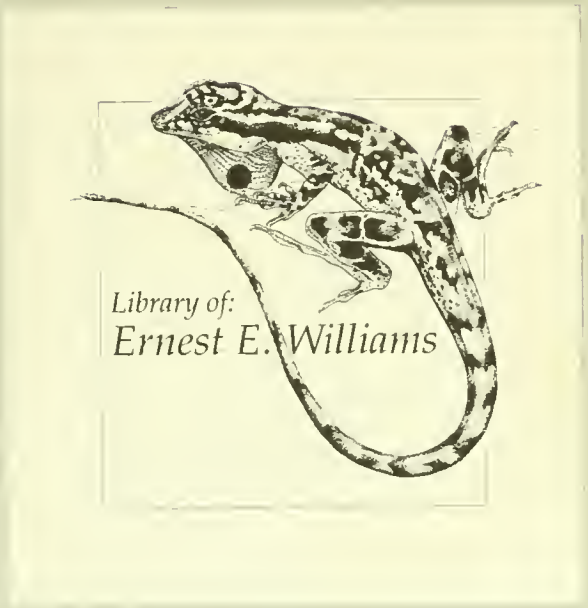

Development of Amblystoma punctatum.

Part I.—External.

By S. F. CLARKE, Ph. D.

1879.

The Development of Amblystoma punctatum--Baird.

PART I, EXTERNAL.

By SAMUEL F. CLARKE, Ph.D.

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In early March of 1878, I obtained in early stages of development a number of eggs which I believed to be those of some Urodele. They were found in considerable numbers in the pools and small streams in and near the woods about Baltimore, during the months of March and April. They occurred in gelatinous masses, Plate 4, Figure 30, which varied greatly in size, were usually more or less oval in shape, and attached to the stem of some aquatic plant or to an overhanging blade of grass.

This year I was so fortunate as to secure living specimens of both sexes of *Amblystoma punctatum* before the females had deposited their eggs. They all did well in confinement; the males furnished an abundance of spermatozoa at the critical moments, the eggs passed through their various phases of development, and a record of the external change is preserved by a series of camera-lucida drawings. The animals derived from the eggs brought to me in the spring of 1878, were studied with considerable care and received considerable attention in respect to their food and surrounding conditions. I was unable, however, to keep them after they reached the abranchiate stage, and in consequence could not determine what form I had been at work upon. I was much pleased then to find upon carefully comparing the eggs laid by the adults in my aquaria this spring, their course of development and their more advanced forms, that they agree in every particular with the eggs and young forms which had occupied my attention in the previous years. By getting the eggs in this way I was able also to obtain the changes during segmentation, which I had not gotten from my previous observations.

The eggs, as I have said, occur in gelatinous masses, and these vary in size from a small bunch of three or four eggs to a large mass containing two hundred eggs and weighing sixteen ounces. When the eggs are deposited in the water they are seen to be covered with a very viscid, tenacious, translucent substance, of

which there seems to be but a thin coating, serving to keep together the eggs which have been deposited in that particular spot. This viscid substance however, rapidly absorbs water and in a few hours forms the bulky gelatinous matrix in which the eggs are contained. During the early stages of development the matrix is of sufficient consistency to hold together when the mass is suspended by a small portion held in the hand. As development progresses however, the mass at first gradually, afterwards more rapidly, loses consistency, so that by the time the embryos have acquired their balancers, the eggs will almost drop out of the matrix when the mass is held out of water in the hand. Each egg is surrounded by two membranous shells and the large space between the two membranes as well as the inner space, is filled with a clear, transparent fluid. The embryo thus situated within two elastic, spherical sacs containing fluid and the whole placed within a mass of yielding gelatinous substance, is well protected against injuries from collision with hard objects and also from becoming the food of voracious fish; for the latter appear to find no satisfaction in drawing into their mouths, portions of this gelatinous material which slips out as often and as rapidly as it is drawn in.

I was interested to find, after carefully watching the process a number of times, that the number of eggs deposited at a time depends upon accident. If the creature is disturbed, as by another individual striking against or touching it, or by the moving or jarring of the dish, she immediately suspends operations, and seeks some more quiet spot for the continuance of her labors. I have seen a single egg deposited and again a bunch containing one hundred and fifty. While the eggs are being extruded the animal usually lies with its anterior limbs extended laterally, while the hind limbs are curved around the opening of the cloaca and appear to assist in holding together the eggs as they are laid.

The males showed no inclination to clasp the females, but quietly deposited quite large masses of an apparently rather thick liquid, opaque white, on the bottom of the dish in which they were kept. Upon examination this liquid was found to consist of spermatozoa moving actively in a liquid. The eggs were found to have adhering to their outer shells, shortly after, a considerable number of these male elements, but I could not succeed after trying a great many times in finding any spermatozoa within even the outer shell.

Most of the eggs were laid during the night, and by nine o'clock the next morning the first segmentation furrow had usually made its appearance. The spermatozoas, Plate 4, Figure 31, are unusually large, averaging .75 millimetre or .03 of an inch in length. They are very slender and acutely pointed at both ends. When first thrown out they often have a remnant of the mother-cell still attached to some portion of them, but on account of their active movements it is soon thrown off. As active movements begin to cease in them, one end is often bent around till it touches and adheres to the body, thus forming a loop of variable shape and dimensions, which has much the appearance, until carefully studied, of an enlarged portion or "head" of the spermatozoöid, Plate 4, Figure 31c, 31d. For ready reference, I give the measurements of the spermatozoa of a number of different amphibia, both Anoura and Urodela.

Rana temporaria, ...	.008 to .011	of a mm.
Pelobates fuscus, ...	.017	" "
Triton, ...	.088	" "
Menopoma allegheniense, ...	.25	" "
Amblystoma punctatum, ...	.75	" "

The first three are taken from Wagner and Leuckart's article,* the others were made by myself.

A few minutes after an egg is deposited there exists between the inner shell or membrane and the yolk, a quantity of gelatinous matter which seems to form, as development goes on, a third, inmost shell, very delicate and hyaline. The yolk lies so close to this inmost shell that it cannot at first be distinguished. As the process of segmentation begins, the yolk-mass is separated by a small space from this inmost shell, when the latter becomes distinctly visible. It remains until the medullary folds are nearly closed in, when it disappears; it being often, if not always, torn apart by the rapidly elongating embryo. At this early period the diameter of the outer shell is about twice that of the inner, and this relative size is maintained with considerable regularity throughout the period of intra-oval life. Both shells now rapidly increase as water is absorbed. By the end of segmentation the shells have reached nearly or quite their largest size, and remain as they then are until the embryo bursts them and makes its way out.

The Cycl of Anat. and Phys., f. 472.

If a freshly laid egg be stripped entirely of its shells and all adhering gelatinous matter, it will be found to be divided into two zones which are almost exact hemispheres, marked out by colors. One hemisphere is black, and the other quite light, almost white. The light portion is not evenly colored; the lightest part of it forms a zone lying next to the dark hemisphere and the darkest portion of the light hemisphere is at the pole, the spot where the vitelline plug is to be formed. This coloration changes as development goes on. Although the lighter hemisphere is not a clear white, it is a sufficiently light color to make the two hemispheres quite sharply defined. It can readily be seen by pricking open an egg and allowing the contents to flow out, that this coloring matter lies on the surface, the inner contents of the egg being uniformly opaque—white. If one of these unfertilized eggs be placed in water, it instantly and always assumes a position with the dark hemisphere up and the light pole down; and as often as it is turned over in any other position it immediately rights itself when the retaining force is removed. As sections show no cavity in the eggs at this period it must be that the density of the unfertilized egg is not uniform and that the lighter colored is always the denser hemisphere. The cavity of Von Baer has not yet appeared, and that moreover would not, probably, be large enough to cause such a difference in density as is indicated by the quickness with which the unrestrained egg always takes this position. After the segmentation-cavity is formed, that portion of the spherical yolk containing this cavity is of course lightest and uppermost; but before segmentation and fertilization, when no cavity exists, this action must be produced by a difference in density of the particles composing the yolk. What can be the object of this arrangement by which the different colored poles are thus placed, it is difficult to conjecture. The darker colored areas would absorb more heat from the sun's rays, which under the usual natural conditions would be beneficial to rapid development. The arrangement is the same also as the protective coloring in many birds and fishes; the upper side dark and the under side light. This might be of some service to them, as fish of large size might eat small bunches of eggs and would attack them from below, as the egg-masses are usually at or near the surface. Goette says in respect to this coloring of Batrachian eggs—

"All the observers of the pigmented, developing Batrachian egg agree in this, that sometime after fertilization they turn themselves always with the dark pole upward, even if it was not the case at first. A sufficient reason for this cannot be found. According to my view, this turning of the yolk is apparent, whether general or in part, since only the pigmentary layer following the influence of the newly determined pole, displaces itself." *

Segmentation commences by the appearance of a furrow on the dark hemisphere which stretches around the egg, the two ends meeting at the light pole, and thus dividing the egg into two hemispheres, each of which contains half of the dark and half of the light hemispheres. The two color areas during the early stages of segmentation are more distinctly outlined than at any other period. The dark area has become a rich dark brown. The second furrow forms a great circle at right-angles to the first, and starts also at the dark pole. After the formation of these four meridional sections, by the two furrows, a third furrow passes around the equator and separates the dark from the light hemisphere very sharply. The third segmentation furrow in Triton and in Bombinator differs from Amblystoma in being not equatorial, but nearer the upward pole. From this point segmentation progresses quite rapidly and at different rates in the two color areas; it being much more rapid in the lighter one. As the segments begin to get quite small, more and more color makes its appearance in the light area until as segmentation is about completed, only a small light area is left at the lighter-colored or downward pole. At the time when the first two furrows are complete there may be seen on the different segments near the light pole, a few small depressions in the substance of the yolk, the "trous vitellins" of Dr. Van Bambeke.† They soon disappear however, being visible for a few hours only. Upon examination, it is readily seen that segmentation progresses much more rapidly in the light hemisphere, and that it is carried on with very little regularity, in either. When the egg has finished segmentation the entire surface has become dark colored with the exception of a small irregular area surrounding the lighter pole and stretching away from it in one direction. The cells or segmentation-masses immediately

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*Vide Der Unke, p. 53.

†Bulletins de l'Académie royale de Belgique, 2me série, Tom XXX, Nr. 7, 1870.

about the pole are larger than the others. Very soon an irregular, slight depression of this polar region occurs, which lasts but a short time; this area becoming again even with the surface of the sphere. But this movement or action has resulted in the formation of a very narrow, even, clearly marked groove, which sharply defines, or makes a distinct boundary to, the polar portion of the white area, and extending on either side along the edge of that portion of the lighter area which stretches away from the pole, gradually fades out. See Plate 1, Figure 1. Now the curved groove becomes less and less widely open in front, (Plate 1, Figure 2), until finally the two ends meet. The groove around the now circular area becomes gradually deeper, the entire surface outside of the circular, polar area has become dark colored; the polar-area itself is composed of large white masses with dark outlines. In this way is formed the "vitelline plug" of Ecker. Plate 1, Figure 3. In a side view of an egg a few hours after the formation of the vitelline plug, one sees that the latter has become raised up from the surface of the egg, giving the appearance of a small white mass resting on or protruding from a dark colored sphere. Plate 1, Figure 4. A front or polar view of the same egg at the same period is shown in Plate 1, Figure 3. The plug retains this prominent position but for a few hours and then begins to sink into the egg; as it does so, the adjoining parts of the egg close around it until there is a very small, circular pit or depression left in the centre of the area formerly occupied by the vitelline plug. While the plug is thus being withdrawn into the egg, there appears on nearly opposite sides of the contracting area occupied by the vitelline plug, the walls of the anal part of the medullary fold. Plate 1, Figure 5. This change has, of course, produced a corresponding change in the outline of the egg, between which and the vitelline membrane there is now quite a well marked space, but which is greatest at the lower pole. The medullary folds extend forward towards the opposite or anterior pole of the egg, quite rapidly, so that by the end of the fourth day after the beginning of the formation of the vitelline plug, a stage represented in Plate 1, Figure 2, the two folds have met at the head end. Plate 1, Figure 6. The cephalic portion of the medullary fold is much widest and thickest and the cephalic ends of the lateral wall of the medullary folds are more widely separated than the anal ends. The space enclosed by the medullary folds is marked through its

longitudinal axis with a slight groove or depression, the medullary groove. The areas lying within the medullary folds on either side of the medullary groove, are the medullary plates, and in some instances, are composed of cells slightly larger and a trifle lighter colored than those of the remainder of the embryo.

The egg has meanwhile been changing shape, not only on the dorsal side, that marked by the medullary folds, but also at the anal end, in such a way that in a profile view of the latter region there is seen a depression or a sinuosity in the outline, showing that the originally spherical ovum is beginning to take on the elongated form of the embryo. Plate 2, Figure 7.

The medullary folds having become continuous, the process of folding in and uniting with each other to form the closed, neural tube advances with great rapidity; the entire process occupying eight or nine hours. The first well-marked change in the folds, after they have become continuous at the cephalic end, takes place at points in the lateral-walls about midway between the cephalic and anal ends, where they grow inwards towards each other, Plate 2, Figure 7; then the large, thick walls of the cephalic end rapidly grow towards one another and unite over the middle line of the medullary groove. Near the anterior ends, the cephalic portion of the folds meet and unite first, the union gradually extending backwards along the median line. At the extreme anterior end of the medullary folds however, a considerable space is left which is the last to remain unclosed. In this way a fusiform space, the *sinus rhomboidalis* comes to be left between the anal end and a point about midway between the anal and cephalic ends, where the folds first grew towards each other. Plate 2, Figure 8. This fusiform space, though, is soon closed over by the advancing folds, and is quickly followed by the closing over of the space left at the cephalic end. At the extreme anal end, the folds remain separate over a small area, the space formerly occupied by the vitelline plug, and form a rounded edge about this small cavity or pit. It becomes a definitely rounded cavity by the time that the first constriction, indicating the throat, is seen. While the neural tube has been thus rapidly forming, the embryo has increased very much in size, and its outline has become very much altered. It is now much more elongated, and both the anal or caudal and cephalic ends are becoming more definitely indicated as they grow away or stretch out from the body of the embryo. The entire

surface of the body is now covered with cilia, by aid of which it keeps up a horizontal rotatory motion upon its axis.

In a ventral view of an embryo, at about this stage, we would also notice this change in form, and we would see that the anal end of the medullary folds extend farther around on the ventral side than the cephalic end. Plate 2, Figure 9.

A constriction now makes its appearance in the throat region, thus defining the head from the body. At the same time, the remainder of the region of the neural canal becomes more distinctly outlined; a swelling or slightly oval prominence appears on each side of the head, the first external indications of the optic vesicles. Plate 2, Figure 10. In a dorsal view, a line running along the centre of the neural canal indicates the line of union of the medullary folds. Plate 2, Figure 11. In a ventral view of the same are seen both the optic vesicles, the ridge of the medullary fold between them, the constriction of the neck and the anus at the posterior end of the neural tube. Plate 2, Figure 12. The embryo having reached this stage, a second groove or furrow appears in the neck-region, so that the throat is now marked off both from the head and from the body. The anterior end of the neural canal or head now bends forward and downward upon itself, so that, by this cranial flexure, the fore-brain, with its optic vesicles, no longer occupies the anterior end of the longitudinal axis. The head has also changed in shape, having no longer a simple rounded outline. In the anterior portion of the neural canal there appear a few transverse swellings, the first indications of the protovertebræ. Plate 2, Figure 13. These latter soon increase in number, additional ones making their appearance posteriorly; the neck region becomes larger; the optic vesicles become more rounded and more prominent. There is next seen projecting from the sides of the neck behind and above the prominence of the optic vesicles, a pair of lobes, one on each side; from these lobes are to be developed the branchiæ. A little posterior to the branchial lobes, there has also appeared another pair of lobes; from these will be developed the anterior limbs. The optic vesicles are still more prominent, and the protovertebræ now appear in a side view to be somewhat removed from the outer edge of the neural canal towards its centre; they are also larger.

Development now progresses at both extremities, and the entire body increases rapidly in size. The head is still farther separated

from the body by the continued growth of the neck region; the branchial and brachial lobes are growing more prominent, and on the median ventral line of the neck between the branchial lobes, or slightly posterior to them, is a single rounded prominence which indicates the pericardial region. The posterior end of the body, owing to the development of the tail, which is stretching away from the body, has become more elongated, and is obtusely pointed.

In a ventral view at this stage, the nasal pits are distinctly seen, as two small, black cavities lying just within or ventral to, the swellings of the optic vesicles. The head is seen to have become much narrower and longer, and the position of the future mouth is indicated by the space existing between the anterior end of the branchial lobes and the curved outline of the extremity of the neural canal. The beginning of the tail also shows distinctly, and its median ridge, at the end of which is the dark cavity of the anus, is now much increased in size. Plate 2, Figure 14. At a period about two days later than that represented by Figure 14, a new lobe or prominence is seen upon each side of the neck between the eye and the branchial lobe; it is much smaller than, and lies just at the anterior extremity of, the long axis of the branchial lobe. Very often it is developed consentaneously with the branchial lobes, instead of making its appearance a day or two later. From these lobes are to be developed structures which, from their resemblance to the balancers of Dipterous insects, have come to be known as the "balancers." The eyes have progressed rapidly during the last day or two, and the nasal pits are more clearly defined. The body of the embryo is now, by a rapid growth of the ventral side, losing the curved outline which it has always had, owing partly to its having been formed upon a sphere, and is now becoming straight; the caudal portion is developing rapidly and vertebræ will soon be seen making their appearance within its substance.

The animal now begins to show active, muscular movements, which consist of a sudden doubling upon itself; a position retained for a few seconds only, when it regains its original position by another sudden and violent movement of the body. A thickened ridge also appears on either side of the anus; these are the walls of the cloaca. Within a day or two, the rapidity of development varying widely in different specimens, the branchial lobes show

traces of division into three portions; the divisions making their appearance first upon the ventral side and running at right angles to the long axis of the lobes. In this way the three pairs of gills are first indicated, and the divisions between the lobes are the first external indications of the branchial clefts. The small rounded lobes anterior to the gills have already become elongated and somewhat resemble their perfect form.

The integument over the pericardial region has become so transparent that the heart can be seen by transmitted light to be pulsating. Up to this period the embryos, since the closing of the medullary folds, have been of a uniform dark brown or brownish-black. Now, a number of large stellate cells filled with black pigment make their appearance along the region of the protovertebræ, from the branchial lobes nearly to the anus; others soon make their appearance in the same region filled with a greenish-yellow pigment and some of the external epithelial cells have the same yellowish-green hue. These pigment cells are very early found upon the brachial lobes and soon extend over all parts of the body. The body of the embryo is now straight and five or six vertebræ have been formed in its rapidly developing tail. Plate 2, Figure 15.

A dorsal view at this stage, or a little earlier than this, before the divisions appear in the branchial lobes, shows the body of the embryo resting on the unabsorbed yolk, of which there is still considerable left. It also shows very well the relative position of the eyes, balancers, branchial and brachial lobes, and the division between the neck and body. This latter differentiation is now becoming more and more evident. A ventral view shows that a deep constriction has taken place on the sides of the neck, thus marking off that region from the rest of the body. In the anterior end of the body region, where it has been made narrow by the lateral constriction, is the pericardial region; the integument is here so thin that the chambers of the heart may readily be distinguished and the pulsations counted.

The divisions of the branchial-lobes, or the branchiæ, as we may now call them, for the blood is by this time circulating in them, and the balancers all grow rapidly in length. The caudal portion of the body also becomes longer, but otherwise there are but few external changes posteriorly, for a day or two. Most of the energy seems to be devoted to the growth of the branchiæ and the balancers. In examining a large number of specimens, it is

at once seen that there is great variation in the progress of development. The position of the balancers too, varies considerably in different individuals of the same age.

Active growth is next shown in the development of the tail and the caudal and dorsal fin; the branchiæ and supporters are also growing rapidly, and a depression on the ventral side, on a line between the eyes, marks the position where the mouth will appear. The heart may still be seen in the pericardial region, though the integument is gradually becoming more opaque. It is now making from forty-eight to fifty pulsations per minute. Plate 3, Figure 16. During the following thirty-six hours, the branchiæ continue to progress rapidly, becoming more and more elongated, and begin now to bud out small processes from the sides. The eye has become much more perfect, and its structure is nearly complete. The balancers have grown with the gills, though they do not equal the latter in length. The caudal fin has become so large that it now performs its functions as the locomotor organ of the body. The animal shows quite active energetic movements in the egg, and if it is allowed to escape into the water by tearing open the membranous shell, it is seen to swim about with great activity, being propelled by vigorous movements of its tail. Watching its movements as it sinks to the bottom of the dish, which is covered with a deposit of fine, light, vegetable débris, we can readily determine the use of the balancers. As the animal approaches the bottom it holds its balancers out from the body so that they point outwards and downwards; owing to this position in which they are held, the animal sinks but a short distance into the light material of the bottom and thus keeps the head and branchiæ above the dirt where they can be readily furnished with a constant supply of pure water. The pericardial region is at the same time kept free from the bottom, so that there is nothing to interfere with the beating of the heart. Plate 3, Figure 17. This arrangement calls to mind the position which the cuttle-fish, Loligo, assumes when at rest; the tail and posterior portion of the body rest directly upon the bottom while the anterior portion is supported entirely by the median ventral pair of arms, only the anterior or distal ends of which furnish a support for the anterior portion of the body; the rest of the arms are arched so that the head and neck are kept from touching the bottom; thus affording free opportunity for the egress and ingress of water to and from the mantle-cavity and free use of the siphon.

A ventral view at this stage shows that the pericardial region is moved slightly further back, the neck region is not so narrow and the neck groove is continuous across the ventral surface. The outline of the mouth is indicated; the gill processes are increasing in size and in number; the balancers are still growing and have become somewhat capitate and the brachial lobes are beginning to increase in size. The head too is now changing shape, becoming much broader.

It is interesting and suggestive to note in a ventral view at this period, the general resemblance to a young dog-fish, especially in the position of the mouth and branchiæ and the shape of the head and body.

For the next two or three days development is most active in the branchiæ and in the tail. The latter increases considerably in length and the dorsal fin grows rapidly. The branchiæ double their length in two or three days and give off numerous processes which grow rapidly and which are arranged in two rows, the members of which point outwards and downwards, diverging from each other. The brachial lobes are developing slowly, being as yet, a pair of simple lobes or processes on the sides of the body just behind the branchiæ and partly covered by the latter. The change in the form of the head continues; it is becoming more rounded in front and broader. From this time until the posterior pair of limbs are being developed there is very little change externally, in the posterior portion of the body. The branchiæ and supporters have now reached their full development; that is, the branchiæ have all their processes budded out and the branchiæ are relatively to the size of the body as large as they ever will be, though absolutely they will still increase in size; the balancers, however, being only embryonic appendages, have attained their largest size; they are capitate and will now decrease in size and disappear as the anterior limbs develop and take upon themselves the function, previously performed by the balancers. Plate 3, Figure 18. After the branchiæ have become as large as those represented in Figure 18, the development of the anterior limbs may be best studied by cutting away the hinder pair of branchiæ. The limb-processes rapidly elongate, pointing backwards and a little downwards and outwards; at first, they are simple rounded processes with an unbroken outline until the length is two or three times the breadth. When they have attained these dimensions a slight indentation is

seen in the distal or free end of the limb, dividing it into two lobes each of which becomes a digit; the outer one, when the limb is directed backward, becoming the first or most anterior digit and the inner one becoming the second. A slight flexure or bend in the limb now makes its appearance which indicates the position of the elbow-joint. The opening of the mouth makes its appearance usually at about this stage or later. Plate 3, Figure 19. Soon after the first two digits are thus marked out, the balancers begin to diminish in size, becoming more and more slender but not decreasing in length. Plate 4, Figure 21. The mouth-groove is now fully indicated, but the opening appears first in the central portion of the groove and extends gradually in both directions, until the mouth has attained its full size. A side view shows that the tail has become longer, the dorsal and ventral fin-like areas have grown rapidly and the rectum is distinctly seen opening into the cloaca; the position of the mouth too has changed, being much farther forward. This condition is reached from the twenty-fourth to the twenty-sixth day after the formation of the vitelline plug.

The anterior limbs continue to grow rapidly; the second digit growing faster and quickly becoming much larger than the first and at the base of the second digit on the inner side of the foot appears a small process which is to develop into the third digit. Plate 4, Figure 24. The balancers are still more slender, the blood has nearly stopped circulating in them and they are of but little use. A central artery and vein are seen in the balancers when they first bud out from the side of the head, and these increase in length with the growth of the balancers; so that when the latter are fully developed the blood may be seen rapidly circulating throughout the length of these appendages; as they grow more and more slender there is less and less blood sent to them, until when they are in the condition represented in Figure 21, Plate 4, there are only a few stray corpuscles to be seen, which slowly work their way in single file to the extremity of the appendage and passing through the capillaries, as slowly wend their way back again. Circulation in the balancers now soon ceases and being of no further use to the animal, these appendages are no longer retained. While watching through the microscope, a specimen which had but one balancer left, and that a very slender one without any blood circulating it, I noticed that the creature would occasionally give a number of quick, violent shakes with its head;

as these were repeated I saw the balancer gradually break off at its base or proximal end and finally becoming entirely free, fall to the bottom of the dish, leaving the animal free of these embryonal appendages, for which it had no farther use. Plate 4, Figure 22. This observation was made upon a specimen twenty-eight days after the formation of the vitelline plug. In examining twenty-five specimens of this same age I found two in which both balancers were still present; three in which one still remained, and twenty in which both had disappeared. In all of these specimens development had progressed to the condition indicated by the presence of the rudiment of the third digit on the anterior limbs. Consentaneous usually, though sometimes a little later than the appearance of the third digit on the anterior limbs, appear a pair of small lobes on either side of the cloaca which are to develop into the posterior limbs. The progress of development in these appendages is like that of the anterior ones. The processes elongate, a slight indentation in the centre of the distal end appears, which increasing in size as the limb grows, forms two digits, the first and second; from near the base of the second, a process buds out which develops into the third digit; from near the base of the third digit buds out the fourth, and from near the base of the fourth buds out the fifth digit of the posterior limbs. The first indication of the first two digits of the posterior limbs occurs at about the same time that the fourth and last digit of the anterior limbs appear. Plate 4, Figures 23 to 28. All the external parts of the animal being now formed, the creature being about sixty days old, it undergoes no external changes beyond a general growth until the branchiæ begin to decrease in size as they are being resorbed. Plate 4, Figure 29. This change takes place in specimens reared in aquaria at about one hundred days from the beginning of segmentation. The process of resorption of the branchiæ begins at their distal ends; the outer branchial-processes become shorter and disappear, the outer portion of the main body of the branchiæ become shorter; then the inner processes disappear and nothing is left but three pairs of small rounded processes which are slowly absorbed; it taking as long usually for this latter part of the process to take place as it does for all the first portion. The whole process occupies from three to five days. Thus in a few days they change from water to air-breathers, from a less to a more highly specialized organization, and leaving the water take up their abode in damp localities upon the land.

To recapitulate briefly. After segmentation there appears around the lower pole of the egg an area made up of large cells, which, at first hemispherical, then oval and finally circular, forms the vitelline plug of Ecker. This plug protrudes from the egg, then sinks into it, while from the diminishing area around the disappearing plug, stretches away the anal portions of the medullary folds with the medullary groove midway between them. The two folds grow forwards and unite near the opposite pole. The medullary folds close in and unite forming the neural tube. The body elongates; is covered with cilia and rotates horizontally upon its axis. The head is marked off and the optic vesicles appear. The branchial lobes and the lobes of the cephalic-balancers appear; soon followed by those of the anterior limbs. The pericardial region is marked off and the pulsations of the heart are visible. The nasal pits and the position of the mouth are indicated. The tail and the dorsal fin grow rapidly and the branchial lobes are divided into three pairs of branchiæ. The branchiæ give off processes, the eyes develop rapidly and the mouth is moving forward. The constriction takes place across the ventral surface of the neck, and the balancers now fully developed become capitate. The branchiæ become fully developed; the balancers become more and more slender as the anterior limbs increase in length, and the blood having ceased to circulate in the balancers they drop off. The anterior limbs now develop with rapidity, the first and second digits being formed first, then the third, and finally the fourth. The first two digits on the posterior limbs are formed as the fourth digit on the anterior limbs is budding out; then the third, fourth and fifth digits are developed in succession. About the one hundredth day after segmentation has begun, the branchiæ are resorbed and the animal enters the adult state.

Such was the case at least in those individuals which, having the most perfect branchiæ and the greatest amount of food, grew and developed most rapidly. Other specimens, however, which were surrounded by less favorable conditions developed more slowly. One which was hatched from the egg about the middle of May, retained its branchiæ until the last week in the following October, over six months, when, as the branchiæ were being resorbed, the animal suddenly disappeared from my aquarium during the night. From the time when the young are hatched to the period of the changing from the branchiate to

the abranchiate condition, the dorsal and lateral surfaces of the animal are of a greenish-yellow hue appearing lighter or darker according to the amount of black pigment existing in the different specimens. In this respect there is considerable variation, though none of the specimens are very dark. In most of them yellow is more dominant than the green. The under surface up to and during the time when the branchiæ are resorbed is white with perhaps a slight tinge of yellow. In giving the course of development nothing has been said of the time when the embryo escapes from the egg; this was done because the time varies so very much. It occurs about the period that the balancers have reached their greatest size; sometimes however when they are only half-developed and again not until after they have begun to grow smaller.

The rate of development seems to be dependent upon a number of conditions. Some of the bunches of eggs are much larger than others, and while all those eggs in a small bunch of ten or fifteen will develop with very nearly equal rapidity, of the various individuals in a bunch of one hundred and fifty or two hundred some may progress twice as fast as others. Those upon the outside of the large bunches advance most rapidly and those nearest the centre the slowest. Temperature also has a marked effect; if the water is too cold it retards them, if too warm it kills them. The purity of the water too has an important influence; some which were supplied with running water growing and developing much more rapidly than others which were in jars where the water was changed but once or twice a day. While in the egg there is but little trouble in keeping them in good condition, but after they have escaped from the eggshells and have absorbed all the yolk-nourishment, I found great trouble in getting them food. I supplied them with various things but did not succeed in pleasing them. Three or four which were placed in an old aquarium where there were a number of snails and a good supply of Protozoa and vegetable growth, grew quite rapidly and did well, while those in my other aquaria developed cannibalistic tendencies, which were shown by their biting each others gills off and the tips of the tails also. A few only escaped mutilation in this way and these few increased in size much more rapidly than their less fortunate brethren. This rapidity of growth appeared to be of great benefit to them, for as soon as their mouths had attained the requisite size they turned upon the smaller members of their family and swallowed them

bodily. This large supply of food-material enabled these larger individuals to increase still more rapidly so that in two weeks from the time they commenced feeding upon their comrades they were ten times the size of one of the smaller ones of the same age, yet undevoured. Thus there was an interesting case of natural selection by survival of the fittest, going on amongst these young forms. Those who by their activity and strength preserved their branchiæ uninjured, develop so much faster than their brethren, as to enable them to pass through all their changes in the water and leave that element to seek for regions where food was more abundant. The power of reproduction of lost parts by this class of animals is so well-known that it seems remarkable that these young forms should not have reproduced their lost and mutilated branchiæ. But, on the contrary, not a single specimen of the many hundreds who suffered such losses, succeeded in restoring the lost parts. This may have been due to the small amount of food with which they were at that time supplied.

The branchial clefts have not been mentioned for the reason that they do not appear until after the branchiæ have become so large as to cover up the places where the clefts and arches make their appearance. It thus being impracticable to satisfactorily decide this point from external observations, it is left for the present and will be solved when I work up the changes in internal structure. For this work upon the internal parts I have preserved a large series of specimens in the various stages of development from which it is hoped, by means of sections, to get quite a complete history of the changes which there take place.

EXPLANATION OF THE PLATES.

The outlines of all the figures were obtained with the aid of the camera lucida.

PLATE 1.

Figures 1 to 5 are enlarged 18 diameters.
Figure 6 is enlarged 21 diameters.

FIGURE 1.—The lower side of an egg which has just completed segmentation; *v. p.* the area of large, light colored cells that are to form the vitelline plug; *v. p. f.* the begin-

FIGURE 1—*Continued.*

ning of the furrow around the plug. As yet the furrow extends not more than half way around the large cell area.

FIGURE 2.—A view of the same side of an egg, a few hours later; the letters as before. The furrow nearly surrounds the large cell area, and the latter is changing shape.

FIGURE 3.—Shows the same side of an egg, in which the fold has completely surrounded the area of large, light colored cells. This area is now circular, and is the vitelline plug of Ecker. Letters as before.

FIGURE 4.—A side view of Figure 3. The egg has contracted, leaving a considerable space between it and the vitelline membrane. This space is greatest and quite irregular in the region of the plug; the latter projects from the surface of the egg; *v. m.* the vitelline membrane. Other letters as before.

FIGURE 5.—A later view of the anal region; *v. p.* the vitelline plug which has nearly disappeared within the egg; *m. f.* the anal portion of the medullary folds stretching away from the area of the vanishing plug; *v. m.* the vitelline membrane; *m. g.* the beginning of the medullary groove.

FIGURE 6.—The dorsal region at a more advanced stage; *v. m.* vitelline membrane; *m. g.* the medullary groove; *m. p.* the medullary plate of one side; *m. f. a.* the anal portion of the medullary fold, and *m. f. c.* the cephalic portion.

PLATE 2.

Figures 7 and 8 are enlarged 30 diameters.
The rest, 9 to 15, are enlarged 12 diameters.

FIGURE 7.—A dorsal view. Letters as before. The embryo has lost the circular outline of the egg and is changing shape rapidly; the medullary folds have assumed an irregular outline, and the point at which they will first unite is already indicated.

FIGURE 8.—A dorsal view of the same specimen, taken two or three hours later. The embryo is rapidly elongating and the medullary folds have united along most of their length. The sinus rhomboidalis is now one of the

FIGURE 8—*Continued.*

most prominent features. *m. f. c.* cephalic portion of medullary folds; *m. p.* medullary plate; *m. g.* medullary groove.

FIGURE 9.—A ventral view, less magnified, of a more advanced stage. *c*, the cephalic end of the medullary tube; *a*, the anal end of same.

FIGURE 10.—Lateral view of same specimen from which figures 7 and 8 were taken. Figure 10 was made twelve hours after figure 8. *n. c.* neural canal; *e*, optic vesicle; *t*, throat region; *a*, anus.

FIGURE 11.—Dorso-lateral view of same specimen as figure 10. *m. f. l.* line of union of medullary folds; *m. f. a.* anal portion of medullary folds. Other letters as before.

FIGURE 12.—Ventral view of specimen from which figures 10 and 11 were made. *cl*, the swollen mass from which the caudal portion is mainly developed. Other letters as before.

FIGURE 13.—Lateral view at a later stage. *e*, optic vesicle; *mb*, midbrain; *bn*, the lobe from which the branchiae are to be developed; *ba*, lobe from which the anterior limb develops; *pr*, the external indications of the protovertebrae; *t*, throat region. From the condition represented in Figure 12 to that of Figure 13 the change of outline, with the exception of the increased cranial flexure, has been slight. The energy has been used in developing special parts, rather than in general growth.

FIGURE 14.—A ventral view. The embryo has been growing rapidly in the last two or three days; is much elongated, and the different regions of the body are acquiring definite limits. Letters as in Figure 13. Unfortunately, there is no reference to the nasal pits in this figure; they are the small, dark, oval depressions lying between the neural tube and optic vesicles. Compare Figure 15.

FIGURE 15.—Lateral view of a specimen considerably more advanced. The entire figure of the adult is quite well outlined; *n. p.* the nasal pit of the right side; *e*, the developing eye; *b*, the rudiment of the balancer of the right side; *n*, the pericardial region, with heart partly showing through; *bn*, the branchial lobe, which is beginning to divide into the three portions from which the branchiae of this side will develop; *ba*, lobe which gives origin to the anterior limb; *vt*, vertebrae; *pb*, black pigment

FIGURE 15—*Continued.*

in connective tissue-like corpuscles, which appear first in the dorsal region; *py*, yellow pigment in small cells resembling ordinary epithelium cells; *df*, dorsal fin; *vf*, ventral fin.

PLATE 3.

Figure 16 is enlarged 12 diameters.
Figures 17 to 20 are enlarged 10 diameters.

FIGURE 16.—A lateral view; *f*, depression in which mouth is formed; *bl*, balancer; *h*, pericardial region; *n. p.* nasal pit; *a. l.* abdomen; *v. f.* ventral fin; *d. f.* dorsal fin; *n. f.* and *n. t.* neural or spinal region. The most rapid centres of growth at this period are the tail, dorsal fin, branchiæ and balancers.

FIGURE 17.—Ventral view a day or two later than that of Figure 16. The region between the nasal pits and the anterior end of the body has been imperfectly represented in the figure. It is simply rounded. The position of the mouth is distinctly indicated by the groove *m*; the throat is clearly marked off from the body by a suture or depression; the balancers are developing rapidly and have become capitate; the branchiae are much elongated and are budding out lateral processes; the lobes of the anterior limbs show signs of active growth once more; *a*, the anus. The heart can no longer be seen through the thickened integument.

FIGURE 18.—A dorsal view; *n. t.* external indication of outline of brain cavity; *ba*, lobe of anterior limb. The caudal region has much increased in length; the branchiae are longer and have acquired numerous processes of considerable length. The limb-lobes are also more elongated.

FIGURE 19.—Represents the anterior end only; *bal*, balancer, now completely developed; *bn*, branchiae; *b. s.* branchial stump, the gill having been cut away to show the anterior limb; *b. a.* the anterior limb. The latter is now much elongated, the elbow-joint is indicated and the first and second digits.

FIGURE 20.—The same as Figure 19, but with the branchiae not cut away.

PLATE 4.

Figures 21 to 28 are enlarged 12 diameters.
Figure 29 is enlarged 14 diameters.
Figure 30 is one-half natural size.
Figure 31 is enlarged 100 diameters.

FIGURE 21.—View of anterior end of body and head; *bl*, balancer; these appendages are now becoming more and more slender, and the circulation in them is diminishing; *bn. s.* the branchial stumps, the branchiae having been cut away; *d1* and *d2*, the first and second digit of the anterior limb; *d3*, the first rudiment of the third digit.

FIGURE 22.—Shows the anterior portion only; *be*, the balancer which has just been shaken off by the animal. The branchiae are now fully developed. The digits of the front limb are elongating.

FIGURE 23.—A part of the hinder portion of the body; *ce*, the cloaca; *pa*, the posterior appendage budding out.

FIGURE 24.—Distal portion of anterior appendage of same specimen; *d1*, *d2* and *d3*, the first, second and third digits.

FIGURE 25.—View of cloacal region a little later; *ce*, cloaca; the distal part of the posterior appendage is bifurcating, giving rise to the first and second digits, *d1* and *d2*.

FIGURE 26.—Anterior appendage of the same; *d1*, *d2* and *d3*, the first, second and third digits; *d4*, the rudiment of the fourth.

FIGURE 27.—The anterior appendage at a later stage and turned in the opposite direction; *d1*, *d2*, *d3* and *d4* the first, second, third and fourth digits.

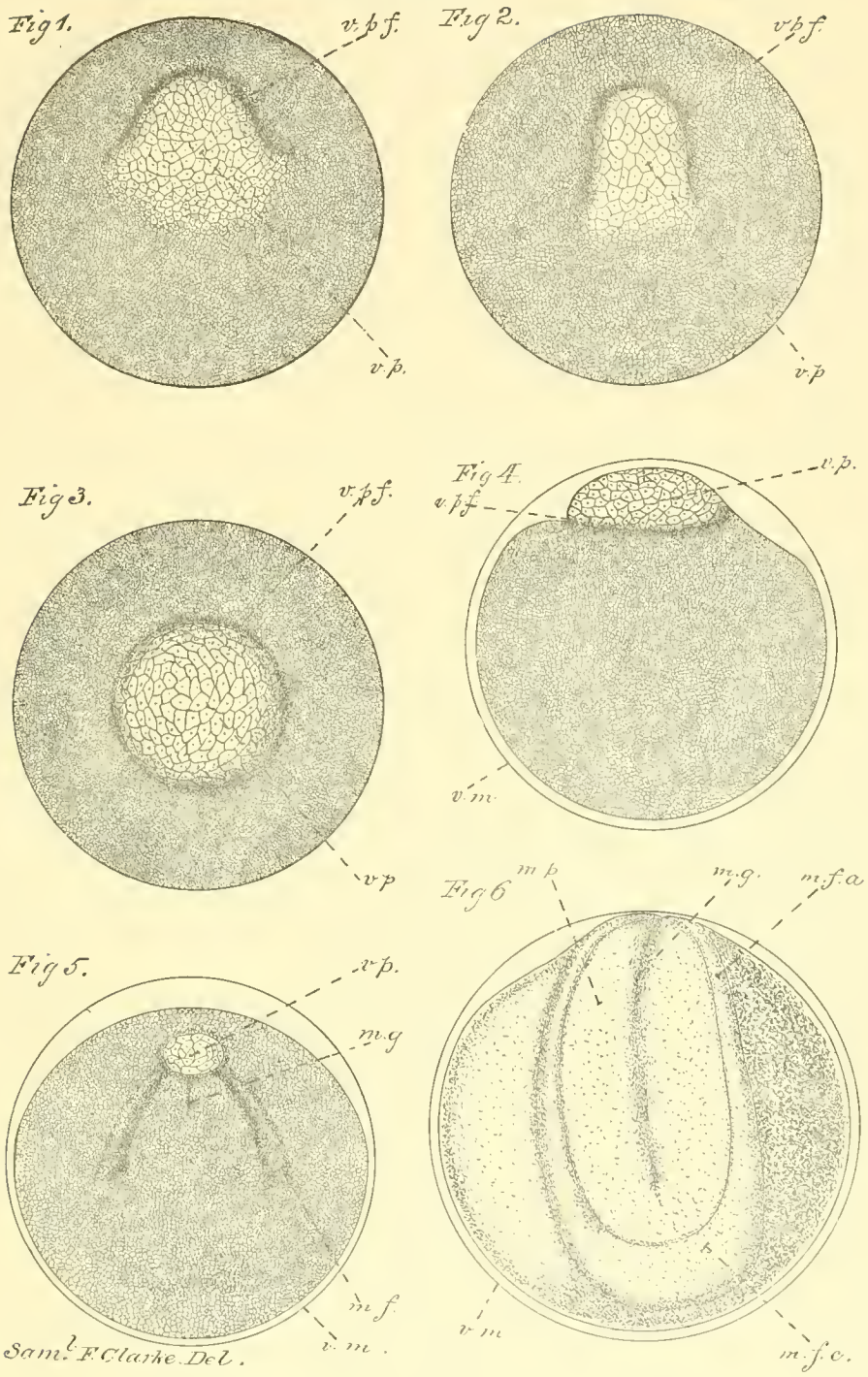
FIGURE 28.—The posterior limb of the same. Letters as before.

FIGURE 29.—A portion of one side of the head and neck; *bn*, the branchiae which are being resorbed. The appendages of the branchiae have already been resorbed, and these rounded stumps will disappear in the course of three or four days.

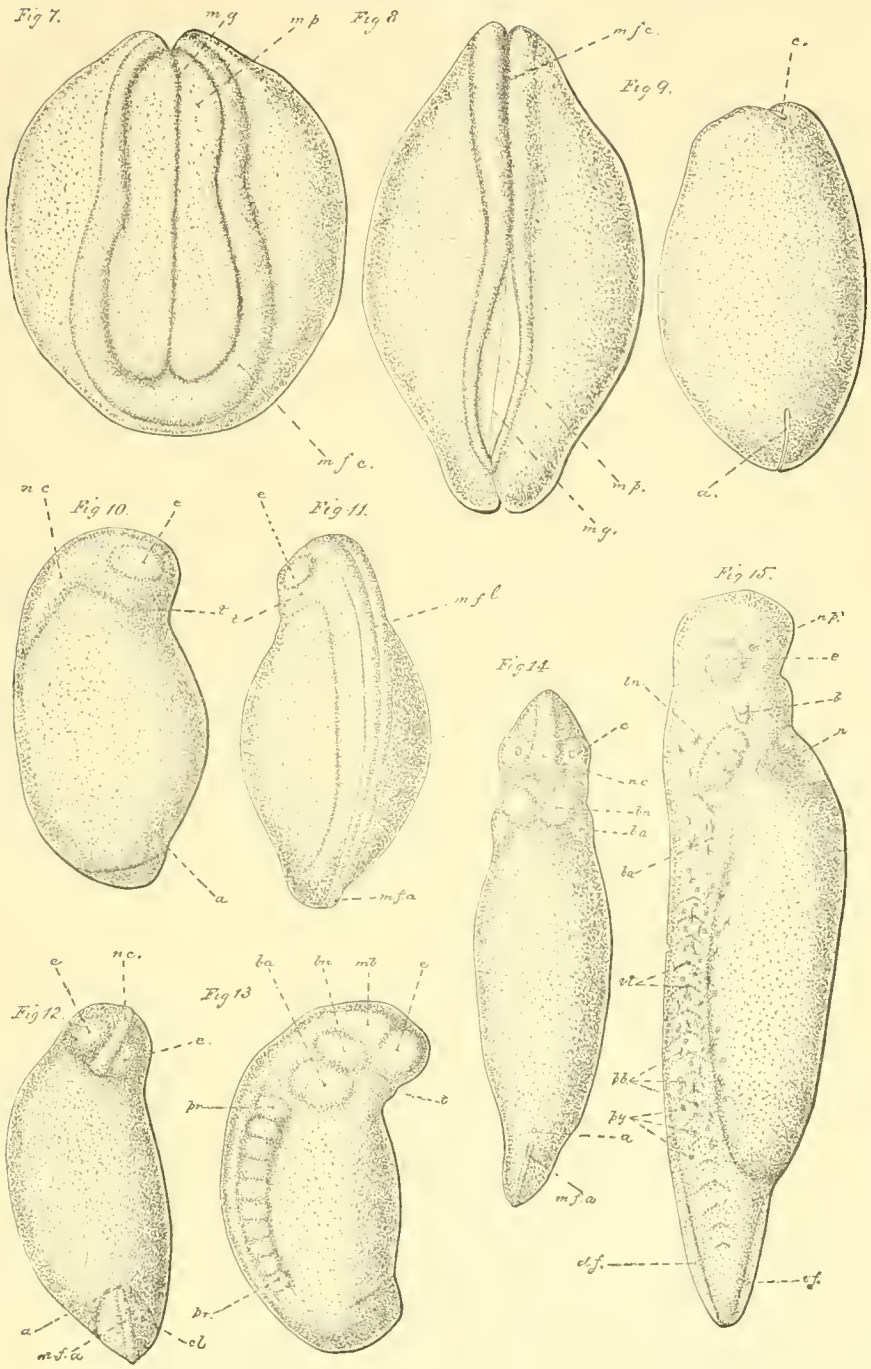
FIGURE 30.—Represents a bunch of eggs attached to a blade of grass. The double membranes about each egg show very plainly. The bunch from which this figure was made, contained over 100 eggs. It is one-half natural size.

FIGURE 31.—Four spermatozoa, enlarged 100 diameters only. *b*, has attached to it a remnant of the mother-cell; *c*, and *d*, have one end bent round so as to form a loop, which condition gives the appearance of the outline of a head.

Development of Amblystoma. **Plate 1.**



Development of Amblystoma. **Plate 2.**



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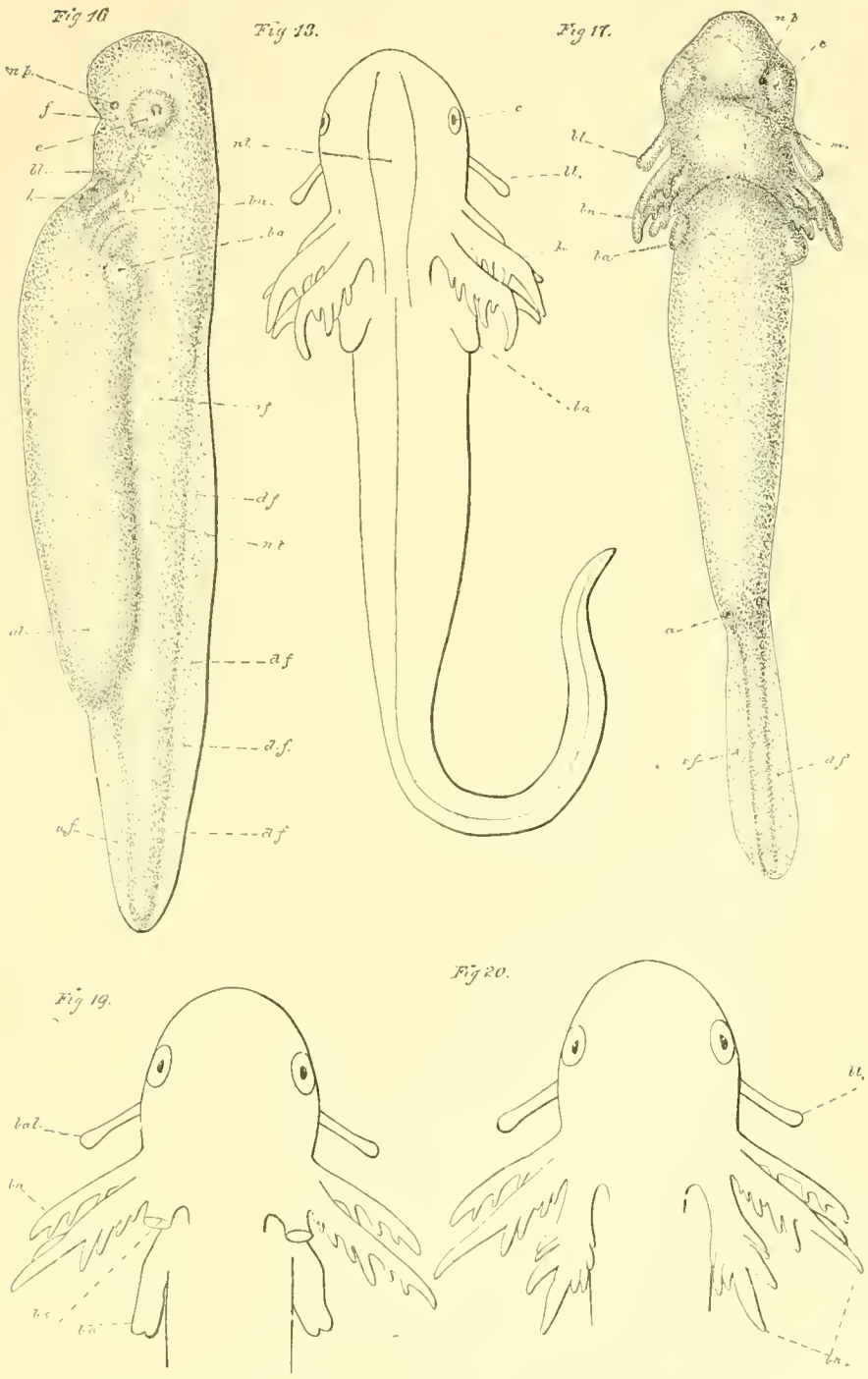
Development of Amblystoma. *Plate 3.*



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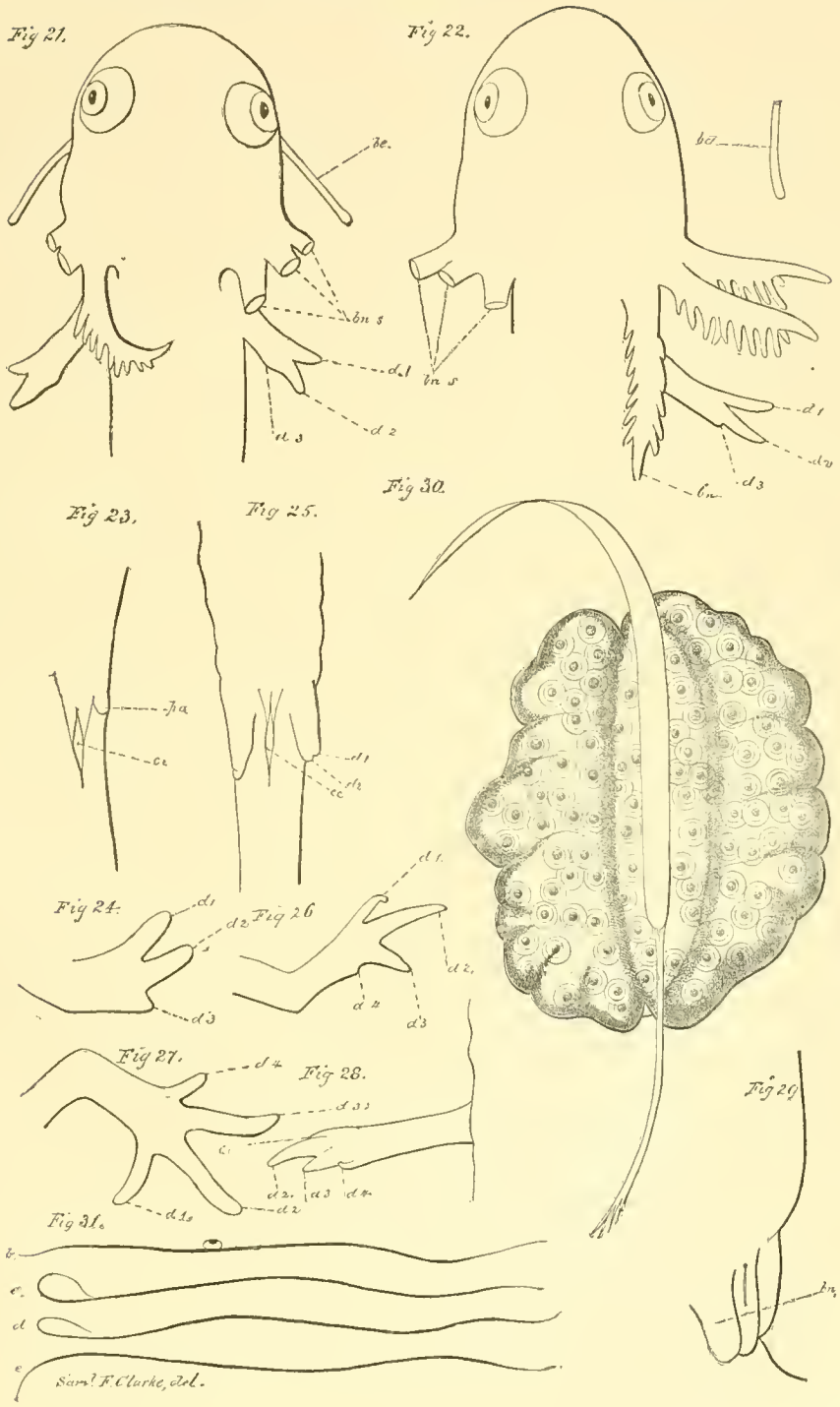
Fig 21.
Fig 22.
Fig 23.
Fig 25.
Fig 30.
Fig 24.
Fig 26
Fig 27.
Fig 28.
Fig 29
Fig 31.
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