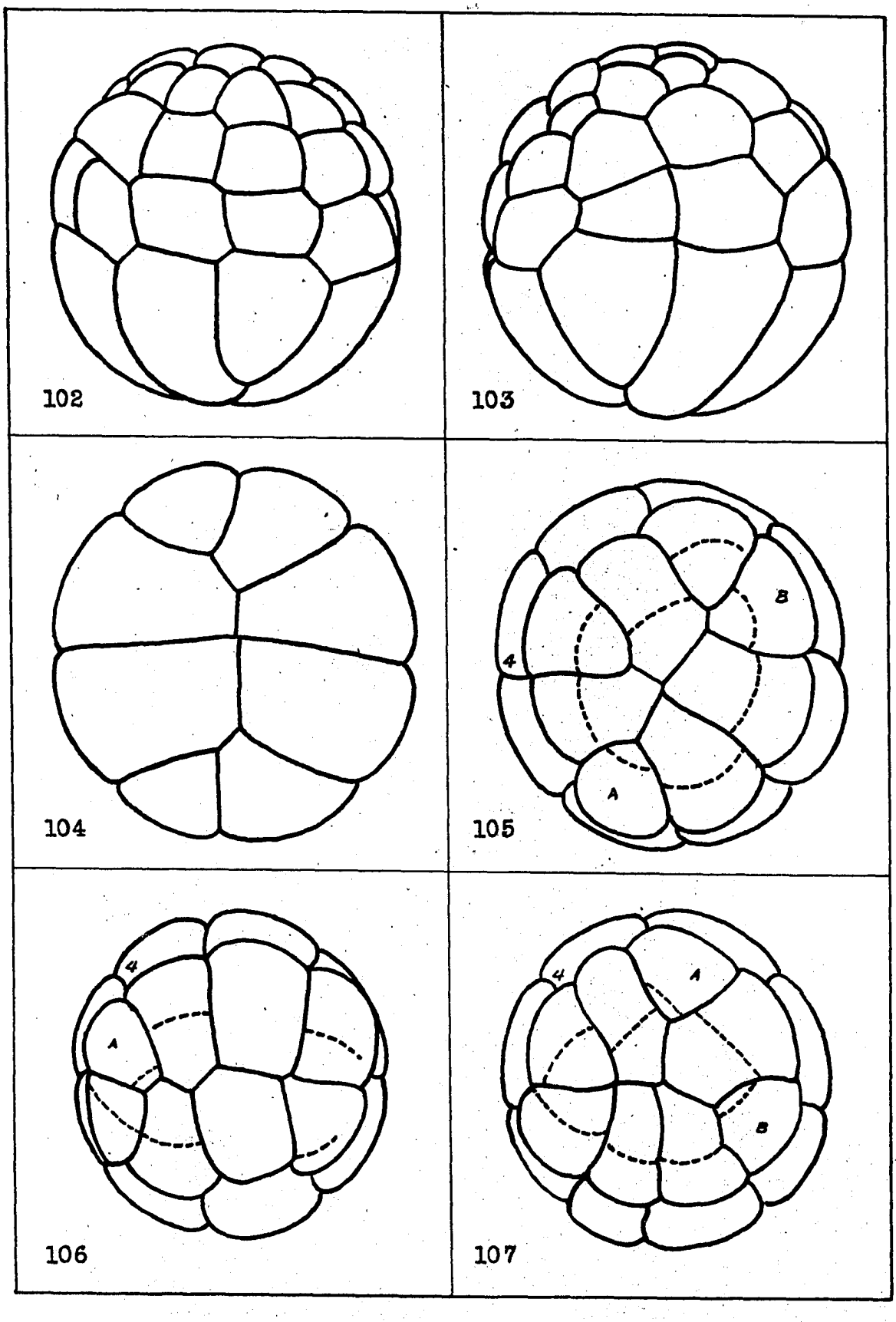

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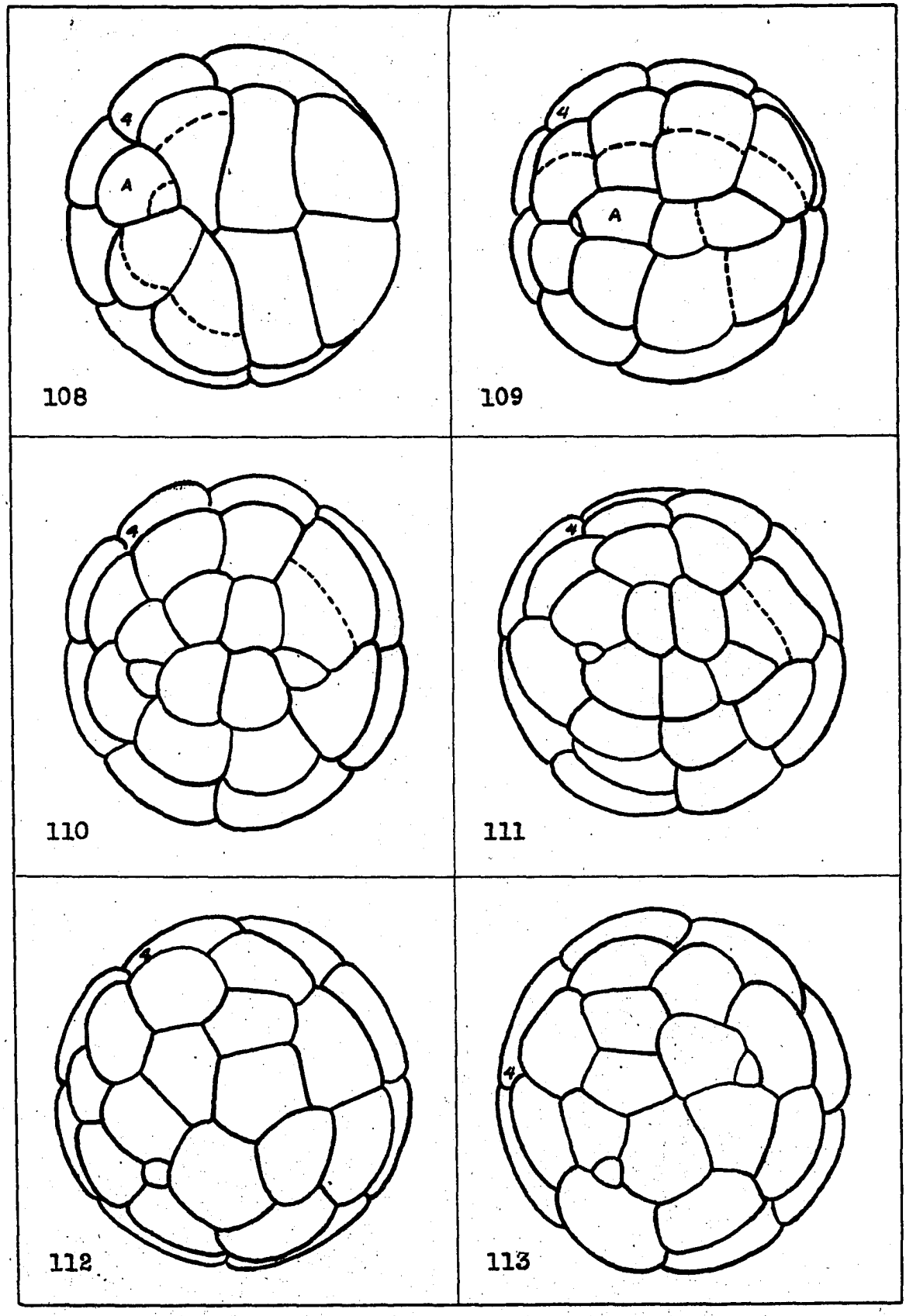

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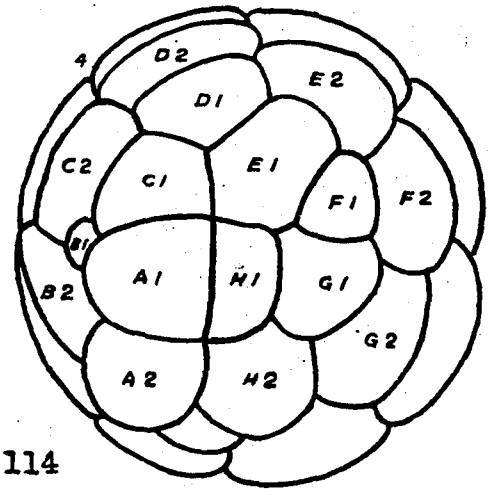

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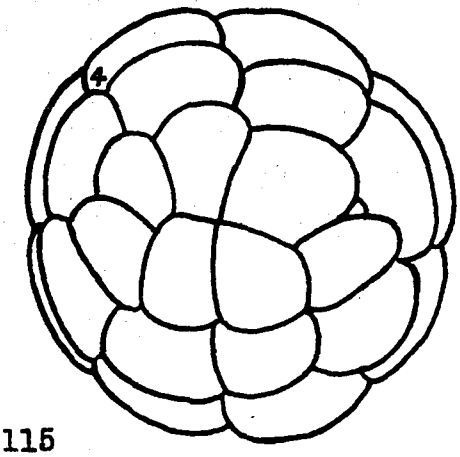

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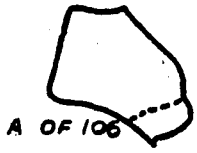

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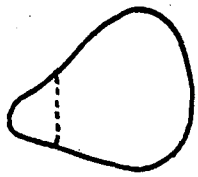

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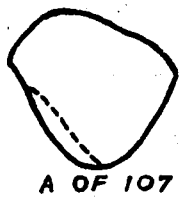

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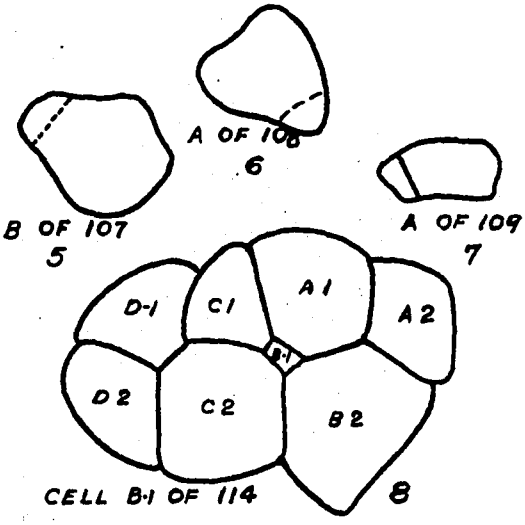

CELL B-1 OF 114
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STAGE 6

Sixth Cleavage

Ten to fifteen minutes after the beginning of the fifth furrows those of the sixth cleavage appear. At this time in many of the blastomeres a small dark spot, plainly visible in both living and fixed embryos, indicates the position of the nucleus with its surrounding mass of brown pigment granules. These dark areas are best seen in dorsal cells of the second and third circlets, although they are sometimes visible also in ventral cells of these two circlets, in cells of the first circlet, and in the macromeres. In later blastulae these dark areas indicating the position of the nucleus can be seen in practically all the external cells and under a sufficiently high magnification their movements during the various phases of the mitotic cycle are easily observed. Appearance of the areas is due to the fact that, with steadily decreasing size of blastomeres, the nuclei are coming to lie nearer the surface; sections show that flattening of cells has not yet occurred.

In Stage 6, as in the preceding ones, the dorsal blastomeres constitute an area of accelerated cell division expressive of the bilaterality of the embryo (Figs. 117 to 120). At the same time, appearance of furrows in the cells of the second and third circlets at a time when few or none are present in cells of the uppermost circlet suggests

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a marginal acceleration of cleavage. Almost all embryos (Figs. 118 to 122) with early sixth furrows exhibit this apparent marginal acceleration, but it is particularly well shown in the embryo represented in Figure 121. However, study of sections shows that in usually from three to four of the central cells of the uppermost circlet the sixth cleavage planes are paratangential or are formed oblique to the surface, and consequently the furrows do not appear in external view. Nuclei of cells of the second and third circlets have been found not to be further advanced as concerns stage of mitosis than those of cells of the first circlet. However,this latter finding cannot be considered as evidence against the occurrence of accelerated marginal division, since, as is pointed out in the discussion of Stage 8, even in those embryos in which there exists undoubted acceleration in formation of cleavage furrows in the dorsal blastomeres, the nuclei of dorsal and ventral cells are not in appreciably different stages of mitosis. On the other hand, the formation in at least some of the central cells of cleavage furrows not visible at the surface leads to the conclusion that there is no real evidence in support of the occurrence of marginal acceleration of cleavage. In Cryptobranchus, Smith (1912) found after the sixth cleavage a deficiency in number of micromeres as compared with the total theoretical expectation, and since his sections showed that no horizontal divisions had occurred in the central region, although some had taken place in the marginal row of micromeres, concluded that cell division was taking place more rapidly in the marginal than in the central region of micromeres-- "a condition which may be the beginning of that accelerated development of the

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margin, the later expression of which is almost wholly internal."

As concerns direction of furrows, the sixth cleavage is predominantly vertical. In the second and third circlets, which at the end of the fifth cleavage consist of cells usually rectangular in surface view and elongated in the latitudinal direction, sixth furrows are almost always vertical, although occasionally oblique. In cells of the uppermost group of micromeres the direction of the cleavage plane varies a great deal. In some cells, particularly in those which are elongated in a meridional direction, it may be oblique to latitudinal; in others it may be vertical, and in still others, as has been stated, the plane of cleavage is either paratangential or oblique to the surface. Furrows in the eight macromeres may be vertical, oblique, or horizontal. In embryos similar to the ones shown in Figures 99 to 104 those four cells which do not extend to the lower pole nearly always divide by a vertical or oblique furrow (Fig. 125), while in the other four furrows are often oblique to horizontal (Figs. 123 and 124). In the latter cells the position of the furrow varies, and the daughter cells may be either equal or markedly unequal in surface view. Again, the four macromeres extending to the pole may divide by a vertical furrow. In a few embryos (Fig. 124) it is indicated that in one or more of the yolk cells a paratangential cleavage plane or one oblique to the surface is formed; study of sections shows that planes of these types do occur at this time in at least some embryos.

Cells which began their inward migration during Stage 5 are seen in the later part of Stage 6 to be almost completely submerged. Sixth furrows often form in such a way as to give rise to cells with small

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surface area which in the later part of the stage begin to move inward. These cells are often located in the central portion of the region of micromeres (Fig. 122). Occasionally they are found also in the second circlet (Figs. 118 and 119), in which case they are long and narrow, or they may (Fig. 117) be formed at the outer margin of the uppermost group of micromeres. Evidently the cells in question are at the sixth cleavage not limited to any one area, but may occur anywhere from the upper pole to the margin of the large yolk cells. Inward migration of cells is of quite common occurrence in Stage 6.

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STAGE 7

Seventh Cleavage

Seventh furrows appear from ten to fifteen minutes after the beginning of the sixth furrows, which are at this time all completed superficially. In most embryos at the beginning of the seventh cleavage the number of cells visible at the surface is less than the theoretical expectation, due to formation of paratangential cleavage planes or planes oblique to the surface and inward movement of cells during the two preceding stages. This discrepancy is noted especially among the central micromeres near the upper pole. In the embryo shown in Figure 126, for instance, there are apparently fifteen macromeres, fifteen cells in the third circlet, sixteen in the second, and only eleven, including the small central and partly submerged cell, in the uppermost group. In this last group of cells, moreover, usually three or four of the central micromeres are noticeably larger in surface area than are the surrounding cells; they are evidently those which at the sixth cleavage were divided by paratangential cleavage furrows.

Seventh furrows vary somewhat as to direction. While some may be vertical and some oblique, the majority of them are latitudinal, all of them showing a tendency to come in at right angles to the longer axis of the cell. As is shown in Figure 126, the dorsal area of accelerated cell division is still evident. No appearance has been seen

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which suggests marginal acceleration of cleavage.

Throughout this stage those cells which began their inward movement during Stage 6 are seen to be well submerged. As has been stated, they lie in various positions, but occur quite commonly at or near the upper pole. No evidence has been seen of formation of cells with very small surface area during the seventh cleavage.

It is to be expected that, since some cells show a tendency to divide more rapidly than others and furrows do not all appear simultaneously, the last division of a group will fall progressively nearer in time to the first of the next group, and the distinction between sets of furrows will eventually be lost. In Scaphiopus, embryos with early seventh furrows show that acceleration of cleavage in the dorsal blastomeres is not contributing to loss of synchronism, since dorsal cells are dividing, as in earlier stages, only slightly in advance of ventral cells. With the seventh cleavage, however, the tendency toward loss of synchronism is manifest in a lag in macromere division more marked than that in earlier stages. This point is illustrated by embryos similar to those shown in Figures 127, 128 and 129. In these three embryos the seventh furrows are apparently completed in all the micromeres and transitional cells at a time when no furrow (Figs. 127 and 128) or only one furrow, and that an early one (Fig. 129), has appeared in the macromeres near the lower pole. Seventh cleavage furrows in these large yolk cells are completed only three to four minutes before the beginning of the eighth cleavage. In some of the macromeres seventh furrows fail to appear at the surface; since sections show that after completion of the seventh cleavage some of

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these cells have been divided by paratangential cleavage planes, it can be assumed such was the case in the cells in question.

After completion of the seventh set of furrows, cleavage becomes quite irregular. Each embryo apparently cleaves according to a pattern of its own, and embryos can no longer be even roughly grouped into types. The tendency toward alternation of vertical and latitudinal sets of furrows becomes disturbed, and cleavage planes occur in almost any direction. Blastomeres can no longer be distinguished as constituents of a particular circlet, and their shape and distribution vary a great deal. They tend to be pentagonal or hexagonal in surface view, rather than triangular or quadrangular as in preceding stages.

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Explanation of Figures

Stages 5 and 7 Sixth and Seventh Cleavages

117-120. Acceleration of cleavage in dorsal cells.

117. One medium and four early furrows in dorsal cells. One cell of the first circlet is being divided into blastomeres of highly unequal surface area.

118. Furrow complete in one dorsal cell, early in four, very early in two. Among ventral cells, furrows medium in two cases, early in four cases. One cell of the second circlet is being divided into parts unequal in surface area.

119. Furrow complete in one dorsal cell of first circlet and in one of second circlet, all other furrows early. Two cells of the second circlet are being divided into blastomeres of unequal surface area.

120. Among dorsal cells, two medium furrows in cells of second circlet, and early furrows in one cell of second circlet and in one of third circlet. Very early furrows in two ventral cells.

121. Apparent marginal acceleration of cleavage. Early to medium furfows in fifteen cells of the second and third circlets, and none in the eight cells of the first circlet nor in the eight macromeres.

122. Apparent marginal acceleration of cleavage. A cell near the upper pole is being divided into blastomeres of unequal surface area.

123 and 124. Sixth cleavages in the lower hemisphere.

125. Macromeres of the embryo shown in Fig. 123.

1. Those of the left side (with reference to gray crescent)

2. Those of the right side (with reference to the gray crescent).

126. Early seventh furrows, slightly further advanced in dorsal than in ventral cells.

127. to 129. Lower hemispheres of three embryos in each of which the seventh cleavage is completed among the micromeres and transitional cells.

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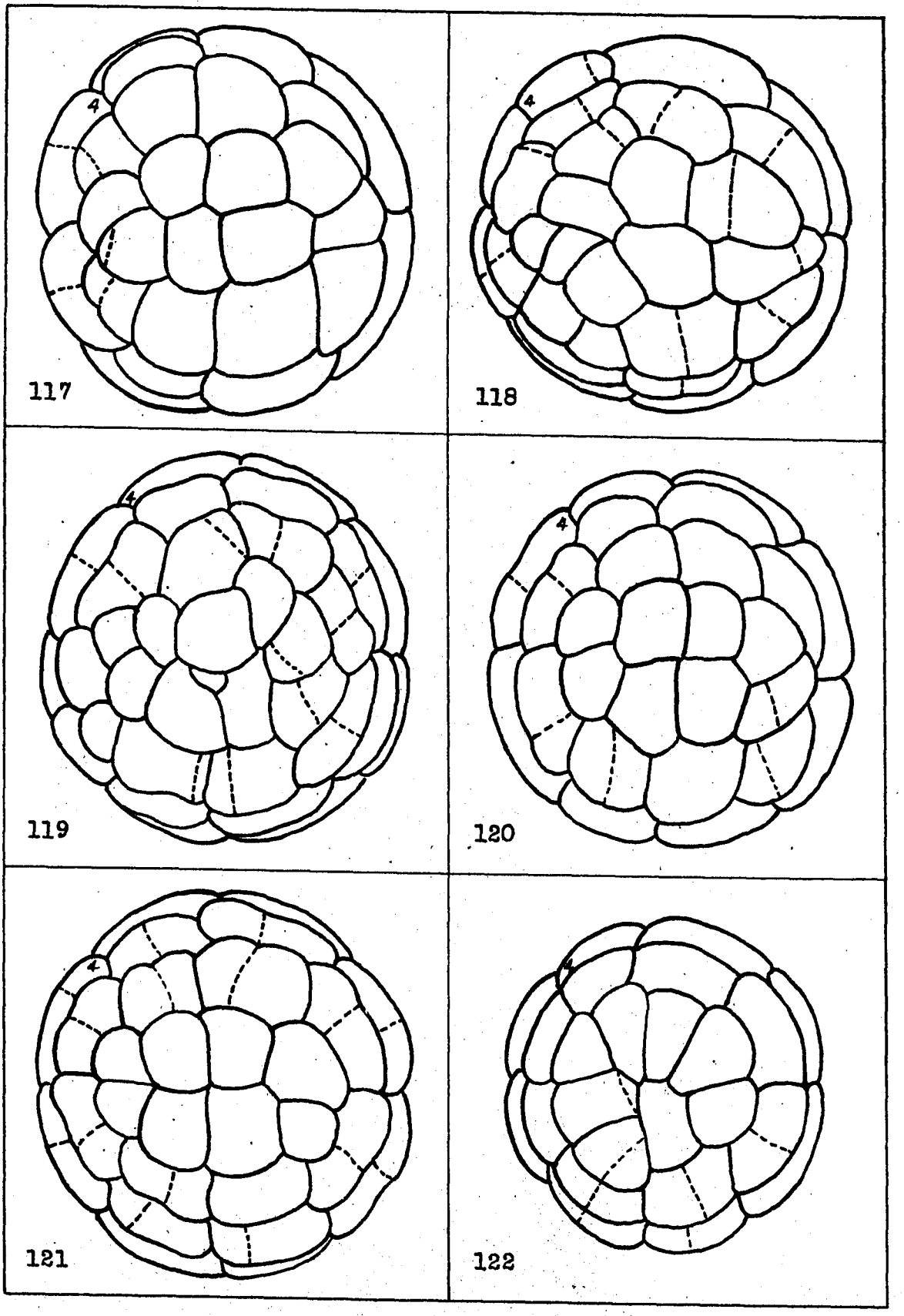

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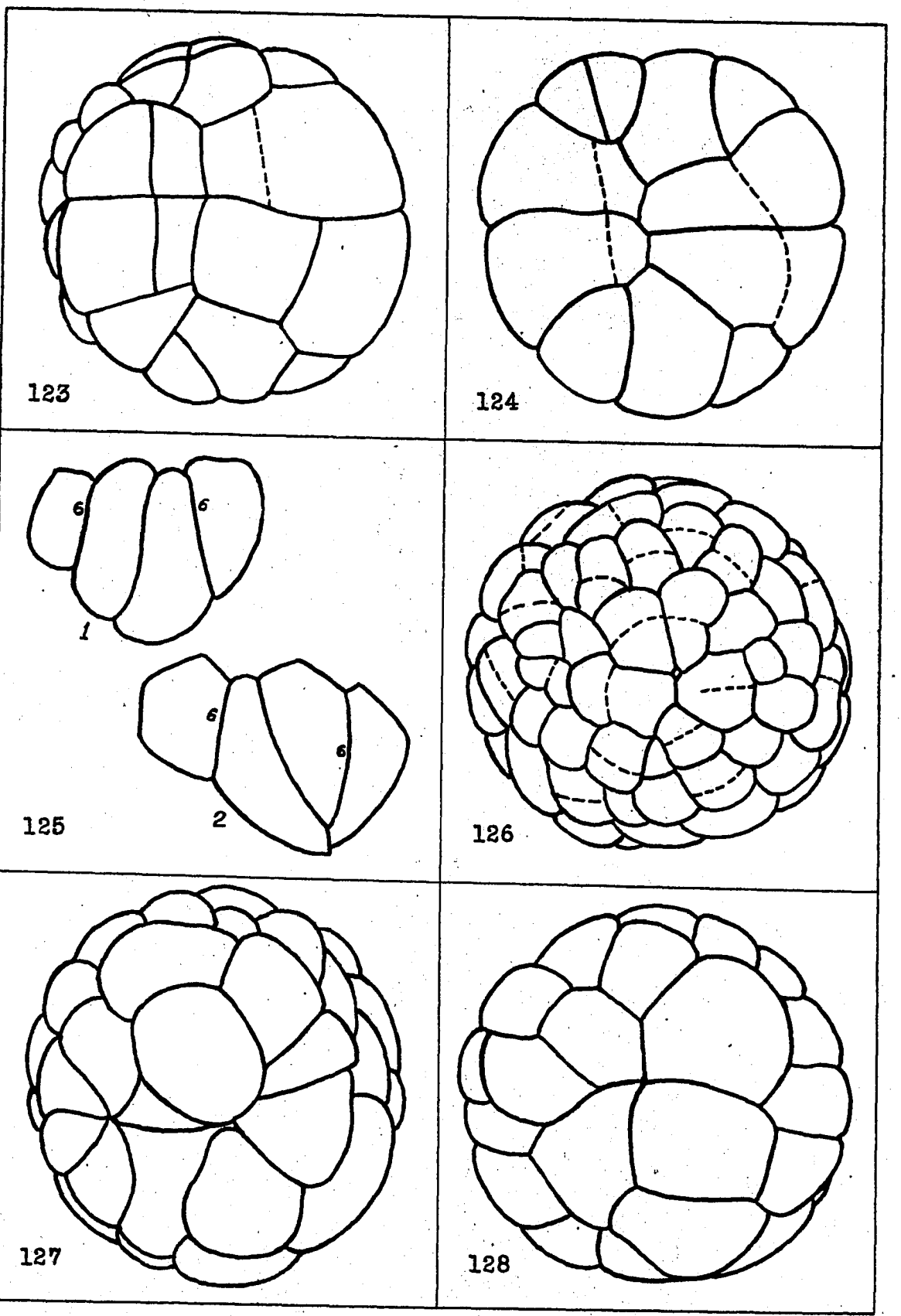

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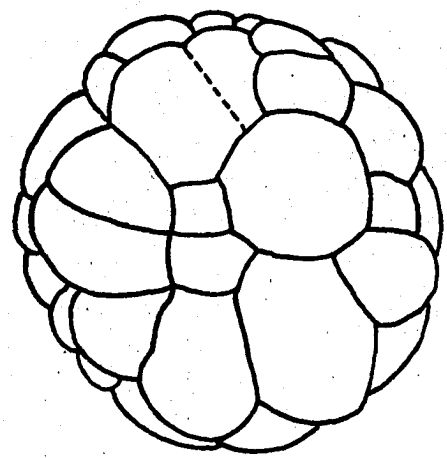

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Stage 8

After completion of the seventh cleavage it becomes convenient for purposes of description to discuss development in terms of arbitrarily fixed stages. Each stage is considered as extending to the beginning of the succeeding stage, although, in each, description is based mainly on embryos in that state of development which initiates the stage. Stages 8, 9, 10, and 11 each extends over several cell generations.

Stage 8 begins, at 2½ to three hours after the egg has been laid, with the first appearance of the eighth set of cleavage furrows. In certain blastulae at the beginning of the stage there are a few large central cells near the upper pole, but in most this is not the case. Embryos with early eighth furrows show that dorsal cells are still cleaving slightly in advance of ventral cells. In several embryos nearly all the dorsal micromeres and dorsal transitional cells are in apparent telophase and early to medium furrows are present; in a number of the ventral micromeres and ventral transitional cells, slightly earlier furrows are present. Macromeres in these embryos show no indications of furrows.

Although in all cleavages from the second to the eighth, inclusive, it has been shown that undoubted acceleration exists in formation of cleavage furrows in the dorsal as compared with the ventral

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blastomeres, there is not during any of these cleavages any appreciable difference in stage of mitosis between nuclei of the dorsal and ventral cells when considered as groups. Such minor differences as have been found to exist are no more than those slight variations which exist among nuclei of cells of either region as considered in itself. No doubt this accounts for the fact that, as far as it has been followed, acceleration of cleavage in dorsal blastomeres does not result in an increasing disturbance of synchronism; at the eighth cleavage, for instance, there is no greater discrepancy between times of appearance and completion of furrows in the dorsal and in the ventral blastomeres than there was at the third or fourth cleavage. That synchronism still exists among the micromeres and transitional cells at the end of Stage 8 is shown by the fact that the nuclei of these cells are, as seen in sections, all in practically the same stage of mitosis. Thus it is obvious that at least up to the end of Stage 8 the smaller size of dorsal cells has not been brought about by the occurrence of more divisions among them than among the ventral cells. In this connection, it may be noted that in the four Amphibia which they studied Jordan and Eycleshymer (1894) found synchronism existing as long as they were able to follow cleavages in the living embryo (up to the tenth set in Ambystoma).

Small cells extend, at the middle of Stage 8, about to the equator, the number of and area covered by them having been increased by addition of small cells cut off at the upper parts of the macromeres. Below lie cells of an intermediate size (about two in any one meridian) and around the lower pole from four to eight larger yolk cells or macro-

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meres. The dorsal portion of the embryo is, as in earlier stages, characterized by the presence of smaller cells and less pigment as compared with the opposite region. In embryos fixed in formalin those cells in the region of the gray crescent have a slightly translucent appearance. During Stage 8 it is no longer possible to distinguish first and second furrows in the upper hemisphere. In the lower hemisphere, however, it is usually possible to locate the intersection of the first two furrows. With the approximate location of the lower pole thus revealed, it can as a rule be determined that on the dorsal side of the embryo, as compared with the ventral, the small cells extend slightly nearer this pole and that the transition between smaller and larger cells is more gradual.

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Stage 9

Stage 9 is reached about one hour after the beginning of the preceding stage. Outlines of the micromeres are still plainly visible at a magnification of fifteen diameters. At the beginning of the stage there are present in any lateral meridian (that is, a meridian in the lateral rather than in the dorsal or ventral part of the blastula) about twenty micromeres, seven transitional cells, and three macromeres. Since it is almost impossible to follow early furrows in cells so small, external features give no clue as to whether the dorsal portion of the embryo still constitutes an area of accelerated cell division nor as to the extent to which synchronism persists.

In all embryos the entire pigmented hemisphere has a splotched appearance due to the fact that, whereas in earlier cleavage stages pigment has extended equally over the surface of the blastomeres and down into the furrows, it now fails to extend down into the furrows or into those portions of the cells immediately adjacent to furrows. This condition arises in the latter half of Stage 8, and persists in succeeding stages. In embryos in which this peculiar distribution of pigment first becomes evident there is an appearance which suggests downward movement of certain cells toward the germ ring, but which may be due only to that shifting and rearrangement of cells which follows cleavages; at the boundary between pigmented and non-pigmented

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cells one often sees in various meridians one or more pigmented cells extending down into the zone of non-pigmented cells and entirely surrounded by these latter except at the top. During Stage 9 the same appearance is noted.

In this stage, as in the preceding one and the two succeeding ones, the dorsal portion of the embryo is marked by the presence of small and lightly pigmented cells, and by the slightly lower extension of small cells and the more gradual transition between small and large cells in the dorsal than in the ventral region. In each of the four stages, this external evidence of bilaterality is paralleled by evidence of bilaterality in the internal structure of blastulae; the dorsal wall of the blastocoele is as a rule thinner than the ventral wall.

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Stage 10

One hour after the beginning of Stage 9, and about five hours after egg-laying, embryos have reached the beginning of Stage 10. Both micromeres and macromeres are much smaller than in the preceding stage, but cell outlines in any part of the blastula can be seen at a magnification of forty diameters. About thirty micromeres, ten transitional cells, and five macromeres appear in surface view in any one lateral meridian. The peculiar distribution of pigment noted in the two preceding stages persists, but cells and their pigmented areas are so much smaller that under low magnifications the blastula has a speckled appearance. That a considerable amount of downward movement of micromeres has taken place is evidenced by the extension of pigmented cells to, and in the later parts of the stage slightly below, the equator. Pigmented cells often extend downward as irregularly shaped and not very sharply delimited bands or tongues separated by areas of light-colored cells. Dorsal micromeres and transitional cells remain smaller and lighter in color than ventral ones.

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Stage 11

Stage 11 (that of the late blastula) immediately precedes gastrulation. It begins about six and one-half hours after egg-laying, and extends to the first appearance of the dorsal lip of the blastopore. Externally, it differs from Stage 10 only in the smaller size of the blastomeres and in the slightly lower extension of pigmented cells. Cell-outlines can still be seen at a magnification of forty diameters, but only with an illumination which throws into relief the surface of the embryo. About fifty micromeres, fifteen transitional cells and eight macromeres appear in a lateral meridian. The "speckled" appearance is retained, but it is noted that the pigment is not always equally distributed among the cells of a certain area, some having a much lighter or darker pigmentation than do the neighboring cells. Dorsal and ventral regions can still be distinguished. Measurement of 52 embryos shows that there has been no appreciable increase in total size throughout the cleavage period; the average diameter is the same as that of the uncleaved egg. The chorion has not increased in diameter, and both inner and outer jelly envelopes remain the same thickness as in the egg just before the first cleavage.

According to Bellamy (1919) the greatest downward movement of the germ ring takes place in the region of the future dorsal lip, indicating that growth in length (between the apical pole and the germ ring)

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is greatest and proceeds most rapidly in the sagittal plane. "At the time of gastrulation some of the material included in the dorsal lip cells is certainly not less than 100° from where it was in the four-cell stage." Dürken (1932) and others have described similar cell movements. In Scaphiopus, at the beginning of Stage 11 pigmented cells extend downward about the same distance in all meridians. Without experimental work, no statement can be made as to whether or not downward movement is greatest among the dorsal cells.

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Stage 12

Stage 12 begins, at seven hours after egg-laying, with the appearance of the dorsal lip of the blastopore, and extends up to the time when, three and one-half hours later, early neural structures first become visible externally.

By means of a very simple experiment it was shown that the method used in locating the dorsal portion of the embryo in this stage, as well as in the four preceding stages, is trustworthy. Twenty-one embryos with the dorsal lip just appearing were selected at random, that is, without reference to whether or not the dorsal region was plainly marked, and were oriented with the animal pole uppermost, so that the blastopore lip was not visible. The embryos were examined one at a time, and that portion characterized by smaller and lighter pigment spots was assumed to be the dorsal region. In all except one case, in which the situation was doubtful, the dorsal lip, when the embryo was turned over, was found to lie in the region which had been assumed to be dorsal.

At the time the first indication of the dorsal lip appears small pigmented cells extend somewhat below the equator. The blastopore lip appears, about 20^{o} below the equator, at the boundary between these cells and the larger light-colored transitional cells, at a place where the transition in cell-size and degree of pigmentation is rather abrupt. It appears first as a shallow irregularly shaped depression varying

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somewhat in appearance in different embryos, but usually elongated in the equatorial direction and about twice as long as wide, with the deepest part of the depression exactly at the border between the two types of cells. The depression, which is at first so slight as to be visible only when the dorsal portion of the embryo lies in partial shadow, involves an area five to six cells in width. At this time the surface of the gastrula both above and below the dorsal lip curves gradually into the depression. Deepening progresses at first with little increase in length of the depression, and the slope of the sides becomes more abrupt. By the time elongation of the blastopore lip is noticeable the latter has assumed a crescentic shape (occasionally it is crescentic at its first appearance). In any case, the lip fifteen minutes after its appearance is well established as a crescent. With elongation the depression narrows and deepens, and the inward slope of transitional cells into it becomes more gentle, while the portion forming the dorsal lip dips abruptly down into the groove. As gastrulation proceeds and the depression narrows until visible only as a cleft, the cells on both sides of it descend abruptly into the cleft. In some embryos in the dorsal and crescentic lip stages there is an accumulation of brown pigment at the bottom of the blastoporic depression and on the cells of the dorsal lip. By the time the crescentic blastopore has become well established the occurrence of pigment in these regions has become more common, and often there are pigment strands converging into the blastopore from some distance above it.

As the blastopore lip continues to extend and approaches a semi-

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circular shape, the boundary between micromeres and transitional cells becomes more sharply marked. The blastoporic groove extends along this boundary line as a cleft which is at first slight but which rapidly deepens. At about thirty minutes after appearance of the dorsal lip the blastopore has assumed a semi-circular shape, and fifteen minutes later it is established as a complete circle. As the blastoporic groove lengthens the circular area composed of non-pigmented cells becomes smaller due to continued downward movement of the germ ring, so that the ventral lip is completed at a lower level than that at which the dorsal invagination first appeared. The blastopore lip at any stage until it becomes circular forms an arc of that circle of steadily decreasing diameter which marks the boundary between micromeres and yolk cells.

One and three-fourths hours after appearance of the dorsal lip, the yolk plug has a diameter only half that in the early circular blastopore stage. At about this time the outer jelly envelope begins to slough off.

During the early phases of gastrulation the entire upper hemisphere has an irregularly rough and bumpy appearance, better marked in some embryos than in others. About the time the blastopore becomes complete there is formed, beginning usually on the dorsal side, a circle of sunken cells (Fig. 152) lying slightly above the equator and approximately opposite the yolk plug. As gastrulation continues, this circle of sunken cells moves toward a point opposite (or slightly ventral to the point opposite) the center of the yolk plug. Decrease in diameter of the circle during this movement results in its complete dis-

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appearance at the time the yolk plug has been reduced to a diameter of about sixteen to twenty degrees. Dissection of embryos and study of sections show that this circle of sunken cells marks the boundary of the roof of the blastocoele; thus it is analogous to the 'Scheidewandfurche' or 'septal furrow' of Cryptobranchus japonicus and Cryptobranchus allegheniensis as described by, respectively, Ishikawa (1908, 1909) and Smith (1912). The area enclosed by the 'septal furrow' may be considered analogous to the 'Keimholensegment' of Ishikawa and to the 'fenestra' of Smith (1912). It is not transparent, nor is its surface cut up into small polygonal areas each comprised of several cells and separated by lines resembling cleavage furrows, as is the case in Cryptobranchus allegheniensis. In both living and fixed embryos of Scaphiopus, the area merely has an irregularly rough and bumpy appearance.

Smith (1912) stated that he has found in the literature no other mentions of similar structures except a description by Hatta (1907) of such a 'boundary groove' in the gastrula of Petromyzon. In Scaphiopus the 'septal furrow', although it takes the form of a circle of sunken cells rather than that of a sharply defined furrow, is well marked in both living and fixed embryos, and in the former its forward and upward movement marking the decreasing extent of the blastocoele is easily followed. I have found no mention of such a furrow in any other anuran gastrula. Both Hatta and Smith have concluded that the furrow is passive in origin and a product of gastrulation. In both Cryptobranchus and Petromyzon, according to Smith, the egg contains considerable yolk, and the roof of the blastocoele is unusually thin; the

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occurrence of the 'septal furrow' he regards as a remarkable case of convergence in purely embryonic characters. In Scaphiopus also the furrow is apparently passive in origin; since the roof of the blastocoele is not unusually thin, it seems possible that the appearance of the furrow may result from the rapidity with which gastrulation takes place.

The blastopore lip remains circular until the diameter of the yolk plug has been reduced to about fifteen degrees, and then becomes ovoid. By this time, rotation of the gastrula has brought the blastopore into a posterior or slightly dorsal-posterior position.

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Stage 13

Three and one-half hours after appearance of the dorsal lip, and about the time the blastopore has become ovoid in shape, the position which the neural groove will occupy is marked out by a line of pigment, not very sharply delimited, extending forward from the blastopore. As the lateral lips of the blastopore approach each other this line becomes slightly sunken to form the neural groove, and the thickened margins of the medullary plate appear. One hour later, by the time the yolk plug is entirely withdrawn and the lateral lips of the blastopore are closely appressed, the neural groove has extended considerably in length and the neural ridges are well marked in their antero-lateral and transverse portions. No elongation has taken place and the embryo is still approximately spherical. The neural ridge is narrower and lower in its transverse portion than it is laterally. Sense plate and gill plate are indicated by slight thickenings. Posteriorly, the borders of the medullary plate are not yet elevated to form ridges. The neural plate is lightly pigmented throughout its entire area, while the various regions of the neural ridge are more heavily pigmented, particularly at the inner boundary. The neural groove is shallow and narrow, and is still marked by a line of pigment; anteriorly it joins (or divides into) two grooves which extend into two slightly sunken and deeply pigmented areas (optic rudiments) located in the inner border

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of the transverse fold. The distance from blastopore to the outer boundary of the transverse fold falls a little short of 180 degrees. Fifteen minutes later (Fig. 153) the optic depressions are deeper, and sense plate and gill plate are further elevated. Evidences of segmentation of the neural plate inside the neural ridge in these embryos, as well as in those in the early part of Stage 14, are expressed faintly if at all.

During stage 13 sloughing off of the outer jelly layer continues, although the layer remains as a cap-like mass adhering to one side of the inner jelly layer; the latter is at this time still adherent to the chorion. The chorion has at Stage 13 increased a little in diameter, and does not surround the embryo as closely as in earlier stages. As development proceeds and growth in length of the embryo occurs, the chorion expands a great deal, and by the time of hatching it has attained a diameter from two to two and one-half times that in the cleavage stages.

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Stage 14

14-1. Stage 14-1 is reached two and one-fourth hours after the beginning of the preceding stage, or one hour later than the stage shown in Figure 153. The embryo (Figs. 130, 131) has elongated slightly in an antero-posterior direction. The neural folds have approached each other to a considerable extent, and their elevation has proceeded posteriorly to the level of the blastopore. Sense plate and gill plate are fairly well defined. The neural groove remains visible as a narrow pigmented line, slightly depressed, extending the full length of the neural plate from blastopore to anterior end. The transverse neural fold has extended posteriorly to a slight extent, overarching the neural plate and contributing to closure of the neural folds; in this process the optic rudiments are becoming covered. In the embryo sketched they are still indicated, and the crescent shaped area at the anterior end of the neural plate and just inside the transverse fold is deeply pigmented. The neural tube is open more widely in its anterior half (head region) than elsewhere.

During Stage 14-1 movement of particles can be seen in the fluid surrounding the embryo, indicating that ciliation is becoming established. In Rana temporaria also, streaming of suspended granules is first seen at about this stage, according to Assheton (1896).

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14-2. Twenty-five minutes later (Fig. 154) the neural folds have come together and fused in the mid-region, but the tube is still open in the region of the head and in that portion extending a little distance anteriorly from the blastopore. The sense plate is better developed, and the gill plate in some embryos shows evidences of becoming divided into an anterior and a posterior part. The embryo has elongated a little more than in Stage 14-1.

14-3. At the end of the next twenty minutes, the neural folds have come together and fused throughout their entire length, and lie closely appressed, with the line of union marked by a deep median groove (Figs. 132, 133). That portion of the neural tube just anterior to the proctodaeal pit is the last to close. The dorsal body-line is in some embryos still almost straight, but in most cases it is slightly concave, head and tail being a little upraised above the mid-dorsal region. A vertical line has appeared indicating the position of the first branchial grove. In the mid-portion of the sense plate just ventral to the end of the line formed by union of the neural folds is a slight depression; this will deepen to form the mouth depression, and in it at a later stage the stomodaeal invagination will appear. In most embryos both the upper and the lower portions of the blastopore remain open.

14-4. Thirty minutes later, the line of fusion of the neural folds is marked only by a slight pigment line. (Figs. 134, 135). The gill plate is more massive, and head and tail upraised a little more.

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The upper opening of the blastopore persists as a deep pit. At this time the chorion has increased somewhat in diameter, and is becoming less taut. A thin layer of the inner jelly envelope still adheres to it. In some embryos the outer envelope remains as a cap-like structure, while in others it has been lost.

Rotation begins in some embryos in Stage 14-3, at about the time head and tail become slightly upcurved. In others it may occur slightly later, but in all cases it is well established by Stage 15-2. Up to about Stage 14-3 embryos lie with the ventral surface down. At about the time rotation begins the head comes to lie slightly higher than the rest of the body, and the embryo, although inclining slightly to one side, is supported mainly on the ventral surface of body and tail. Ciliary beat is evidently in such a direction as to move the head up and over, but gravity prevents the completion of such a movement. As a result of the two forces, the embryo moves in a clockwise or counterclockwise direction, with the head rising higher and higher, until at a certain point it becomes overbalanced and topples to one side, toward the outside of the circuit. The circuit is then repeated, and toppling occurs each time at about the same point. Embryos rotating in a clockwise direction fall on the right side after upraising of the head, while those rotating in a counterclockwise direction fall on the left side.

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Explanation of Figures

130. Stage 14-1. Dorsal view.

131. Stage 14-1. Lateral view.

132. Stage 14-3. Dorsal view.

133. Stage 14-3. Lateral view.

134. Stage 14-4. Dorsal view.

135. Stage 14-4. Lateral view.

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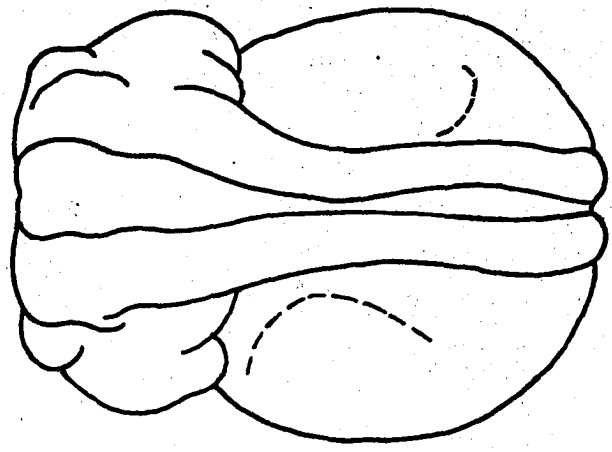

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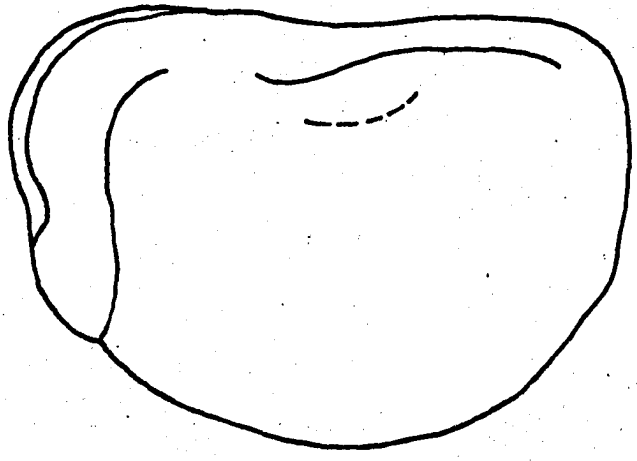

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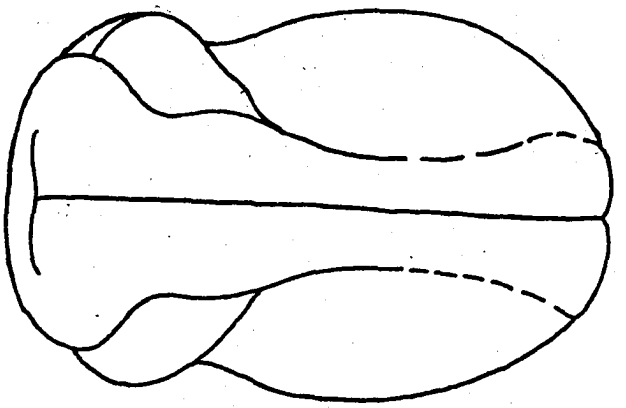

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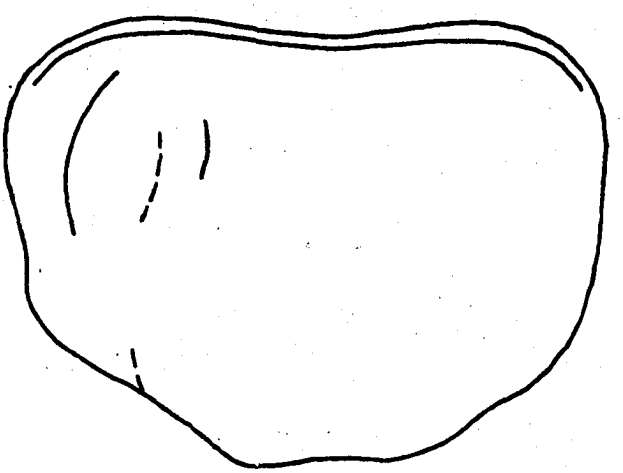

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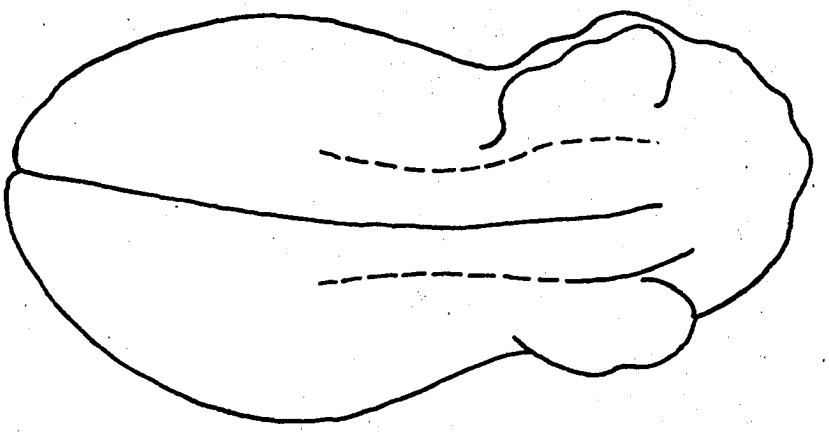

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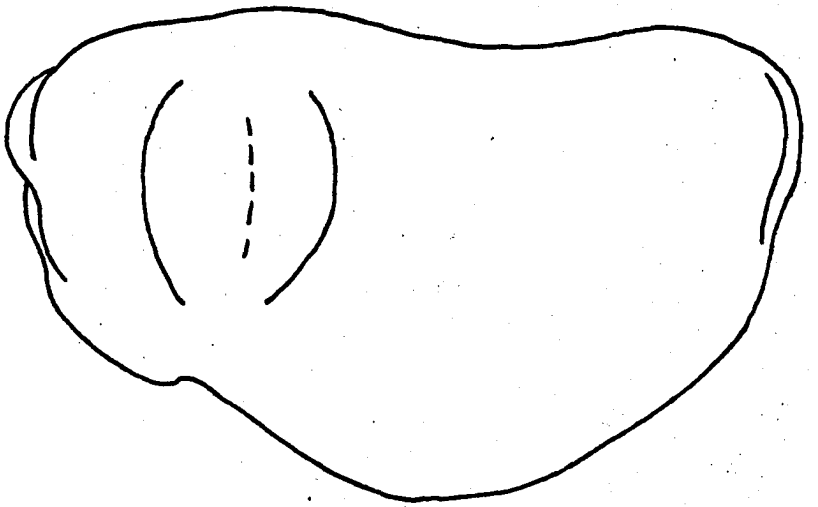

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Stage 15

15-1. Stage 15-1 (Fig. 155) is reached sixty minutes after the beginning of Stage 14-4, or fifteen hours after egg-laying. A second external branchial groove is present as a vertical depression posterior to the first one. The position of pronephoros and pronephric duct are indicated externally by raised areas. Meseblastic somites are beginning to be faintly indicated in the mid-dorsal region. The mouth depression has deepened and elongated dorso-ventrally, and the position which the adhesive organ will occupy is marked by a slight accumulation of pigment, as yet not very definitely delimited. Elevation of head and tail are better marked. The line of fusion of the neural folds is still indicated by a narrow band of pigment. At the posterior end of this band the proctodaeal pit is now seen as a slightly elongated depression.

15-2. One-half hour later, embryos have reached the stage shown in Figures 136, 137 and 138. The body has elongated more, and the chorion has expanded; inside the latter the embryo is still straight, that is, length of body and diameter of chorion are about the same. The tail length is between one-fourth and one-third the length of the body. The elevation marking the position of the pronephros is higher and better marked, and that indicating the duct extends further post-

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eriorly. The outlines of the three primary brain vesicles are indicated. The auditory sacs or otocysts appear as crescent-shaped spots on the sides of the head. Lateral to and slightly above the mouth depression is a pair of invaginations marking the location of the olfactory organs. The head of the embryo, as viewed from the front, is now about twice as long as wide, and the mouth depression extends over about half its length, reaching ventrally almost to the adhesive organ. The latter is now definitely established as a widely U-shaped depression more heavily pigmented than the surrounding ectoderm. From the mid-dorsal region to about the tip of the tail the ectoderm overlying the neural tube is drawn into a thin ridge, the first indication of the dorsal fin or web of the tail. Elongation of the tail has increased the distance from its tip to the proctodaeal pit, giving to the latter, comparatively speaking, a more ventral position. The cap-like outer jelly envelope is still retained in the case of some embryos.

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Explanation of Figures

136. Stage 15-2. Dorsal view.

137. Stage 15-2. Lateral view.

138. Stage 15-2. Antero-ventral view.

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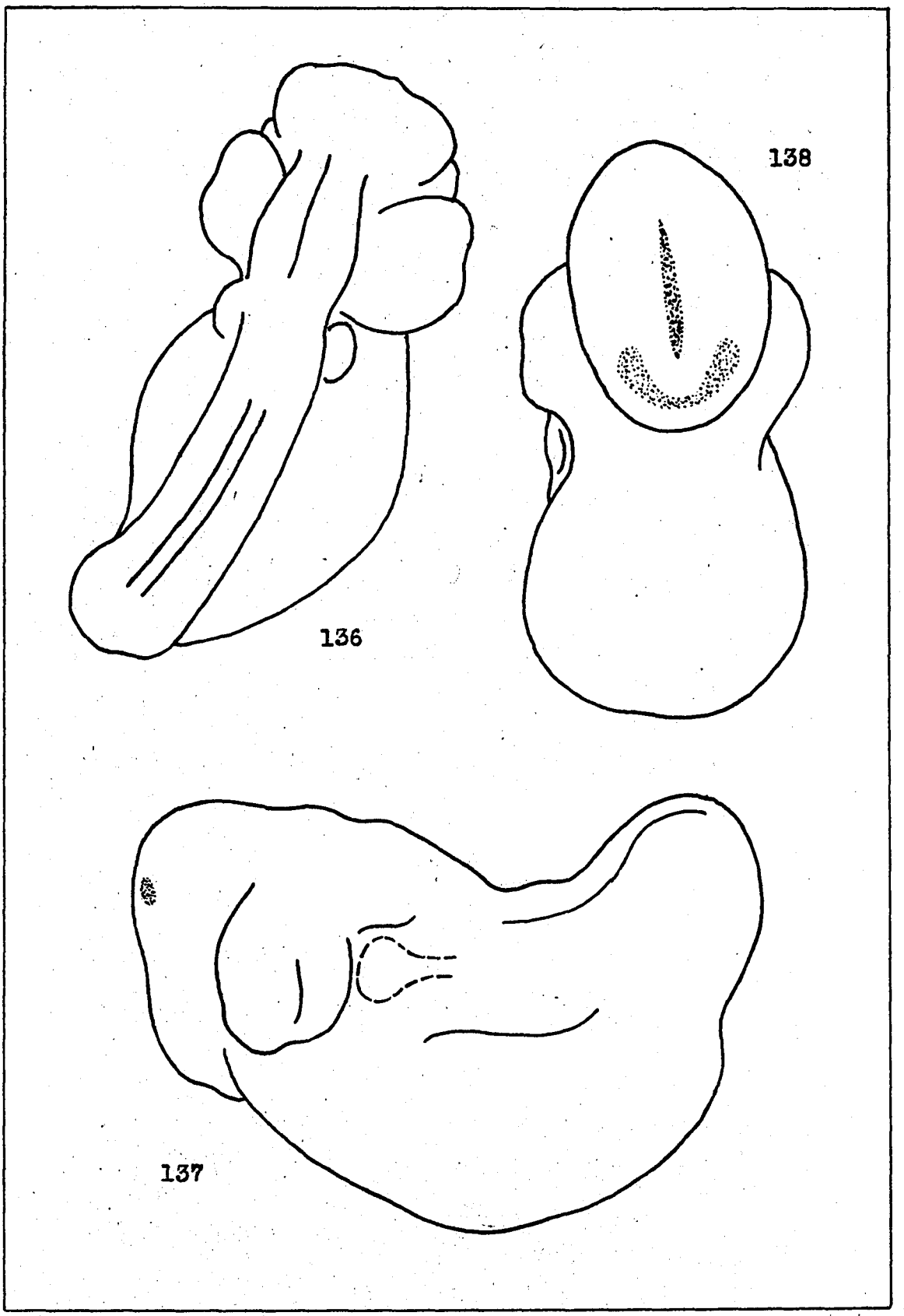

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Stage 16

One and one-half hours later, at seventeen hours after egg-laying, embryos appear as that shown in Figures 139, 140, and 156. The tail has elongated more, and is now about one-third the length of the body. The total body length has slightly exceeded the diameter of the chorion, so that as the embryo lies within the membrane the tail is somewhat curved. Due to the sharp upcurve of the tail, the dorsal line of the body is quite concave, while the ventral line is still strongly convex. The ventral edge of the tail from just above the proctodaeal pit almost to the tip is becoming flattened laterally in an early stage of fin formation. The mouth depression has deepened. From the crest of the head, as viewed from the front, there extends downward a pigment area in wide wishbone-shaped formation; each of the two ends of this area joins one of the depressed olfactory placodes, which are also heavily pigmented. Although the point has not been studied in sections, it seems likely that this pigmented area may indicate the position of those glands the secretion of which softens the chorion before hatching takes place. The eyes appear as swollen dark-colored areas lying slightly posterior to the olfactory placodes.

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Explanation of Figures

139. Stage 16. Lateral view.

140. Stage 16. Antero-lateral view of head.

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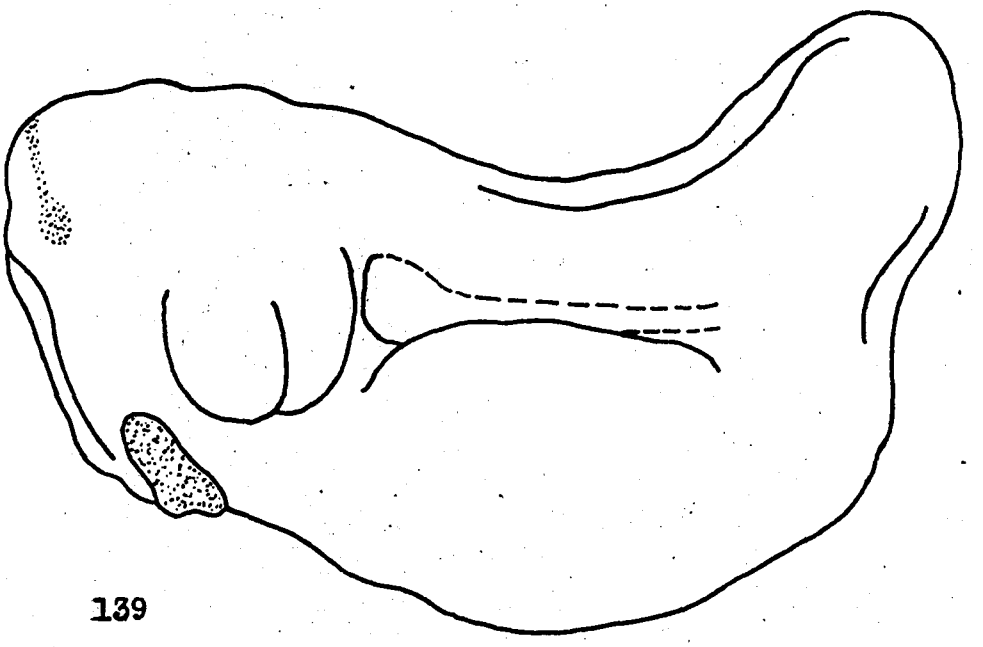

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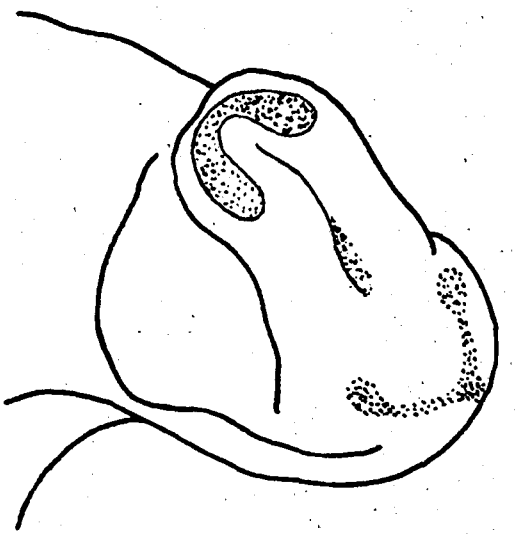

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Stage 17

Three hours later, at twenty hours after egg-laying, elongation has proceeded to such an extent that both head and tail are curved to one side or the other as the embryo lies in the chorion. (Fig. 141). The tail is about one-half the body length, and the angle of elevation of tail above body is less sharp. A third branchial groove is faintly indicated posterior to the first two. The body is slender from side to side, and deep-dorso-ventrally. (Fig. 142). The head (Fig. 143) is much longer and narrower, with the olfactory placodes deepening into pits, the mouth depression deeper, especially in its upper half, and the adhesive organ more ventrally located.

A certain amount of variation exists in the manner of rotation. Due no doubt to the change in body shape, embryos as a rule now lie on one side, with head and tail upcurved, and rotate usually in a clockwise direction if lying on the right and counterclockwise if on the left side. The head lies at a much higher level than the tail. Although at one point in the circuit of rotation the head rises higher than at other points, there is no pronounced toppling. At times embryos may, while turned slightly to one side, rest mostly upon the ventral portion of the body and rotate much as in earlier stages.

At the beginning of Stage 17 the first spontaneous muscular movements appear. The earliest movement consists of a slight contraction,

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with wrinkling of the integument, in the dorso-lateral neck region. A little later the head may be moved to one side once to several times. Shortly afterward, the tail also is capable of these movements, and head and tail may be flexed at the same time, both in the direction of curvature of the embryo. Muscular movements are at first slow, but soon become more rapid. In this stage they have not been observed to be strong enough to reverse the direction of curvature of either head or tail.

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Stage 18

Stage 18, (Figs. 144, 145, and 157), which is reached three and one-half hours after the beginning of Stage 17, is the one immediately preceding hatching. The embryo has elongated a little more, and both head and tail are curved as it lies in the chorion. The tail, which is now a little more than half the body length, no longer projects upward, but forms a straight line with the dorsal body surface. Its thin dorsal portion, which will become the tail fin, curves up well above the tail proper. The nasal pits have deepened, and are heavily pigmented. Both mid-and hind-gut have elongated. Bud-like structures on the surface of the first and second branchial (second and third visceral) arches form the first indication of outgrowth of the external gills. The adhesive organ is better developed. Muscular movements are stronger and more rapid. Head and tail may be moved separately or at the same time. Movements in the early part of this stage may be sudden, but are not violent, as they become in the later part of the stage and just before hatching.

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Explanation of Figures

141. Stage 17. Dorsal view.

142. Stage 17. Lateral view.

143. Stage 17. Antero-lateral view of head.

144. Stage 18. Lateral view.

145. Stage 18. Antero-ventral view of head.

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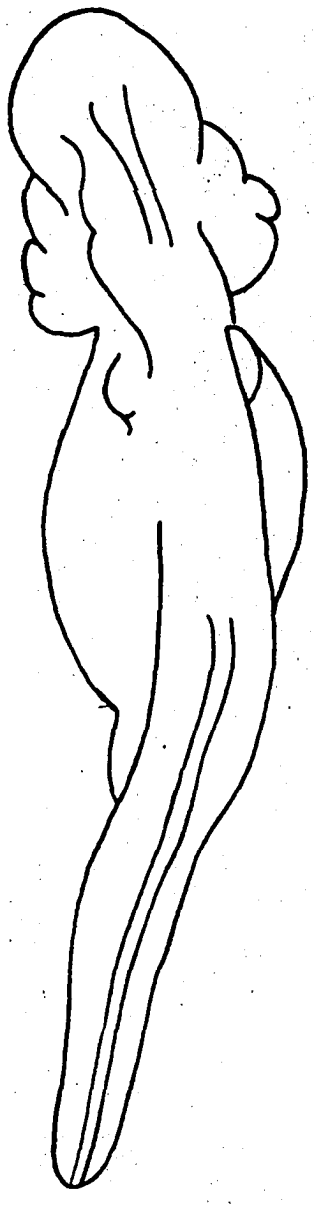

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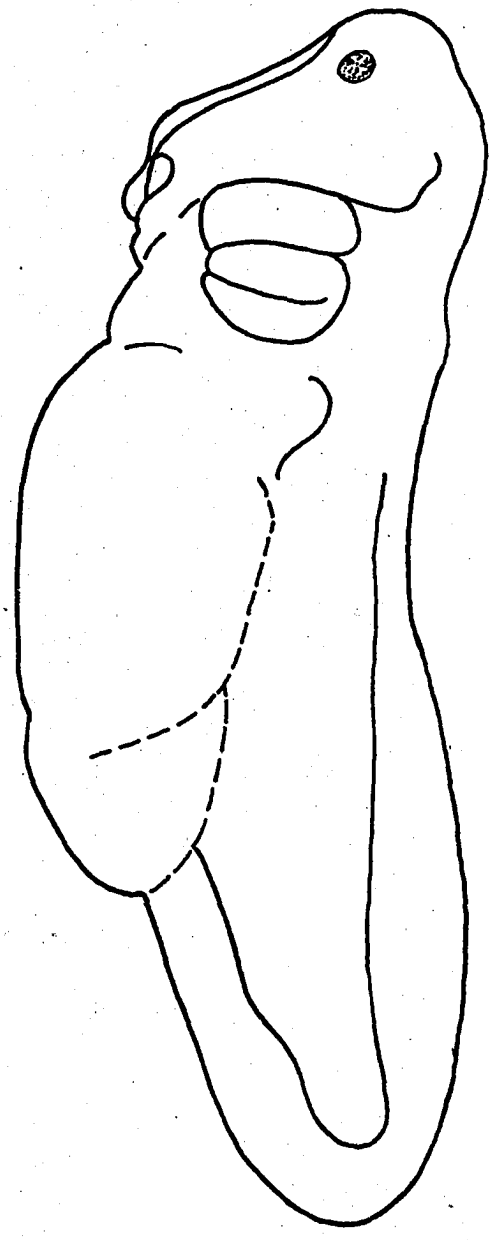

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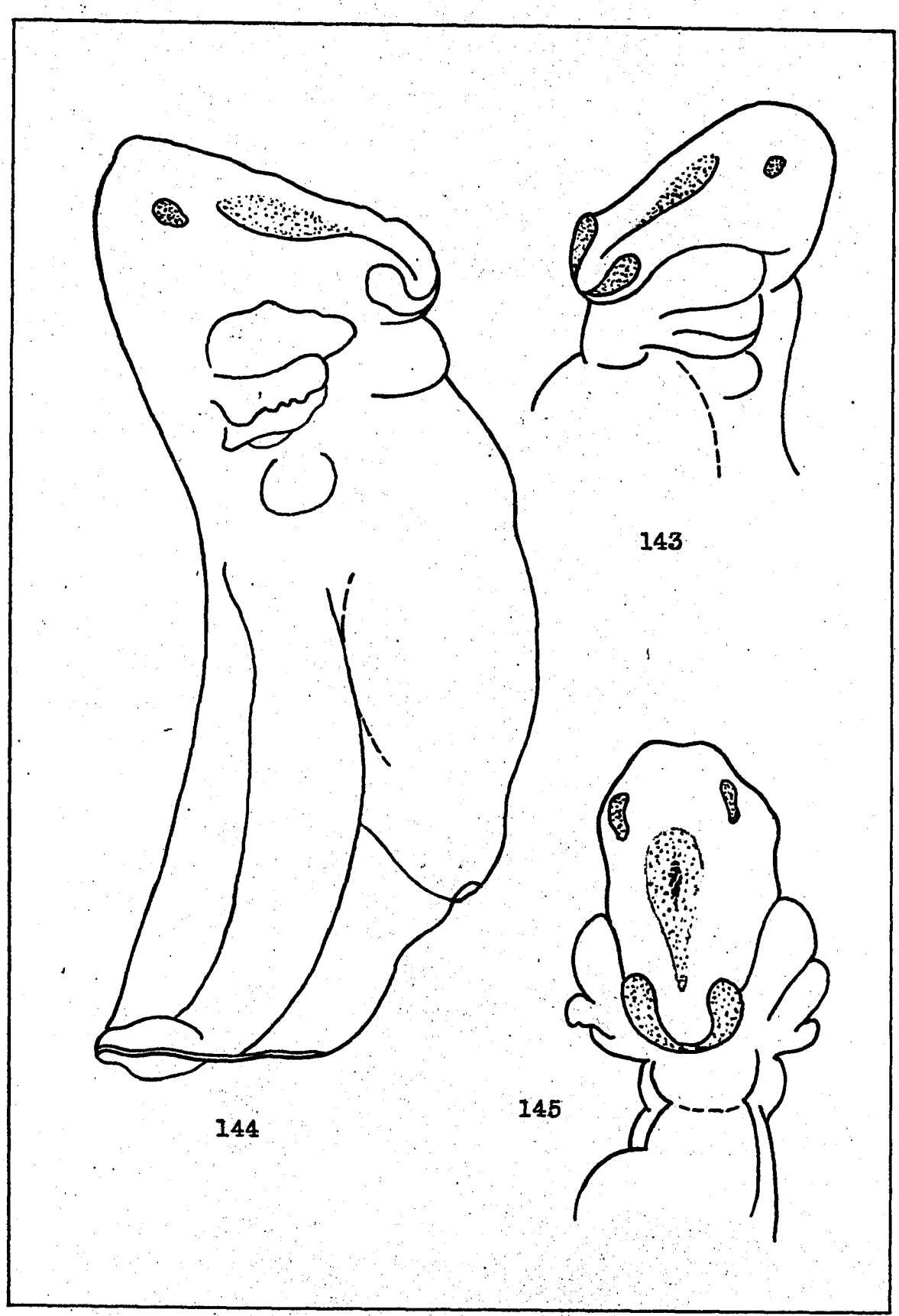

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Stage 19

At twenty-eight hours of age embryos have reached the stage of development shown in Figure 158. In most cases hatching takes place at this stage, although in some embryos it may occur in the later part of the preceding stage, and in still others not until the later part of Stage 19.

The embryo chosen as representative of Stage 19 is five millimeters in length. On the first and second branchial arches the outgrowths (early gill filaments) are longer than in the preceding stage. The third branchial arch is more pronounced, but as yet shows no indication of formation of gill filaments. The stomodaeal pit or mouth depression is still long in the dorso-ventral direction, and is quite deep. At its greatest depth a translucent appearance indicates the thinness of the oral plate. The depression has assumed an elongated diamond shape, with the ventral part longer and narrower than the dorsal, and the lateral portions representing the corners of the larval lips. The body is still deep dorso-ventrally, as compared with the width laterally. Muscle segments are plainly indicated from the region above the pronephric body back through the proximal two-thirds of the tail. The fusion between rectal and proctodaeal walls has not yet become perforated. Heart beat is visible at this time, and gill circulation becomes evident soon afterward, as the gill filaments become more nearly trans-

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parent.

Rotation continues as the time of hatching approaches, but becomes noticeably slower. Embryos lie part of the time on the side and rotate as in Stage 17. Muscular movements become much stronger and quite violent; they consist mostly of flexure of head and tail toward each other in a sudden movement, during which the two may touch. Youngstrom (1938), in a study of the development of behavior and reaction capacities in Anura, found some animals holding for one-half minute or more a deep 'U' contraction, comparable to the coil stage in Ambystoma. In Scaphiopus this does not occur. At times, contraction of muscles on that side of the body opposite the direction of curvature may be strong enough to bring about a reversal of curvature. If during this process the curvature of the head is reversed before the intial flexure is completed in the caudal region the 'S reaction' (Coghill, 1908) is produced, although the position is not maintained for more than one or two seconds. For some time before hatching muscular movements resemble those made in swimming, except as they are limited by restraint from the chorion. Embryos artifically freed from the chorion in the latter half of Stage 16 are capable of swimming for a short distance.

As the time of hatching approaches the chorion, which now has a diameter two to two and one-half times that in the cleavage stages, becomes soft and loses its tautness. It can easily be dented in by pressure, flattens somewhat through contact with the surface upon which it rests, and is sometimes accidentally broken and removed when either living or fixed embryos are drawn into a large-mouthed pipette. Softening of the capsule and initiation of hatching are believed by a

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number of authors to be due to secretion from the frontal gland. A secretion of this type has been shown by Bles (1906) in Xenopus and by Jaensch (1921) in two species of Rana to initiate the hatching process. Fahrenholz (1925) figures the secreting cells as forming a distinct organ in Alytes. Noble (1926) has described similar cells in Alytes obstetricans and in several other forms, among them Scaphiopus holbrookii. In Alytes he found the cells most numerous in a band between the eyes and in an A-shaped cluster on the snout. In Scaphiopus holbrookii, the swollen and apparently secreting cells are found not only in the frontal gland region but also between this band and the adhesive organs. He has stated: "In all higher frogs which have tadpoles hatching into the water the frontal organ probably functions as the releasing mechanism. This frontal organ is frequently indistinct and without a histological examination its limits cannot be determined." Since the frontal organ has been found in Scaphiopus holbrookii, it seems likely that it occurs also in S. bombifrons, and that its secretion is responsible for the marked softening of the chorion which precedes hatching.

The hatching process has been observed in a number of embryos. As the embryo rotates slowly, the entire anterior surface of the head is in contact throughout the cycle of rotation, with the chorion, which is bulged outward, the deepest part of the bulge being that caused by the dome-shaped top of the head. The curved tail may also cause a bulge in the chorion, so that the latter has an elongated shape. Immediately before hatching, rotation becomes very slow, and in some embryos may almost stop. As a rule emergence takes place in the following way. The embryo is almost at rest, with the dome-shaped top of the head making

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a pronounced outward bulge in the chorion. The dome of the head then begins to emerge, and the rest of the body, propelled by ciliary action, follows slowly and gradually. In one-half minute or less the curved tail suddenly emerges and the embryo falls to the bottom of the dish. No muscular movements have been observed to take place during emergence of this type. In nearly every case the adhesive organ becomes attached to the chorion during emergence. The hole left in the chorion is as large as or larger than the body of the embryo, and its edges are clean-cut, as if torn. I have not been able to observe the first formation of the opening, since the chorion where it is stretched tightly over the surface of the head is almost invisible in surface view. It seems likely that gradual pressure coupled with a process of dissolution of the membrane makes the first small hole, which is enlarged mechanically by tearing as the head and body pass through.

In some cases violent threshing and butting movements of the embryo make the first break in the membrane; when this happens the latter immediately wrinkles and collapses. If the break occurs near the head, the latter soon begins to emerge slowly. Other embryos have been observed to break the membrane through movements of the tail; the tear may be quite large, and eventually movements of the embryo bring about its escape. One embryo before hatching had its adhesive organ attached by a strand of gelatinous material to the inner surface of the chorion, and was rotating around the point of attachment; finally, in threshing about, it broke the membrane with its tail.

Newly hatched larvae fail to straighten out at once, but for a

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short time remain lying on the side with head and tail upcurved. In this position and when not attached to the chorion, they progress slowly along the bottom of the culture dish by means of ciliary movement. Immediately after hatching they can progress a short distance by swimming movements, mostly while lying on one side, although they are capable of swimming for a second or two with the ventral surface down. Larvae that are attached to the membrane also make vigorous swimming movements, and within an hour or two most of them have freed themselves. In the latter half of the stage, larvae are capable of swimming to the surface of the water in a finger bowl or culture dish; as they do so, they spiral either to the left or to the right.

According to Savage (1937), it is commonly believed that tadpoles after hatching feed on the envelopes, to which they attach themselves. Obviously this is impossible, as the mouth is at this time not open. Savage found that tadpoles of several species seem to develop faster when allowed to remain on the envelopes, but concluded that the underlying cause is not likely to be found to be nutritive. He has pointed out, in connection with increase in size before opening of the mouth, that an animal forming cavities and tubular organs out of solid and less aqueous material must increase in size. It has, however, been shown that if dissolved organic material is present in high amounts in the water, tadpoles at a later stage than Savage's can absorb nutriment from solution (Krogh, 1931). In Scaphiopus, attachment to the chorion takes place only incidentally to hatching. Free larvae have never been observed to attach themselves, while those that are attached free themselves, as as has been stated, within an hour or two.

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Stage 20

Two and one-half hours after hatching, presuming this occurs at the beginning of the preceding stage, most larvae are from .5 to 5.5 mm. in length. They still lie most of the time on the side. Although they swim for a short distance with the ventral surface down, they are still not capable of orienting themselves while at rest, and fall to one side or the other. Occasionally one finds a larva at this stage still unhatched. The eye, lying behind and slightly above the nasal pit, remains visible only as a dark area. External gills are well developed on the first three branchial arches. The mouth depression has widened laterally and shortened dorso-ventrally, and its outlines approach toward the shape of the larval lips. The oral plate has not yet been perforated. The adhesive organ is slightly better developed than in the preceding stage.

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Stage 21

At five hours after hatching, with body lengths varying from 5.5 to 6.5 mm., the swimming ability is better developed, but larvae are still unable to orient themselves while at rest. The eyes are plainly evident in surface view, and at the beginning of the stage dark pigment is present on the dorsal part of the body. During the middle and later parts of Stage 21 many of the larvae attach themselves to objects, such as the side of the dish, by means of the adhesive organ. In this position they rest on the ventral part of the body, but at other times they lie on the side. At the beginning of the stage the lips are assuming the characteristic tadpole shape, and around the mouth small protuberances indicate the beginning of papilla formation. Mouth and anus have not yet opened. The opercular or gular fold is just beginning to form.

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Stage 22

Stage 22. (Figure 159) is reached fifteen hours after hatching. Length has increased but little, most larvae measuring about 6.5 mm. Pigmentation is better marked. The gular fold has formed across the entire ventral surface as well as above and in front of the external gills. Both mouth and anal openings are present, the larval lips are well formed, and papillae are larger. The eyes are well developed, and the nostrils deep. The gut shows one transverse fold. The adhesive organ has begun to retrogress, and larvae no longer attach themselves by it. Larvae can now orient themselves on the ventral surface while at rest, showing only a slight tendency to fall to one side or the other. The lateral body width is almost as great as the dorso-ventral depth.

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Stage 23

At twenty-four hours after hatching, larvae vary from 6.5 to 7.5 mm. in length. Specimens in exactly the same stage of development may vary considerably in length and in other measurements. The opercular fold has extended further posteriorly. Pigmentation is now well developed on the dorsal surface of the body. Larval jaws show at this time the beginning of cornification. The papillae around the mouth are larger than in the preceding stage, and the fold on which they are located protrudes. The hind limb bud is evident as a small rounded protuberance.

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Stage 24

Stage 24 (Figs. 160 and 161) is reached about thirty hours after hatching, or fifty-eight hours after egg-laying. The larva photographed is 8.5 mm. in length, although in the majority of specimens at this stage of development the length is somewhat less. The opercular fold has covered the gills on the right side, but not those on the left. The row of papillae surrounding the mouth is completely formed, but teeth have not yet appeared. The integument is beginning to become transparent, and jaw muscles can be seen through the ventral body wall. The gut shows more coils than in the preceding stage. Cornification of the larval jaws is better developed, and both algae and liver are taken as food. At thirty-three hours after hatching a number of living tadpoles have been found in which all of the tail except a ragged stub had apparently been eaten off by other tadpoles; smaller specimens are the ones most often attacked. Dead tadpoles during this as well as later stages are also eaten. Evidently the tadpoles take either animal or vegetable food as soon as the jaws are well enough developed to permit feeding.

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Stage 25

Stage 25 begins at the age of sixty-four hours. Body shape is practically the same as in the preceding stage, but there has been some increase in size; in a specimen chosen as representative, the body, tail and total legnths are, respectively, 3¼, 5½, and 8 3/4 mm. The gular fold has by this time completed its growth, and the spiracle is present as a latero-ventral opening. The larval jaws are well cornified, but the teeth have not yet appeared. Pigmentation is a little more dense than in the preceding stage. The integument is now quite transparent, and, through the ventral body wall, jaw muscles, heart, gut, etc. are plainly visible.

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Later Larval Development and Metamorphosis

Larval development from Stage 25 to the onset of metamorphosis is a matter mainly of change in size and shape of body, degree of development of limbs, etc; accordingly, satisfactory criteria are lacking for division of the period into stages. Moreover, those variations in rate of development which have been noted in earlier stages become even better marked during this period; in each group of tadpoles of exactly the same age there are marked differences in size, and, in addition, tadpoles otherwise in a comparable stage of development vary somewhat in length and size of body. The measurements given in Table IV are those of single specimens chosen as representative of the various age groups. Since by the twentieth day tadpoles have as a rule attained their maximum body size, measurements are given only up to that time.

Table IV

Growth in Length of Tadpoles of S. bombifrons Cope from the Fourth to the Twentieth Days. Age is computed from the time of egg laying.

Age in Days	Length in Millimeters		
	Body	Tail	Total
4	4.5	6.5	11
6	6	9	15
8	7.5	10	17.5

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Table IV. cont'd

Age in Days	Length in Millimeters		
	Body	Tail	Total
10	8	12	20
14	14.5	19	33.5
17	18	26	44
20	22	30	52

By the twentieth day, the body length of tadpoles varies from 20 to 23 mm., tail length from 30 to 39 mm., and total length from 50 to 62 mm. In some cases the maximum total length is not reached until four or five days later, as the tail may continue to lengthen up to that time. Body and tail lengths are not necessarily correlated.

At the fourth day, chromatophores have appeared on the hind leg, a slight constriction indicates the position of the joint between metatarsals and tibia and fibula, and the distal portion of the limb is broadened and flattened. At the seventeenth day, the rudiments of all five digits are visible, and a second joint is indicated proximal to the first one. By the twenty-second day, the digits of the foot are longer, and the limb as a whole is much larger and better developed; the metatarsal tubercle is well marked, but shows no indication of cornification. On the twenty-third day, the tubercle is partially cornified on its free edge, and by the twenty-fifth day it is completely cornified.

Teeth first appear on the sixth day. By the twenty-second day the mouth is widening from side to side, and, although larval teeth

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and jaws are still present, the tadpoles feed much less voraciously than in earlier stages. The horny larval jaws are lost between the twenty-fifth and the twenty-eighth day; tadpoles have been observed to feed up until the time when both mandibles have completely disappeared.

Formation of eyelids is indicated at the twenty-second day by the presence of small ridges of integument; by the twenty-third day, each eye is surrounded by a definite fold of integument. Two days later, the nictitating membrane is evident and the eyes are definitely protuberant. By the twenty-third day, the ridges which in earlier stages surround the nostril have flattened down.

By the twenty-third day, the integument has become slightly rugose over the entire dorsal surface of the body.

At about the twenty-eighth day, one or both of the fore limbs emerges, the left usually appearing first and the right at any time up to one day later. At this time, the horny larval jaws or mandibles have been lost, the skin is definitely rugose and is assuming the color markings of the adult, and resorption of the tail fin has begun--that is, both dorsal and ventral portions of the caudal fin are reduced in size and somewhat thickened and opaque, and the total length of the tail has been reduced a little. The tongue is well developed, the mouth wide, and the muzzle has the "pug-dog" shape characteristic of that of the adult. All five digits of the hind foot show the beginning of cornification at the tips. As soon as both front legs have emerged the young toads spend part of their time out of the water, and may partially bury themselves in sand.

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During the day on which the arms emerge, the length of the tail is reduced 8-11 mm. On the three following days, the reductions in length are, respectively, 5 to 7 mm., 3 to 5 mm., and 1 to 3 mm.

By the thirty-second day, metamorphosis is practically completed. Of the tail there remains only a whitish stub, 1-3 mm. in length. Color markings on the dorsal surface of body and legs are those of the adult. The burrowing instinct is fully developed, and the young toads bury themselves in sand or in soft soil. Small insects are taken as food (Trowbridge and Trowbridge, 1937). During the next four or five days the tail stub disappears entirely.

There is a certain amount of variation in the time at which the fore legs emerge, and a corresponding variation in the time at which metamorphosis is completed. As has been indicated, in many specimens it is completed at about the thirty-second day. In a few cases the front legs have emerged as early as the twenty-sixth day. By the thirty-fifth day, metamorphosis is completed in all except a few individuals which were small in size and retarded in stage of development. Retarded individuals metamorphose later and usually at a somewhat smaller size than do the other tadpoles. Adolph (1931) found that tadpoles of Rana pipiens and R. sylvatica retarded by crowding metamorphosed at a small size but at a later time than did the control group. In Scaphiopus, it has been observed that the larval period may be greatly prolonged by retardation of growth due to insufficient food.

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DISCUSSION

Degree of Regularity of Cleavage

Few studies are available for a comparison of the degree of regularity of cleavage in the various Salientia, since investigators have for the greater part described only the 'typical' condition, and, beyond stating that irregularities are common, have failed to make any more specific statements. With the exception of those studies (Morgan and Tsuda, 1894, Morgan and Boring, 1903, Brachet, 1906, Jenkinson, 1906, etc.) which are concerned with variation in the plane of the first or second cleavage with reference to that of the gray crescent, the study made by Jordan and Eycleshymer (1894) on the cleavage of the amphibian ova is, to the writer's knowledge, the only one in which an attempt has been made to determine the extent and frequency of cleavage variations. As a result of their study, Jordan and Eycleshymer concluded that irregularity in the early cleavage stages of the amphibian embryo is the rule, and regularity the exception.

In Scaphiopus, on the other hand, extremes of variation and of irregularity have rarely been found to take place. The most irregular and bizarre cleavages have been noticed in many cases to appear only in those embryos which are abnormal and in which development ceases and death occurs soon afterward. As a result, the atypical cleavages of

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extreme types can be assumed to signify the abnormality and approaching death of the embryos in which they occur. Embryos of this type almost without exception are spotted and slightly swollen in appearance, a characteristic which one learns to associate with abnormality, and which is distinguishable also in fixed embryos. It is unfortunate that, due to time limitations imposed by the speed of development, no detailed observations have been made on the fate of embryos in which highly atypical cleavages occur; in order to attack the problem, one could perhaps make use of the method of Rugh (1934) to obtain embryos in small lots and at times other than the breeding season.

As stated earlier, in fixed material embryos which are moribund can be distinguished by their appearance, and it is in these that the most aberrant cleavages appear. Embryos of this type have not been included in the study of cleavage, except in a few cases in which the situation has been explained. For example, in connection with the second cleavage, it has been pointed out that Figures 10 to 14 illustrate examples of those aberrant cleavages which signify the abnormality and approaching death of embryos. In the same section it is also noted that in the embryo shown in Figure 6 there is a striking and probably not truly representative difference in degree of development of second furrows in the dorsal and in the ventral blastomere; the embryo in question had to a slight extent that aspect which can be described as "spoiled".

Although it is quite difficult to differentiate in every case between those cleavages that merely depart somewhat from the typical condition and those which indicate abnormality and presage the death of an embryo, the appearance of an embryo at the time an aberrant cleavage

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occurs is usually sufficient to enable one to recognize and to interpret the condition. In this connection, it may be pointed out that an interesting situation was noted in the latter part of March, 1938, when eggs were obtained after an unusually early breeding congress. Large numbers of eggs failed to cleave at all, and a number of others died after either the first, second, or third cleavage; as a rule only one or possibly two aberrant cleavages precede death of the embryo. In this year, most embryos that completed the third cleavage continued development to form perfect tadpoles, although in many the cleavages were slightly more irregular than had been the case with other lots of eggs. Moreover, a greater number of abnormalities among larvae were observed in this year than in other years. Although an attempt was made to remove spoiled eggs and embryos from living ones, this could not always be done as completely or as soon as it should have been, and one is led to wonder whether products of deterioration might have affected development; this factor of course was not responsible for the fact that many eggs failed to cleave at all or that embryos died after one of the first three cleavages.

With the several exceptions already stated, only embryos that were normal in appearance have been chosen to illustrate variations in cleavage. With material so chosen, it becomes evident that cleavage in Scaphiopus is not subject to as great variations as in some other Anura. The first two cleavages have in every case been observed to be vertical, and variations have been shown to exist only in such minor matters as the points of departure of second from first furrows, formation of acute and obtuse angles at both upper and lower poles, variation in the relative

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sizes of dorsal and ventral cells, etc. As concerns the third cleavage, Jordan and Eycleshymer found that in Rana palustris about half the embryos failed to form a true "first equatorial" plane in all four quadrants. Out of 69 embryos, only 29 showed truly horizontal third furrows; in some of the remaining 40, equatorial furrows appeared in three quadrants, and a true vertical in the fourth, in others three planes were vertical and one horizontal, while in still others the whole third set of furrows was vertical. In Scaphiopus, on the other hand, no true vertical third furrows have been observed-- that is, furrows with the upper end located at or near the upper pole. In 80 out of a series of 122 embryos, the third furrow was considered horizontal in all four quadrants. In 32, one or more of the furrows was found to be slightly oblique, so that it joined the vertical first or second furrow at some distance from the adjacent third furrow; however, the obliquity was not so great but what the furrows could still be considered horizontal in all quadrants. In ten embryos, the tendency toward obliquity had been carried further, and usually one or occasionally two of the third furrows had taken a direction intermediate between horizontal and vertical. Even those two embryos of this last group which showed the closest approach to a vertical third furrow had one end of the furrow located in or near the position normal for a third furrow. In this connection, it was shown that a relationship exists between presence or absence of a polar furrow and obliquity of third cleavage furrows.

Jordan and Eycleshymer stated also that, by the fourth cleavage, there is almost invariably an end of any constancy whatever in the relative position of blastomeres. In Scaphiopus, this is certainly not the

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case. Fourth furrows have been found in the majority of cases to be vertical, although occasionally (Figs. 51 to 54) one or more of them may be oblique or may approach a horizontal direction. After completion of the fourth furrows, most embryos show a more or less definite bilateral arrangement of micromeres and macromeres; many of these are of the types shown in Figures 63-65, 67, 69, 71, 73, and 74, and others of the types shown in Figures 75-77. Again, arrangement of cells may be roughly radial (Figs. 83, 84, and 148), and in a few cases other variations exist in configurations of cells. Since nearly all embryos can be classed into types, it becomes evident that regularity still exists to a certain extent.

Even at the fifth cleavage, the direction of furrows is in Scaphiopus fairly regular; the furrows are nearly always latitudinal and approximately at right angles to the plane of the fourth division, and in most embryos they are so in all sixteen blastomeres. Occasionally one or more of them may be oblique to vertical. An irregularity in direction of a cleavage plane as a rule results in irregularity in the immediately succeeding plane; for instance, in those rather rare cases in which one or more of the fourth planes approached a horizontal direction, fifth furrows in the cells in question are vertical (Fig. 97). At the completion of the fifth furrows, the thirty-two blastomeres are arranged in four more or less definite horizontal rows or circlets.

At the sixth cleavage, furrows in the second and third circlets of cells are nearly always vertical, although occasionally oblique. In the first and fourth circlets, the direction of cleavage planes varies a great deal. During this as well as the preceding cleavage, regularity of arrangement of cells is somewhat modified by the formation of furrows

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oblique to the surface and by the inward migration of blastomeres. During the sixth and succeeding cleavages the situation is further affected by formation of paratangential cleavage planes which divide cells into external and wholly internal blastomeres. At the seventh cleavage, the number of cells visible at the surface is less than the theoretical expectation; the direction of furrows is predominantly latitudinal, although there is a considerable amount of variation. In later cleavages the tendency toward alternation in direction of planes of division is disturbed to an even greater extent, and cleavage becomes quite irregular.

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Establishment of Bilaterality

In the higher animals, the most obvious feature of organization is that expressed by the term bilateral symmetry. Since bilaterality is a feature of fundamental importance in the organization of the embryo, it may be expected to appear very early in ontogeny. The problem of tracing its origin is one of considerable interest, and one particularly wishes to determine at what time and in what manner it is first expressed.

Amphibian eggs, like many others, appear when unfertilized to be radially symmetrical around the primary axis of the ovum, which may be considered as a line passing from the upper or active pole through the center of the egg to the lower or inactive pole. The active pole is early indicated by such phenomena as "secretory activity, accelerated yolk metabolism, formation of pigment, position of nucleus, expulsion of polar bodies, etc." (Eycleshymer, 1915), or, in general, by a high rate of metabolic processes (Bellamy, 1919). It is at or near this pole that the first cleavage furrow makes its appearance. Although its location varies somewhat in the different Amphibia, and there are divergences of opinion as to its position even in a single group (see for example Eycleshymer, 1915, Pfluger, 1883, Roux, 1888, O. Hertwig, 1892, Morgan, 1894, and Goodale, 1911), the future head end of an embryo can be determined rather early in development. On the other hand, the median

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plane of the body may lie in any one of a number of meridians, and the question arises as to which one of these meridians will represent the median plane, that is, what structure, appearance, or phenomenon will foreshadow the definite bilaterality of the embryo.

Attempts to show that the amphibian egg exhibits bilaterality from the beginning of oogenesis have failed to produce any conclusive evidence in support of the view. However, it has been determined by numerous observers (Roux, 1883, 1885, Schultze, 1900, Morgan and Boring, 1903, Weigmann, 1927, and others) and is quite generally accepted that after fertilization and before cleavage the egg of the frog possesses a bilaterality which foreshadows the definitive bilateral symmetry of the embryo and which is expressed superficially in the formation of the gray crescent. Roux (1887) described the gray crescent as forming on the side of the egg opposite the point of sperm entrance, as did also Schultze (1900), although the latter concluded the crescent arises in a preformed region. However, external influences acting at the time of fertilization may exert an influence in determining direction of the median plane (Jenkinson, 1909, Weigmann, 1927). The latter found that, in Rana fusca and Rana esculenta, although the gray crescent fixes the position of the dorsal side and therefore determines bilaterality, gravity as well as compression between glass plates can influence the direction of bilaterality; in compressed eggs, the median plane is shown to be independent of the fertilization meridian. According to Herlant (1911), since in pricked frogs' eggs the gray crescent forms without relation to the point of pricking, the egg probably has an initial bilaterality of its own which is overridden by the stimulus introduced by

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the spermatozoon; Loeb (1921) raised over twenty parthenogenetic frogs to an advanced and some to an adult stage. All these facts indicate that there is no essential relationship between the fertilization meridian and the establishment of bilaterality. In this connection Lillie (1919) has made the following statements:

> The relationship which has been shown to exist in certain cases must therefore depend upon a certain time relationship in the course of the two processes. The influences radiating from the spermatozoon establish a gradient from its original excentric position, which may influence the direction of the plane of symmetry in which there is also a gradient, if its determination is synchronous, as in the frog.

Although in certain species of amphibia there is an approach to coincidence between either first or second cleavage furrow and the median plane, it is becoming recognized that there is no direct relationship between the direction of the early furrows and the establishment of bilaterality. Newport (1851) first observed that in the majority of cases the plane of the first cleavage coincides with the median line of the embryo, and numerous observers later confirmed his observation. Roux (1885), Schultze (1887), Morgan and Tsuda (1894), Kopsch (1900), Brachet (1906) and others have demonstrated that, on the other hand, there are cases in which the two planes do not coincide, and it is now quite generally recognized that bilaterality is established by the position of the gray crescent, and that coincidence between the plane of the first or second furrow and the median plane of the embryo exists only when the plane of the furrow in question coincides with that of the crescent. Brachet (1903, 1905) demonstrated by experiment that each of the first two blastomeres of the frog embryo can produce an entire embryo only when the plane of the first furrow coincides with the plane of bilater-

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ality previously determined and indicated by the position of the gray crescent. McClendon (1909, 1910) concluded that each of the first two blastomeres of the egg of the tree-frog Chorophilus triseriatus is totipotent only when the first cleavage furrow bisects the gray crescent. Eycleshymer (1904) found in the living egg of Necturus no fixed relation between the early cleavage furrows and the median plane of the embryo. In Cryptobranchus, Smith (1915), using a variety of methods, found no constant relation between the first cleavage furrow and the median plane of the gastrula. Jordan and Eycleshymer (1894) from observations on the living segmenting eggs of Ambystoma punctatum, Diemyctylus viridescens, Rana palustris, and Bufo variabilis concluded:

> The first and second cleavage planes undergo, even in the earlier stages, extensive torsion. Everything indicates that the extent of this shifting increases greatly in later stages. This led us to conclude that the earlier cleavage planes and the embryonic axes have no vital connection and that the coincidence where it exists is of no fundamental significance.

Eycleshymer's later (1904) study of cleavage in the living egg of Necturus revealed the same type of irregularity. Similar variations have been observed in practically all classes of vertebrates.

> The inevitable conclusion from such a mass of evidence cannot be other than that neither the position or direction of cleavage grooves has the slightest significance as far as the setting apart of definite embryonic areas is concerned. (Eycleshymer, 1915).

A number of authors consider that sooner or later bilaterality becomes evident in the cleavage pattern of the embryo as a result of increased cellular activity on one side of the micromere region. This area of accelerated cell division lies on the side on which the dorsal lip of the blastopore will appear, and, in those forms in which bilaterality is

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indicated in the presence of the gray crescent, is located in the region of the crescent.

> With the recognition of these areas of accelerated cellular activity, the one at the active pole, indicating the position of the future head of the embryo, the other at the side of the egg, indicating the position of the forthcoming blastopore, it necessarily follows that the median plane of the embryo must coincide with a line passing through the centers of the two. (Eycleshymer, 1915).

Assheton (1905) maintained that two such main centers of growth play an important part in the organization of the vertebrate embryo, and that recognition of them is essential to a correct appreciation of embryonic processes. As Eycleshymer pointed out, while there are minor differences of opinion as to the extent and time of origin of this area, there is no question as to its location or significance. Eycleshymer continued:

> When these observations were first published in 1898, many questioned the existence of such a secondary area of cellular activity. Yet a search through the literature showed that such an area had been observed in many groups of vertebrates. Lwoff found such an area at the posterior end of the embryo of Amphioxus. The figures of the segmenting blastodiscs of Elasmobranchs, given by Balfour, Ruckert, Gerbe and Sobotta all show that in these forms such an area is present. In the Reptilia, Vay's studies on Tropidonotus show that an area of small cells represents the posterior end of the embryo. v. Koelliker first called attention to such an area in the blastodisc of the chick and suggested that it determines the position of the posterior end of the embryo. The later investigations of Duval and Kionka leave no doubt as to the frequent and probably constant appearance of this area in the locality which later becomes the posterior end of the embryo.

Eycleshymer (1898) found this area of accelerated cell division occurring in Ambystoma in the late blastula state. In Necturus he found (Eycleshymer, 1904) that as early as the fourth or fifth cleavage the cells on one side begin to divide more rapidly than any others, excepting those of the primary area. "It was possible to predict in this form the

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median plane of the forthcoming embryo at an extremely early state of cleavage." These two areas, occurring respectively in the late and early amphibian blastula, he regarded as genetically continuous.

De Bussy (1905) stated that in the blastodisc of an embryo of Cryptobranchus japenicus with forty micromeres he observed no secondary center of accelerated cell division, although Smith (1912) has called attention to the fact that his drawing of the embryo strongly suggests the existence of such a center. In Cryptobranchus alleghenlensis, Smith (1922) observed eccentric development of the micromeres in both the early and late blastula stages; the former bears no constant relation to the direction of the median plane of the embryo, but the latter he considered as undoubtedly an expression of the definitive bilateral symmetry of the embryo.

Schultze, in 1900, described the bilateral organization of the late blastula of the frog. Ishikawa (1908, 1909) described a similar condition in the embryo of the giant salamander of Japan (Megalobatrachus sieboldii). Morgan and Boring (1903) noted that the pigmented cells on the gray crescent side of the frog embryo are slightly smaller from the beginning than the other pigmented cells. Morgan and Tsuda (1894) found that in the eggs of Rana the less densely pigmented half very early in segmentation shows signs of a more rapid development and growth than the darker and pigmented side, and determined that the blastopore appears first on the less pigmented and further developed side of the egg.

Notes and drawings of developing Bufo eggs made by C. O. Whitman in June, 1894, (see Eycleshymer, 1915), show that he was able to distinguish the secondary area of accelerated cell division as early as the

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advent of the second furrows.

> When the first cleavage groove runs in the plane of symmetry the second cleavage grooves are at right angles and appear at about the same time in both halves as shown in Figs. 3 and 4. When the first cleavage groove is transverse to the plane of symmetry the second cleavage grooves do not appear at the same time, but the one on the lighter side of the upper hemisphere appears first.... The blastomeres on the posterior (blastoporic) side are smaller than on the anterior side, from the very first. It is the blastoporic side that takes the lead in division and the cells are smaller here all the way up to the time when the blastopore appears.

In Scaphiopus, bilaterality is undoubtedly established at the beginning of gastrulation; that invagination which initiates formation of the blastopore lip appears first, as in other Anura, on the dorsal side of the embryo. Consequently, any character or phenomenon which in earlier stages is found to be in constant connection with or persistently characteristic of the dorsal region may be considered an indicator of bilaterality. Since the dorsal region is characterized and in part distinguished by the presence of the gray crescent throughout development from fertilization up to the formation of the dorsal lip, appearance of the crescent may be taken as the first evidence of establishment of bilaterality.

In connection with the relationship of cleavage planes to that of the crescent, it is shown in the figures illustrating the cleavage stages that although in many cases (Figs. 1 to 3, 7, 8, etc.) the plane of the first cleavage coincides with the middle of the gray crescent, and in fewer cases the second furrow bisects the crescent,(Figs. 4, 5, etc.) there are at times varying degrees of departure from coincidence. (Figs. 17, 83, 95 and 105). Moreover, it would be impossible, in view of the torsion of furrows and shifting of cells which follows cleavages, for

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either one of the two first furrows to separate the material of the embryo into two parts, one of which would give rise to the right and the other to the left side of the forthcoming animal. Torsion of cleavage furrows has been shown in the early cleavages to be more marked in those embryos in which a polar furrow forms at the second cleavage (Figs. 9, 18, 31, 35, etc.), but it occurs to a considerable extent also in those embryos in which no polar furrow is formed. In later stages (Figs. 60, 83, 97, etc.) torsion of furrows becomes even more marked. From these considerations, it becomes obvious that any coincidence which may exist between the location of the early cleavage furrows and the median plane of the embryo is only incidental, and that direction of furrows has no significance as concerns indication of bilaterality.

It has been shown in the discussion of and figures for the various cleavage stages that at every cleavage from the second to the eighth, inclusive, the dorsal region or region of the gray crescent is characterized by an acceleration of cell division. The occurrence of this eccentric area of accelerated cell division may be considered a phenomenon always associated with the region of the gray crescent, and therefore an expression of the bilaterality of the embryo. So far as I have been able to determine, Whitman (see Eycleshymer, 1915) is the only other investigator in the field of amphibian embryology who has described the appearance of the dorsal eccentric area of accelerated cell division as early as the beginning of the second cleavage. Eycleshymer found that the area in question becomes evident in Necturus at the fourth or fifth cleavage. Smith (1922) accepted as evidence that cell division had proceeded a little more rapidly on one side of

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the embryo than on the other the facts that the cells in the former region were smaller in surface view and more numerous, and that the cleavage furrows were in this region more uniformly complete; he was able to distinguish the area of accelerated cell division during his Stage 5 (fifth cleavage). In late segmentation immediately preceding gastrulation he considered that the cleavage pattern as seen in surface view enables one to predict the side on which the blastopore is to appear, since on this side a more rapid multiplication of cells has occurred and the micromeres and transitional cells as a rule approach nearer the vegetal pole. A number of investigators have merely stated that the dorsal cells take the lead in division and are from the first smaller than the ventral cells. In the present paper, figures are given showing the actual earlier appearance or earlier completion (or both) of furrows in the dorsal cells at each cleavage from the second to the seventh.

In Scaphiopus the dorsal portion of the embryo is further characterized by the occurrence of cells smaller in size than those in the ventral portion of the same embryo. The same situation has been described in practically every other Anuran the development of which has been studied. It has been shown in the discussion of Stage 8 that at least up to the end of that stage the smaller size of the dorsal cells is due not to the occurrence of more divisions among them, but merely to the fact that they are smaller from the beginning than are the ventral cells. During succeeding stages up to the time of appearance of the dorsal lip of the blastopore, the dorsal portion of the embryo, and particularly that part of it lying near the median plane of the gray

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crescent, is distinguished by the presence of cells smaller in size and more lightly pigmented than those in corresponding positions on the opposite side of the blastula, and by the slightly lower extension of small cells and the more gradual transition between small and large cells in the dorsal region. These features of the dorsal portion of the blastula are a constant characteristic up to the time of appearance of the dorsal lip, and constitute a continuous evidence of differentiation indicative of bilaterality.

The internal structure of early and late blastulae, as well as of embryos in the various cleavage stages, gives a further evidence of bilaterality of the embryo. The blastocoele, from the time of its first indication at the eight-cell stage up to the beginning of gastrulation, is, as a rule, eccentrically located, due to the fact that the dorsal wall is slightly thinner than the ventral wall. Since synchronism in cell division among the micromeres and transitional cells has been shown to persist at least until the end of Stage 8, it is obvious that during this period the eccentric position of the blastocoele is due only to the fact that dorsal cells are from the first smaller than ventral cells. What the underlying causes of inequality in thickness of the dorsal and ventral walls may be during Stages 9, 10, and 11, is not definitely known; certainly the situation is due partly to the same fact as in earlier stages, but, since it is not known to what extent thickness of the walls is affected by cell movements during these stages, the entire complex of causes is not understood. At any rate, this internal evidence of bilaterality corresponds with the external evidences manifest in the presence of the gray crescent, the small size

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of dorsal cells, and the area of accelerated cell division known to exist throughout the early cleavages.

In short, it can be stated that bilaterality of the embryo is first indicated by the position of the gray crescent, and that it is further evidenced in external aspect by the occurrence of a dorsal area of accelerated cell division and by certain morphological features which characterize the dorsal portion of the embryo, and in internal aspect by inequality in the thickness of dorsal and ventral walls of the blastula.

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Developmental Rate

Differences in developmental rate among animals have been described and their causes discussed by numerous authors. The following quotation and statement of the problem is from Stockard (1921).

> "It is a generally known fact that the eggs of different species do not progress at the same rate of development even during comparable stages. The lengths of time between fertilization and the first cleavage and the rates at which the early cleavages follow one another may differ decidedly among the eggs of even closely related forms. These differences in developmental rate are probably fundamentally connected with differences in chemical structure of the egg substances, and in particular with the different rates of oxidation of certain stuffs. It is a well-known chemical fact that very slight differences in composition between substances may cause very great differences in their oxidation capacities.
>
> The efforts on the part of numerous embryologists to associate the differences in rate of cleavage and time required to attain certain stages of development with the size of the egg, the amount and position of the yolk substances, or even the types of cleavage have not been satisfactory. Certain meroblastic eggs develop much faster than certain holoblastic ones, while other holoblastic eggs have a rate of cleavage far more rapid than the meroblastic types. All of the so-called laws of cleavage rates based on morphological differences among egg types have been found to fail so decidedly when applied in general that one is forced to seek more deep-seated causes for the differences in developmental rate.
>
> At the present time we can only state that such causes probably reside in the differences in chemical make-up of the several species of eggs... The rate of development certainly depends, particularly during later stages, on the amount of food available, but the supply of oxygen and the degree of temperature at which development is taking place have a far more striking influence on the rate. Cessation of development also occurs much more promptly from absence of oxygen or sudden changes in temperature than from any other natural modifications which happen in the environment. These facts point decidedly to the rate of development as being dependent upon kind and rate of chemical change, most particularly upon rate of oxidation. The egg probably has a definite coefficient of metabolism dependent upon the inter-

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> action of its specific chemical structure and the given environment in which it normally develops. The rate of development results from both the internal qualities of the egg and the nature of the surrounding environment.
>
> The present extremely crude state of our knowledge of the chemistry of development will permit of no more satisfactory statements of the principles underlying differences in developmental rate than those which have been attempted above."

Moore (1933), after finding that eggs of Dendraster fertilized with sperm of Strongylocentrotus have the cleavage tempo of their own species, and that nucleated and non-nucleated fragments when fertilized show time intervals for cleavage identical to those for the whole egg, concluded that neither sperm nor egg nucleus has any effect on segmentation tempo, but that reactions of cytoplasm alone determine it; he suggested that cleavage reaction depends upon a substance of granular character in the cytoplasm.

As concerns those differences in developmental rate which occur among the Amphibia, Jordan and Eycheshymer (1894) have shown that the inter-cleavage periods in urodelan eggs are much longer than the corresponding periods in anuran eggs, and that the whole course of urdoelan and anuran development is marked by a similar disparity in time. They concluded that the inter-cleavage period does not depend on the size of the egg, although the quantity of yolk exerts an influence upon the speed with which furrows cut their way to the lower pole. "We see no escape, therefore, from the conclusion that in this instance the rapidity of cell division depends upon the innate and inherited tendencies of the cytoplasm and nucleus, rather than upon the size of the ovum."

In Scaphiopus, the rate of development throughout the entire embryonic and larval period is more rapid than that which has been described from any other amphibian. The cleavage divisions in part-

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icular take place with surprising speed; they are among the most rapid cell divisions ever observed. The following table (Table V) provides a comparison of cleavage rates in several Amphibia and in one teleost; the latter, Brachydanio rerio, is included only because its cleavage rate, like that of Scaphiopus, is extremely rapid.

Sources of data used in Table V are as follows:

Diemyctylus viridescens, Ambystoma punctatum, Rana palustris, and Bufo variabilis-- Jordan and Eycleshymer (1894)

Chorphilus triseriatus-- Wilson (1896)

Rana sylvatica-- Pollister and Moore (1937)

"The Frog"QQ Reed (1904)

Pelobates fuscus-- van Bambeke (1868)

Brachydanio rerio-- unpublished manuscript by Roosen-Runge, of Brown University.

A number of other data are available on cleavage rate in Anura, although they are not complete enough to be included in a table. For instance, Newport (1851, 1853, 1854) determined that the egg of the frog begins to segment in from four to five hours after fertilization, and that divisions take place at intervals of about one hour. It is commonly stated, in generalized descriptions of the embryology of the frog, that cleavages occur at intervals of about one hour.

Table V shows, as one would expect, that there is a correlation between rate of cleavage and length of the period between fertilization and the appearance of the first furrow; this is particularly well illustrated by comparison of the data for urodelan and for anuran embryos. In Scaphiopus and in the teleost Branhydanio, with short intercleavage periods, the time elapsed before the first cleavage is also short.

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TABLE V

Duration of Cleavage Divisions in the Early Emb
Seven Anurans, and One Teleost

	Diemyctylus viridescens	Ambystoma punctatum	Rana palustris	Bufo variabilis	Chorophilus triseriatus
Fertilization to first cleavage	10 hours	10 hrs	4-5 hrs	4-5 hrs	
First Completed					
Interval between 1st and 2nd	2 hours	1 hr.50 M	1hr.15 M.	1 hr.5 M.	40-45 min.
Second Completed					
Interval between 2nd and 3rd	1 hr.45 M.	1 hr.55 M.	1hr.15 M.	1 hour	30 min.
Third Completed					15-18 Min.
Interval between 3rd and 4th	1 hr. 40 M.	2 hours	1 hour	1 hour	15-20 Min.
Fourth Completed					
Interval between 4th and 5th	1 hr. 50 M.	1 hr. 40 M.		1 hour	
Fifth Completed					
Interval between 5th and 6th	(2 hrs.45 M)	(1hr.35 M.)			
Sixth Completed					
Interval between 6th and 7th	(2hrs. 45M)	(1hr. 25M.)			
Seventh Completed					
Interval between 7th and 8th		(1hr. 25M.)			
Interval between 8th and 9th		(1hr. 25M.)			
Interval between 9th and 10th		(1hr. 30M.)			
Interval between 10th and 11th					
Interval between 11thand 12th					
Interval between 12th and 13th					
Temperature	18° C	18° C	18° C	18° C	

(Time in parentheses refer to individual cases, not

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TABLE V

ions in the Early Embryos of Two Urodeles,
, and One Teleost

labilis	Chorophilus triseriatus	Rana sylvatica	"The Frog"	Pelobates fuscus	Scaphiopus bombifrons	Brachydanio rerio
hrs		2hrs. 30M		3 hrs	30-50 min.	25 min.
				1 hr.	9-14 min.	
.5 M.	40-45 min.	30 Min Plus	50 M.	1½ hr.	10-15 Min.	20 Min.
				30 M.	9-14 Min.	
ur	30 min.	1hr. 30 M.	1 hr.	1½ hr.	10-15 Min.	19½ Min.
	15-18 Min.			10 M.	4¥5 Min.	
ur	15-20 Min.	30 M.plus	55 M.		10-15 Min.	19 Min.
					5-6 Min.	
ur			45 M.		10-15 Min.	18 min.
					5-6 Min.	
			55 M.		10-15 Min.	17½ Min.
					6-8 Min.	
			50 M.		10-15 Min.	18½ Min.
					7-11 Min.	
			45 M.		10-15 Min.	19 Min.
			55 M.			
			55 M.			20½ Min.
			1 hr.			20 Min.
			50 M.			
			1hr.15M.			
C		18° C		12-14° C	23-25° C	25° C

individual cases, not averages)

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It has not been definitely determined whether or not each embryo, in the case of Scaphiopus, possesses a cleavage rhythm of its own, and whether the rhythm varies much among different embryos. The sets of furrows as a rule appear at ten to fifteen minute intervals, although occasionally one of sixteen or seventeen minutes is observed. At one time, when the temperature was allowed to drop to 19° C., intervals up to 22 minutes elapsed between some of the sets of furrows. The rhythm remains practically constant as far as the cleavages have been studied. The data obtained by Reed (1904) also indicate a fairly constant rhythm during the early cleavages, although it is suggested that by the time the twelfth cleavage is completed the division rate is slowing down a little.

A comparison of the rate of cleavage of Scaphiopus and that of Pelobates is of especial interest, since the two are closely related forms. Van Bambeke's data shows that, in the latter, the intercleavage period is about one and one-half hours, while three hours elapse between fertilization and the first cleavage. However, comparison is complicated by the fact that the embryos of Pelobates were developing at a temperature of 12°-14° C., and those of Scaphiopus at one of 23°-25° C.

In Chorophilus triseriatus, according to Wilson's data, cleavage is quite rapid; intervals of 40-45, 30, and 15-20 minutes occur between, respectively, the first and second, second and third, and third and fourth sets of furrows. Wilson believed that development in the embryos he studied was more rapid than normal, since they had been kept at a temperature of 0° C. for eight hours before observation was begun.

Bragg (1938) has found that cleavages takes place rapidly in embryos of Bufo cognatus, although the rate does not equal that in Scaphiopus. "An embryo in the two-celled stage when first observed passed through

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two divisions in forty-five minutes. Some embryos reached the thirty-two or sixty-four cell stages in one and one-half hours."

The shortness of the intercleavage period in Scaphiopus is paralleled by the rapidity with which furrows are completed. Jordan and Eycleshymer (1894) found that although size of egg and quantity of yolk do not affect the intercleavage period, they do have an unmistakable influence on the speed with which furrows cut their way to the lower pole. From this standpoint, it is particularly interesting to note that in Scaphiopus both the first and the second furrows cut their way to the lower pole and are completed externally in from nine to fourteen minutes, even though the egg is not particularly small one and contains no less yolk than do those of several Anura in which a much longer time is required for completion of furrows. The average diameter of eggs of Scaphiopus bombifrons is 1.5 mm. The average diameter of eggs of Scaphiopus bombifrons is 1.5 mm. Bragg (1937) has reported that the average size of eggs of Bufo cognatus is 1.18 mm., and Wright (1933) has stated that eggs of Rana palustris and Rana sylvatica are, respectively, 1.6 mm. and 1.8 to 2.4 mm. in diameter.

It was stated in a previous paper (Trowbridge and Trowbridge, 1937) that as the time spent in cleavage decreases, that spent in rest increases. It has since been concluded, however, that the cleavages occur at rather regular intervals, and that the phenomenon noted has no real significance. Each of the first two cleavages, as is shown in Table V, requires from nine to fourteen minutes for its completion, due, no doubt, to the fact that each must traverse the embryo from upper to lower poles. The third cleavage is completed in any one cell in from two to three minutes after its first appearance, but, since the furrows form more rapidly in dorsal than in ventral cells, from four to five minutes are

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required for completion of the set of furrows. As cleavage continues, the cells that are to be divided become smaller, and, even though the lag in division in macromeres causes the entire period consumed in cleavage to be extended, it is still not as long as in the case of the first two cleavages. By the time of completion of the seventh set of furrows, lag in division in the macromeres is more markedly prolonging the period consumed in cleavage.

Later as well as earlier phases in the developmental process of Scaphiopus are completed more rapidly than in other Anura. The dorsal lip of the blastopore appears at seven hours after egg-laying. Thirty minutes later the lip has assumed a semi-circular shape, and at the end of another fifteen minutes it exists as a complete circle. About one and three-fourths hours after the beginning of gastrulation, the blastopore has a diameter only half that in the early circular blastopore stage. Three and one-half hours after appearance of the dorsal lip, the yolk plug has been reduced to a diameter of fifteen or twenty degrees, the blastopore lip has become ovoid in shape, and gastrulation is practically completed. Early neural structures appear externally at about this time. One hour later, the yolk plug is entirely hidden and the lateral lips of the blastopore are closely appressed. In Pelobates fuscus, the blastopore lip appears at 43½ hours after egg-laying. The same stage is reached in Rana sylvatica at nineteen hours, and in Chorophilus triseriatus at twelve hours. According to Bragg (1938), in Bufo cognatus: "Young blastulae advanced to yolk-plug stages in five hours at a temperature of 25-30° C., although many developed at a slower rate under closely comparable conditions. Gastrulation is a much slower process than cleavage and it often takes several hours for the yolk-plug to become overgrown completely."

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In Chorophilus triseriatus, gastrulation begins at twelve hours after the first cleavage, and the blastopore closes at the fifteenth hour. Pfluger in 1883 described the first trace of the dorsal lip of the blastopore as appearing in Bufo cinereus at eighteen hours after fertilization; at twenty-three hours the lip of the blastopore had assumed a crescentic and at twenty-four hours a semicircular shape. One-half hour later, the blastopore was established as a circle. At 31½ hours, rotation of the gastrula had brought the blastopore into a posterior position.

In Scaphiopus, the position of the neural groove is at ten and one-half hours after fertilization (that is, at three and one-half hours after the appearance of the dorsal lip) marked out by a line of pigment. Three hours later, the neural folds have come together and fused throughout their entire length. In Rana sylvatica, fourteen hours elapse between appearance of the neural groove and closure of the neural folds.

Hatching takes place in Scaphiopus at the age of twenty-eight hours. In Rana sylvatica, it does not occur until an age of more than ninety hours. According to Bragg (1936), larvae of Bufo cognatus hatch at about fifty-three hours. Hinckley (1882) indicated that the larvae of a tree-toad (species not stated) which she studied escaped from the membranes at forty-eight hours. Wilson (1896) stated that in Chorophilus triseriatus hatching occurs during the second or third day, or by the end of the third day.

Larvae of Scaphiopus bombifrons attain their maximum size of body at about the twentieth day. The fore legs emerge at about the twenty-eighth day, and by the thirty-second day metamorphosis is completed.

There is a possibility that an unusually rapid rate of development might result in the telescoping of certain stages, or in the occurrence

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of cases of heterochrony. However the present study has not brought to light any phenomena of this type. On the other hand, it seems that the rapid rate of development is obvious in all phases of embryonic and larval life, and that it is not more strikingly expressed in connection with any one structure or set of structures than with others.

Anuran embryos and larvae show differences not only in the rate of development of morphological features, but also in the time of development of various reaction capacities. Specimens of Scaphiopus bombifrons have been observed to be remarkably active and vigorous during the early larval life; this characteristic is particularly striking when comparison is made with other tadpoles. Spontaneous muscular movements begin at the age of about twenty hours, and within an hour or two become quite strong; as the time of hatching approaches, movements resemble those made in swimming, and at times become so violent as to result in rupture of the chorion. Swimming ability is developed early, and embryos freed from the membrand during the latter half of Stage 18 (two to two and one-half hours before hatching would normally occur) are able to swim for short distances. In Bufo cognatus, although active muscular movements are evident before hatching, the young tadpoles do not swim much until about ten hours after hatching (Bragg, 1936). Embryos of Bufo woodhousii woodhousii reared in the laboratory by Dr. Bragg and Mr. V. O. Johnson have been observed to be even later in the development of reaction capacities; according to Youngstrom (1938), embryos of Bufo Woodhousii woodhousii are at hatching pre-motile. A table (Table I) given by the latter author illustrates differences in the time of development of reaction capacities in a number of other Anura; although Acris gryllus and Pseudacris triseriata are free-swimming at the time of hatching, Rana areolata and Rana pipiens

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are non-motile or at the stage of beginning motility, and Rana clamitans and Rana catesbeiana are at the stage of beginning motility.

After hatching, larvae of B. cognatus, when not attached to the jelly, lie on the lateral surface and do not swim much until about ten hours after hatching! When about thirty hours old, all swim in a spiral path. At about seventh hours, they begin to lose the tendency to spiral and then swing widely from side to side with only an occasional turning over. At about ninety hours after hatching, all spiralling ceases (Bragg, 1936). Larvae of Scaphiopus are markedly more active. Those which become attached to the chorion during hatching usually free themselves in an hour or two; those not so attached swim immediately. One hour after hatching they are able to swim in a spiral path to the surface of the water in a finger bowl or culture dish. At fifteen hours after hatching, larvae can orient themselves and swim with the ventral surface down, showing only a slight tendency to fall to one side or the other.

It seems possible that the great energy and vigor of movement exhibited by larvae of Scaphiopus may, at least to a certain extent, be correlated with their predaceous habits.

Dr. Bragg, in a study (in manuscript) of amphibian deutoplasm and its relation to embryonic and larval development, has found that in Scaphiopus the yolk seems to be used at a slightly earlier period than in the other four forms studied (Bufo cognatus Say, B. Woodhousii woodhousii (Girard), Rana sphenocephala (Cope), and Triturus torosus). He has suggested that this earlier utilization of yolk may be correlated with the exceptionally high rate of development, although both he and the writer

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believe that it may also be correlated with the high degree of muscular activity.

The question may be raised as to whether the rate of development recorded for Scaphiopus bombifrons at a temperature of 23° to 25° C. is the so-called normal rate, since it is a well known fact that external conditions affect rate of development. In this connection, Stockard (1921), King (1903), and Hertwig (1894, 1896, 1897, and 1899) have shown or pointed out that although a wide range of decrease in developmental rate can be experimentally obtained in the eggs and embryos of Anura by exposure to low temperatures, acceleration of the usuallrate by high temperatures takes place only to a limited degree under natural conditions and but slight increases in developmental rate have been experimentally obtained. Hertwig (1897) defined the optimum temperature as "die Temeratur bei welcher sich der Entwicklungsprocess bei allen Iiern mit der grossten Beschleunigung ohne eine auffallige Storung und Abweichung von der Norm vollzeiht." The optimum temperature has been found to be not far from 28°C. for unsegmented eggs of Bufo lentiginosus, and 30° to 31°C. for later stages (King, 1903). For Rana fusca, the optimum is 20° C. for the unsegmented egg and 24°C. for embryos in later stages of development (Hertwig, 1899). King believed it may be possible to show that the temperature most favorable for the development of anuran eggs depends to a certain extent on the time of year at which the eggs are deposited, and Hoadley and Brill (1937) have reviewed literature showing that there is a correlation between the breeding season of an amphibian and the behavior of its eggs under various temperature conditions. According to the latter, Dr. Gertrud Hermes has found that Rana pipiens, Rana sylvatica, and Bufo

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americanus, which have slightly different breeding seasons, also show differences in their responses to various temperature conditions; a temperature of 29° C. permits normal development in Bufo americanus, but not in the other two. If this relationship does exist, one would expect the optimum temperature for the development of eggs, embryos, and larvae of Scaphiopus to be higher than 23° to 25° C., since the eggs are as a rule laid late in the spring or even during the summer and develop at the relatively high temperatures usually prevailing at these times. Temperature readings taken in pools near Norman show that embryos and larvae at times develop in nature at a higher temperature than that maintained in the laboratory. On May 10, 1936, embryos just at the point of hatching, and somewhat advanced in stage of development than embryos raised in the laboratory and almost certainly of the same age, were taken from a buffalo wallow in which the water temperature was 28° C. On April 31, 1938, the water temperature in a pool in which spadefoot tadpoles were developing was found to be 30° C. Between June 19 and June 25, 1937, Dr. Bragg secured in pools containing spadefoot tadpoles water temperature readings varying from 30° to 37° C. No doubt in warm sunny weather the temperature of water in the type of pools in which eggs of Scaphiopus are deposited is higher, at least during the afternoons, than that at which laboratory specimens developed; at night one would expect it to be somewhat lower.

Stockard (1921), as well as others, has emphasized the fact that oxygen supply, like temperature, has a marked effect on rate of development; while increase in this factor accelerates the normal rate but slightly, reduction may bring about a marked decrease in developmental rate. In an attempt to guard against crowding and insufficiency of oxygen supply for embryos and larvae developing in the laboratory, only a

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relatively small number of specimens was kept in any one culture dish, and the water was changed from once to three or four times daily. Developmental rate under these conditions was practically the same as that of specimens kept in large cement tanks.

Supply of food is known to have a marked effect on the rate of growth of tadpoles after hatching. All laboratory-reared specimens were supplied with an abundance of both animal and vegetable food. As is stated in another section, comparison of tadpoles brought into the laboratory at an age of about three weeks with those of the same group remaining in the pool indicates that in the former the onset of metamorphosis was reached a little earlier than in the latter, possibly due to the feeding of beef liver. Even so, tadpoles reared in the laboratory agree quite closely as concerns time of metamorphosis with those transforming in their natural habitat (see section on Life History). Moreover, the rate of growth of laboratory-reared specimens is similar to that recorded by Gilmore (1924) for tadpoles of the same species developing in pools.

In view of these facts and observations, it is concluded that, while it may be impossible to state exactly what the so-called normal rate of development may be for a species, the time relationships observed and recorded in the development of Scaphiopus bombifrons are as truly representative as it is possible to make them.

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Formation of a 'Septal Furrow' During Gastrulation

It has been pointed out that during gastrulation the boundary of the roof of the blastocoele is indicated in external view by the presence of a circle of sunken cells analogous to the 'boundary groove' of Petromyzon and to the 'Scheidewandfurche' or 'Septal furrow' of Cryptobranchus japanicus and C. alleghenienesis. Apparently in each form in which it has been described the septal furrow is passive in origin and is a product of gastrulation. In both Petromyzon and Cryptobranchus, the egg contains considerable yolk, and the roof of the blastocoele is unusually thin. In Petromyzon, the groove is exaggerated to such an extent that in some cases it constricts the embryo into an hour-glass form. In Cryptobranchus, it takes the form of a definite furrow enclosing the fenestra, a clear and nearly transparent area which, in living embryos and in those fixed in Smith's fluid containing twice the usual amount of potassium bichromate, it is cut up into small polygonal areas each composed of several cells and separated by lines resembling cleavage furrows. In Scaphiopus, the entire upper hemisphere assumes an irregularly rough and bumpy appearance during the early phases of gastrulation, and after the septal furrow has been established this appearance is retained in the area corresponding to the fenestra. Since the roof of the blastocoele is not unusually thin, and since the septal furrow has not been observed in any other amuran gastrula, it seems likely that its appearance may be due to

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the speed with which gastrulation takes place. The formation of a septal furrow is in itself of no great importance, but its occurrence in the gastrula of three forms no more closely related than are Petromyzon, Cryptobranchus, and Scaphiopus is an interesting example of convergence in purely embryonic characters.

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SUMMARY

1. The embryonic and larval development of Scaphiopus bombifrons Cope is described in greater part with reference to external features and in smaller part with reference to internal structure. The early developmental period is, for purposes of description, divided into stages based upon age and upon morphological features.

2. A complete time record derived from study of living material is presented in connection with the description of stages.

3. Development in all phases of embryonic and larval life has been found to be exceedingly rapid. For example, (1) the early cleavages appear at ten to fifteen minute intervals, (2) gastrulation begins at seven hours after fertilization and is completed four and one-half hours later, (3) the neural tube is completely closed three hours after the first appearance of the neural groove, (4) hatching takes place at the age of twenty-eight hours, and (5) metamorphosis is completed on the thirty-second day.

4. Throughout the early cleavage stages there can be distinguished a dorsal eccentric area of accelerated cell division indicative of the bilaterality of the embryo; this area first appears at the second cleavage, and is evident at all succeeding cleavages as far as they can be followed. The superficial cleavage pattern of later blastuale exhibits a bilateral symmetry which corresponds with the definitive

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bilaterality of the embryo and which is paralleled by internal evidences of bilaterality.

5. Transition of the roof and wall of the blastocoele from a single to a several cell-layered condition begins with inward migration of cells at either the fifth or the sixth cleavage. Formation of furrows which divide cells into external and wholly internal blastomeres first takes place at the sixth cleavage.

6. During gastrulation the roof of the blastocoele is bounded superficially by a circle of sunken cells which is analogous to the 'septal furrow' of Cryptobranchus or the 'boundary groove' of Petromyzon; so far as is now known, Scaphiopus is, in the occurrence of this phenomenon, unique among the Anura.

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Explanation of Figures

146. Miscellaneous cleavage stages. Smith and formalin fixation. X11.

147. Stage 4. Earlier completion of fourth furrows in dorsal micromeres. Gray crescent at lower left. Formalin fixation. X35.

148. Stage 4. Micromeres in a roughly radial arrangement. Gray crescent at left. Formalin fixation. X20.

149. Stage 5. Earlier appearance of fifth furrows in dorsal micromeres. Gray crescent below. Formalin fixation. X15.

150. Stage 5. Earlier completion of fifth furrows in dorsal micromeres. Gray crescent at left. Formalin fixation. X15.

151. Stage 5. Embryo showing a small partially submerged cell after completion of the fifth cleavage. Gray crescent at upper left. Formalin fixation. X28.

152. Stage 12. Embryo showing during gastrulation a circle of sunken cells in the upper hemisphere. Smith fixation. X10.

153. Stage 13 (one and one-fourth hours after beginning of the stage). Smith fixation. X22.

154. Stage 14-2. Smith fixation. X22.

155. Stage 15-1. Smith fixation. X15.

156. Stage 16. Formalin fixation. X13.

157. Stage 18. Formalin fixation. X12.

158. Stage 19. Newly hatched larva. Bouin fixation. X13.

159. Stage 22. Formalin fixation. X12.

160. Stage 24. Formalin fixation. X13.

161. Stage 24. Same larva as in Fig. 160. X13.

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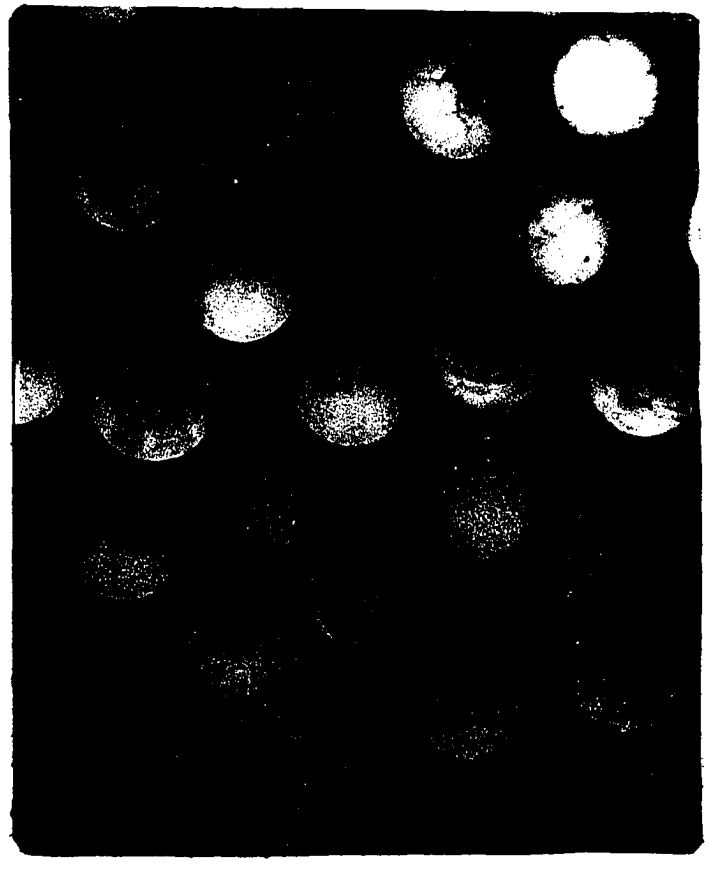

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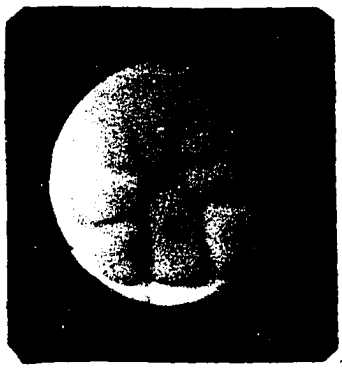

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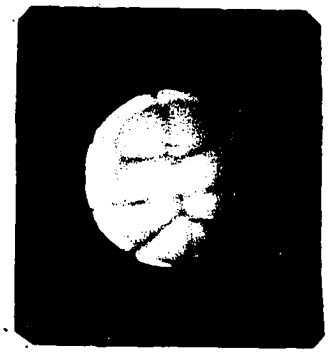

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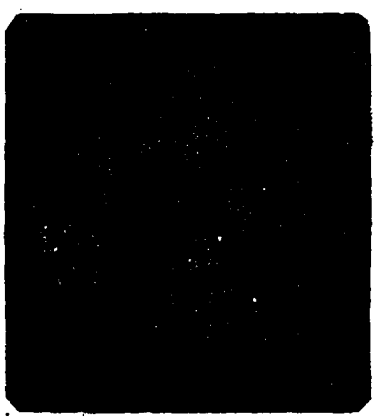

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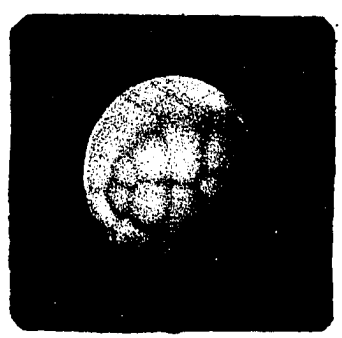

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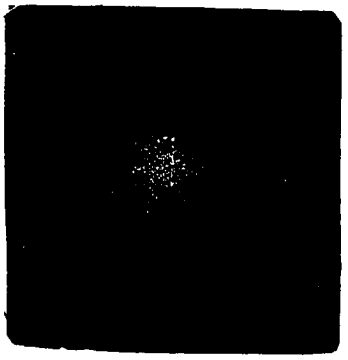

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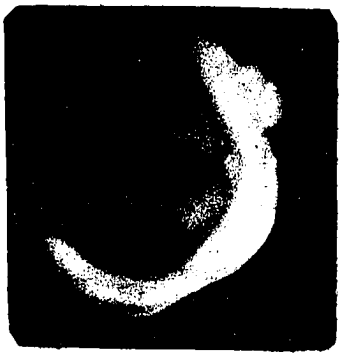

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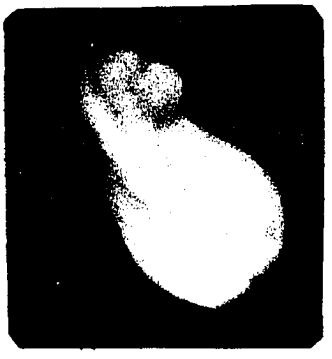

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