THE DEVELOPMENT OF ARTIFICIALLY PRODUCED CYCLOPEAN FISH—"THE MAGNESIUM EMBRYO"

BY

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WITH ONE PLATE AND SIXTY-THREE TEXT FIGURES

INTRODUCTION

Development is the resultant of the interaction between the inherent tendencies contained within the egg substance itself and the external conditions which surround and act upon this substance. The usual interaction of these factors gives rise to normal animal forms. When, however, either factor is changed an unusual form results; in the one case there arises a germinal variant, and in the other an anomaly occurs as a response to the strange external environment. The product of development, the formed animal, is then to a certain extent a creature of its environment. On the other hand the importance of the internal factors must be recognized although modern experimental work oftentimes points in a direction which would indicate that these factors may be largely modified in their influences by the external conditions.

Most monstrosities or abnormalities in development are due to the action of external factors, either mechanical, as pressure, or chemical. Mammals, birds and reptiles, with their complex embryonic membranes, offer many opportunities for the production of secondary abnormalities arising from unfavorable mechanical or physical conditions. Fœtal amputations and scars, membrane fusions distorting facial development, and many other such deformaties are in most cases probably due to secondary influences on development. Besides these there are deformities of a different nature, such as the excessive monsters, *monstra in excessu*, in which certain organs have over developed or produced supernumerary parts; and contrasted with them are defective monsters which fail to complete themselves and are therefore less than a normal individual. It is with this latter class, *monstra in defectu*, that the present study is concerned. These defective individuals may be grouped into two sub-classes: first those in which certain organs fail to complete themselves, as in cleft palate, hare-lip, arrests in the development of the heart and other parts of the circulatory system. Second, individuals in which certain paired organs occur singly or without mates. True Cyclocephali or cyclops monsters find their place in this last group.

Cyclops monsters have long been known to occur in man and other mammals and are described in many of the earliest medical works. In these beings the one eye is in the middle line of the face and often shows external evidence of a double composition. The nose which normally arises above the eyes and grows down between them as the face develops is here mechanically prevented from descending by the presence of the median eye in its path. The fœtus, therefore, has a proboscis-like nose above the eye. The brain and other parts of the body are sometimes deformed though they may be normal.

Among the lower vertebrates true cyclops monsters have been recorded by Spemann ('04) as resulting from mechanical injuries to the eggs of the amphibian, Triton tæniatus. These monsters were double-headed with one or both heads showing the cyclopean defect and were not of the usual single cyclopean type found in man and other mammals.

Two years ago (1907) I carried out experiments in which I was able to produce typical single cyclopean fish. This was the first record of the occurrence of cyclopia among fishes. It is also the first case of consistently producing vertebrate monsters such as are known in nature by changing the chemical environment in which the eggs develop. These embryos are in main details similar to the mammalian cyclops, having a single median eye and anteriorly placed double nasal pits.

The monsters were produced by allowing the eggs to develop in sea-water in which there was an excess of $MgCl_2$. Cyclopia occurred in a large percentage (at times 50 per cent) of the embryos. The discovery was made so late in the spawning season that it was impossible to investigate the details of the cyclopean defect or rear the embryos to hatching in order to observe their ability to swim or to see. The method of production, however, offered such an exceptional opportunity to obtain abundant material for studying all stages of development and degrees of cyclopia that this more extended survey was undertaken.

The following account includes a comparative study of cyclopean embryos from the earliest appearance of the optic vesicle to the perfectly formed free-swimming fish with a functional cyclopean eye.

The experiments were conducted in the Marine Biological Laboratory at Woods Holl, Mass., during the past summer, while occupying one of the rooms of the Wistar Institute.

MATERIAL AND METHOD.

As in my previous experiments, the eggs used were those of the teleost fish, Fundulus heteroclitus.

The method of producing the defect was much the same as that previously employed although expanded and modified in many ways. During the early part of the season it was difficult to find solutions of the proper strengths and the eggs were either killed or unaffected. After a few experiments, however, a strength of $MgCl_2$ in sea-water was found that gave a large percentage of cyclopia, in many cases again causing 50 per cent of the eggs to form such individuals. This was a $\frac{19}{60}$ M solution prepared as follows: 19 cc. of a molecular solution of $MgCl_2$ in distilled water was added to 41 cc. of sea-water. This is not then an actual $\frac{19}{60}$ M $MgCl_2$ solution but it is $\frac{19}{60}$ parts molecular $MgCl_2$. Making the solution in this way adds to the sea-water, water lacking all of its constituents except the Mg and thus increases in a greater proportion the excess of Mg present.

Cyclopia occurred in a series of similarly prepared solutions ranging as follows: $\frac{16}{60}$ M, $\frac{17}{60}$ M, $\frac{18}{60}$ M, $\frac{19}{60}$ M, $\frac{20}{60}$ M, $\frac{21}{60}$ M and $\frac{22}{60}$ M $MgCl_2$. *A point of importance is that the proportion of cyclops embryos produced gradually rises in this series up to the $\frac{19}{60}$ M solution and then falls off again. To illustrate concretely, in Experiment VII the $\frac{16}{60}$ M solution caused 12 per cent of the eggs to form cyclopean embryos, the $\frac{17}{60}$ M gave 30 per cent, the $\frac{18}{60}$ M 22 per cent, while $\frac{19}{60}$ M gave 50 per cent with the cyclopean defect. Continuing the series, the $\frac{20}{60}$ M falls off to 30 per cent and the $\frac{21}{60}$ M gives 23 per cent, while in the $\frac{22}{60}$ M no cyclopia occurred and the eggs were all killed. It must be born in mind that these percentages are for the eggs that formed embryos and not for the total number of eggs first put into the solution. The peculiar fact is, that in a series of $MgCl_2$ solutions we reach a place where a maximum number of cyclopean embryos occur and in strengths both weaker and stronger than this the number of cyclopean individuals is less. If the defect is due to osmotic pressure, we should not expect a greater pressure to bring about a more normal development. If the action is chemical, we do not usually reach a chemically effective dose and find that a greater dose is less effective. It might be argued that below the point of maximum occurrence of the cyclopean defect, the solutions are insufficient to effect any but the weaker embryos, so that a small number of cyclops appear; above this point the solutions are so strong that all except the hardiest embryos die in early stages and those surviving are so resistant that only a few give the cyclopean defect.

At the maximum point the normal or ordinary individuals, which predominate, would be affected, and here the greatest number of cyclopean embryos occur.

As I previously mentioned, the $MgCl_2$ is found to be rather toxic to these eggs during the earlier stages of development. Many die at this time, but in the medium strength solutions 70 to 80 per cent live and form embryos and in the weaker solutions often more than 90 per cent live. After the early embryo is formed, however, the high death rate falls and a dead embryo is of rare occurrence in any of the solutions. Many embryos were kept in the solutions thirty days and some hatched in strengths as strong as $\frac{18}{60}$ M. If, on the other hand, the eggs are removed from the solutions when sixty or seventy hours old, when the cyclopean condition is readily distinguishable, and placed in sea-water they grow much better and many hatch normally. Some of the cyclopean fish came out on the twelfth day after fertilization, though usually they were much slower in emerging. The control embryos hatch in from eleven to twenty days, depending chiefly upon the temperature.

Solutions of $MgCl_2$ in Distilled Water

Distilled water solutions of $MgCl_2$ of several strengths; $\frac{10}{60}$ M, $\frac{11}{60}$ M, $\frac{12}{60}$ M, $\frac{13}{60}$ M, $\frac{14}{60}$ M and $\frac{15}{60}$ M were not effective. The eggs either died during early stages or developed into embryos with two normal eyes. I had found (1906) that salts of lithium induce the same typical defects in Fundulus eggs in both sea-water and distilled water solutions. Such solutions have opposite conditions of pressure, being in one case hypertonic and in the other hypotonic and thus remove all question of osmotic effects as a cause. It was hoped that Mg might also act in the two solutions which would have made it certain that the direct action of the magnesium ion is responsible for the cyclopean condition of these embryos. The problem of cyclopean formation seems, however, to be more complex. It involves the action of magnesium in the presence of certain or all of the sea-water salts.

Solutions of $MgSO_4$ and $Mg(NO_3)_2$ in Sea-water

Sea-water solutions of $MgSO_4$ prepared in a similar manner to the $MgCl_2$ solutions above were employed. The following strengths $\frac{1}{8}$ M, $\frac{10}{60}$ M, $\frac{12}{60}$ M, $\frac{15}{60}$ M, $\frac{18}{60}$ M, $\frac{19}{60}$ M, $\frac{20}{60}$ M, $\frac{22}{60}$ M, $\frac{23}{60}$ M, $\frac{24}{60}$ M, $\frac{25}{60}$ M, and $\frac{27}{60}$ M were ineffective, the eggs in these solutions developing normally with very few deaths at any stage.

$Mg(NO_3)_2$ solutions in sea-water caused typical cyclopia indistinguishable in all respects from that produced in $MgCl_2$. The following strengths were used: $\frac{13}{60}$ M, $\frac{14}{60}$ M, $\frac{15}{60}$ M, $\frac{16}{60}$ M, $\frac{17}{60}$ M, $\frac{18}{60}$ M, $\frac{19}{60}$ M, and $\frac{20}{60}$ M. These $Mg(NO_3)_2$ solutions also killed many embryos during the early stages of development. Cyclopia occurred in from 4 per cent to 40 per cent of the eggs in $\frac{20}{60}$ M, $\frac{19}{60}$ M, $\frac{18}{60}$ M, $\frac{16}{60}$ M, and $\frac{13}{60}$ M. These strengths are comparable to those most effective for $MgCl_2$, both as to the amount of magnesium present and as to osmotic pressure.

Mixtures of $MgCl_2$ + NaCl; $MgSO_4$ + NaCl; and Mg $(NO_3)_2$ + NaCl

Mixtures of $MgCl_2$ and NaCl were added to sea-water as follows: 12 cc. of a molecular solution of $MgCl_2$ was added to 12 cc. of NaCl, and 36 cc. of sea-water was then taken to make the entire quantity up to 60 cc. This solution will be spoken of as $\frac{1}{5}$ M + $\frac{1}{5}$ M, the first term referring to the $MgCl_2$ present and the second to the NaCl. On this basis the following mixtures were used: $\frac{1}{5}$ M + $\frac{1}{5}$ M, $\frac{1}{4}$ M + $\frac{1}{5}$ M, $\frac{3}{10}$ M + $\frac{1}{5}$ M, $\frac{7}{30}$ M + $\frac{1}{5}$ M, in which the $MgCl_2$ was varied and the NaCl kept constant, and $\frac{1}{4}$ M + $\frac{1}{6}$ M, $\frac{1}{4}$ M + $\frac{1}{4}$ M, $\frac{6}{20}$ M + $\frac{1}{4}$ M, $\frac{1}{4}$ M + $\frac{1}{3}$ M, in which the amount of NaCl was varied also.

Such mixtures caused the development of cyclopia, the best results were obtained in $\frac{1}{4}$ M + $\frac{1}{5}$ M, where as times as many as 25 per cent occurred. The $\frac{4}{15}$ M + $\frac{1}{5}$ M gave in one case 30 per cent of cyclopia. The $\frac{7}{30}$ M + $\frac{1}{5}$ M gave 11 per cent. It will be seen that the amount of $MgCl_2$ present in these mixtures is less than that necessary to cause similar results when used alone. This is a peculiar fact and one for which I know of no explanation. Similar results (Stockard '07b) were found with mixtures of salts in distilled water where the final pressure was less than that of sea-water, the normal medium of the eggs. It is also true that if such substances as the sugars be added to a salt solution, a smaller dose of the salt becomes effective in the presence of the sugar. Morgan ('06) first called attention to this peculiar fact in studying the effects of solutions upon developing frogs' eggs. This would seem to indicate that the effects were due to osmotic pressure conditions and by slightly raising the pressure with another element the effective agent was assisted in its action, but my lithium experiments (1906 and 1907b) are against such a view.

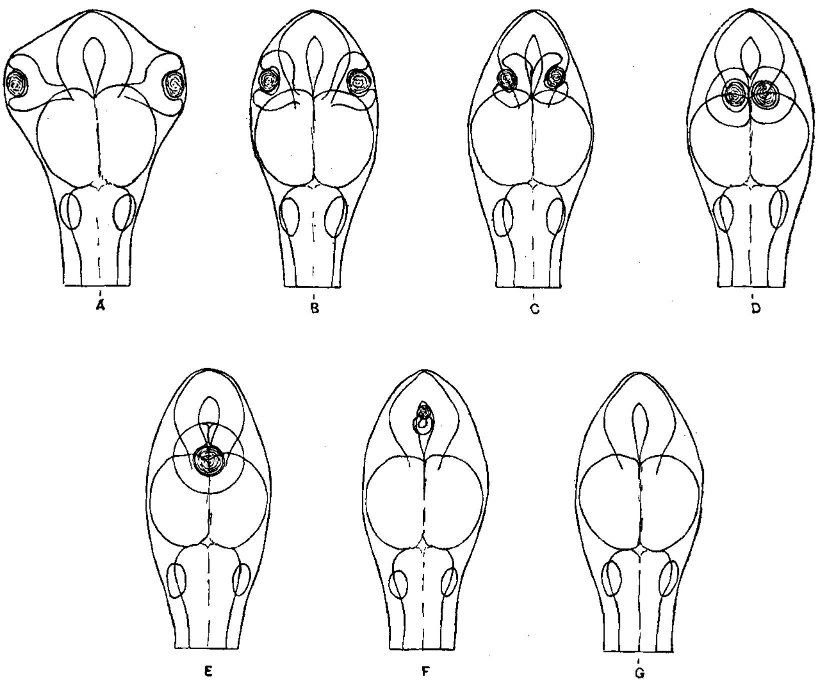
A number of mixtures of $MgSO_4$ and NaCl were tried, all giving negative results. Mixtures of $Mg(NO_3)_2$ and NaCl as follows were used: $\frac{1}{4}$ M + $\frac{1}{6}$ M, $\frac{4}{15}$ M + $\frac{1}{5}$ M and $\frac{7}{30}$ M + $\frac{1}{5}$ M. The first two caused eggs to develop cyclopia. These are mixtures closely similar to the effective $MgCl_2$ and NaCl solutions.

We conclude that cyclopean monsters are produced in Fundulus eggs by the action of sea-water solutions of $MgCl_2$, $Mg(NO_3)_2$ and mixtures of $MgCl_2$ and NaCl and $Mg(NO_3)_2$ and NaCl. No other solutions of the many I have tried during three summers gave similar effects. Other salt solutions and sugar solutions exerting practically the same osmotic pressure also fail to cause cyclopia.

Another argument opposed to the view that osmotic pressure is the cause is the fact that Fundulus embryos are so resistant to changes in pressure. Since two Mg salts give similar results when used in sea-water solutions, it seems probable that the action of Mg, either directly or indirectly, is responsible for the result. Eggs have been subjected to this action before the first cleavage, during the two-cell stage and just before going into four cells, with similar results. No attempt was made to determine at how late a stage the cyclopean condition could still be caused, though it could doubtless be induced after the eggs had passed much beyond the four cell stage. The fact is that cyclopia may be caused in an egg which has started its development normally and which would have given a two-eyed embryo. The idea of a germinal origin of the defect in this case seems excluded. Cyclopia in this instance is the result of unusual external conditions.

CYCLOCEPHALI AND "MONSTRA MONOPHTHALMICA ASYMMETRICA"

The magnesium solutions induce the formation of two distinct types of eye monstrosities. The first type is the typical cyclopean monsters, which exhibits a series of individuals showing various degrees of cyclopia. Beginning with a normal individual having eyes in their usual position, we find others in which the eyes are slightly inclined forward and somewhat closer together than usual; or the eyes are still more approximated and occupy an unusually anterior position. (See the diagram, Fig. 1). Next in the series are individuals with their eyes approximated but still distinctly separate, having two optic nerves and two eyeballs with their choroid coats in intimate approximation. We next find the true cyclopean eye which still shows a double nature having two optic nerves; the retina has a paired arrangement and either one or two lenses may occur, depending upon the degree of distinctness of the two components. This eye generally occupies a ventro-median position and looks forward, inclining slightly downward. The eye in others is completely single, showing no indication of a compound structure; it has one optic nerve, a single retinal arrangement, one lens and one pupil. This is the perfection of cyclopia and many embryos possessing such an eye are apparently normal in other respects, except the mouth and nose. They have a typically bilateral brain and are perfectly capable of free-swimming movements. Passing beyond this stage of cyclopia, we find embryos which have gone to the extreme and show only a defective antero-median eye. In some individuals the eye is represented merely by a choroid vesicle. The step beyond this is the entire absence of the eye. Diagram Fig. 1 gives a schematic illustration of the various degrees in the cyclopean series thus outlined. The histological conditions shown by such a series will be considered beyond. It is important to understand that this series is made up of different individuals showing various degrees of cyclopia and that a cyclopean monster does not pass through these steps in its development. The cyclopean defect is foreshadowed in its final condition when the optic vesicle first separates itself from the brain.



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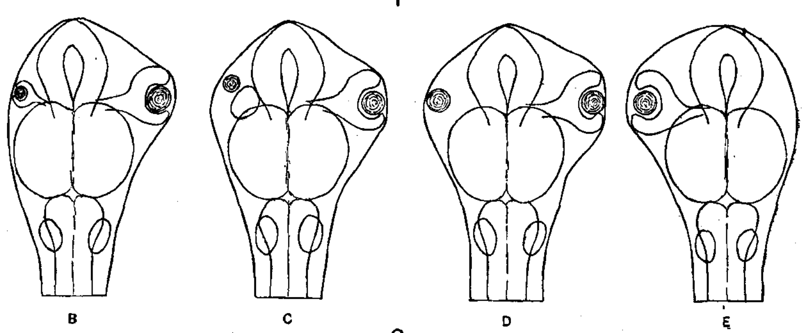

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Fig. 1 Diagrams of the various conditions of the cyclopean defect as shown by the "Magnesium embryos," from the normal *A* to complete absence of the eye *G*.

Fig. 2 Diagram of the monstrum monophthalmicum asymmetricum series, from one defective eye *B* to complete absence of one eye *E*.

The second type of optic defect caused by magnesium is a new monstrosity and may be termed *Monstrum monophthalmicum asymmetricum*, the monster with one asymmetrical eye. It has only one perfect eye which represents one of the normal pair and occupies the usual lateral position. This eye is in all cases perfect while its mate may be indicated by either a small eye, by a mere cellular mass representing an optic cup, or all indications of the second optic cup may be wanting. (See Fig. 2.) This peculiar one-eyed condition exists in many of the embryos in the magnesium solutions. Had such a defect resulted from a mechanical operation, it would probably have been interpreted to mean that one eye anlage was injured and the other not. With the solutions, however, we get a clear case of the gradual dropping out of one eye by comparing different individuals, and here as in cyclopia the defect is present from the earliest appearance of the eye, and is not due to a gradual degeneration, or arrest during development. A study of sections of these embryos makes the conditions clearer.

a The Living Cyclopean Embryos from the First Indication of the Defect to the Time of Hatching

The optic vesicles appear in most eggs when about thirty hours old; at this time the blastopore is just closing and the embryo is well mapped out on the embryonic shield. Many attempts were made to select cyclopean individuals at this stage but it could not be done with a great degree of certainty, since some embryos are always slow in giving off the optic vesicles and these at times appear to have only one, but when examined some hours later are found to be normal. A number of eggs were selected, however, at thirty hours old which proved to be cyclopean on later examination.

At about forty hours the defect is plainly detectable so that one may arrange the eggs very accurately into two groups, the cyclopean individuals and the normal. After such a separation, none of the normal embryos ever exhibited the cyclopean defect in later stages, although kept in Mg solutions. A number of such tests as this in connection with the study of sections convinced me that the cyclopean condition existed as such from the first appearance of the optic vesicles, and no subsequent fusion of the two optic vesicles or cups took place after that time.

A forty-two hour embryo is shown in Fig. 3. It is seen to be well formed and the optic vesicles are clearly outlined on either side of the head. Fig. 4 illustrates a cyclopean individual of the same age. The single optic vesicle occupying a ventro-median position is shown through the transparent embryo. This young individual with its newly formed optic vesicle shows a typical cyclopean condition, and no indication is seen of two separate elements that would later fuse. Other embryos at this age have abnormally twisted cephalic regions and show no indication of eyes, although the cyclopean eye might easily be concealed by the bent brain (Fig. 5). Such embryos at later periods are found to be cyclopean and to have narrow tubular brains showing more or less abnormal bendings.

When the embryos are about three days old, the brain has expanded and presents a distinctly bilateral appearance; the optic cups are well developed and the lenses are partially formed (Fig. 6). A cyclops monster at this time has a well formed body and the brain is often normal, though in Fig. 7 it is inclined toward the narrow tubular condition and is anteriorly twisted. The ventromedian eye is clearly seen through the brain and the outline of its lens is distinct. A somewhat younger, sixty-five hour, embryo is shown in Fig. 8 with a superficially perfect brain and two optic cups intimately approximated. The telencephalon is seen to protrude beyond the eyes, as is the case in the normal individual (Fig. 6).

Three four-day embryos are shown by Figs. 9, 10 and 11. The brain and spinal cord at this time are clearly mapped out by a coarse pigmentation, the two hemisphere-like portions (corpora bigemina) of the mid-brain are distinctly formed and the eyes are large with the lens clearly outlined within the cup. A cyclopean monster with a perfectly formed large ventro-median eye is illustrated by Fig. 10. Comparing its brain and other parts with the normal (Fig. 9), one fails to find any important deviations. The abnormal condition of the narrow tubular brained cyclops, *Camera lucida sketches of living embryos from $MgCl_2$ solutions*

Fig. 3 A normal embryo of forty-two hours, the two optic vesicles present.

Fig. 4 A typical cyclopean individual of forty-two hours. The single median eye (*O. V.*) is represented in circular outline.

Fig. 5 An embryo of same age, twisted brain, no optic vesicle shown.

Fig. 6 Normal seventy-two hour embryo.

Fig. 7 Cyclops of same age. The eye, *op.c*, in ventro-median position.

Fig. 8 Sixty-five hour embryo, two ventrally approximated optic cups.

Fig. 9 Normal four day embryo—bilateral brain outlined.

Fig. 10 Four day cyclops, large ventro-median eye and typically bilateral brain, *op.c*, the eye.

Fig. 11 Four day cyclops with narrow tubular central nervous system.

Figs. 12 and 13 Five day cyclops, narrow tubular brain with waist-like constrictions dividing them into fore, mid and hind-brain regions. Ventro-median eye.

Fig. 14 Five day cyclops with ventro-median eye and dorsally humped brain.

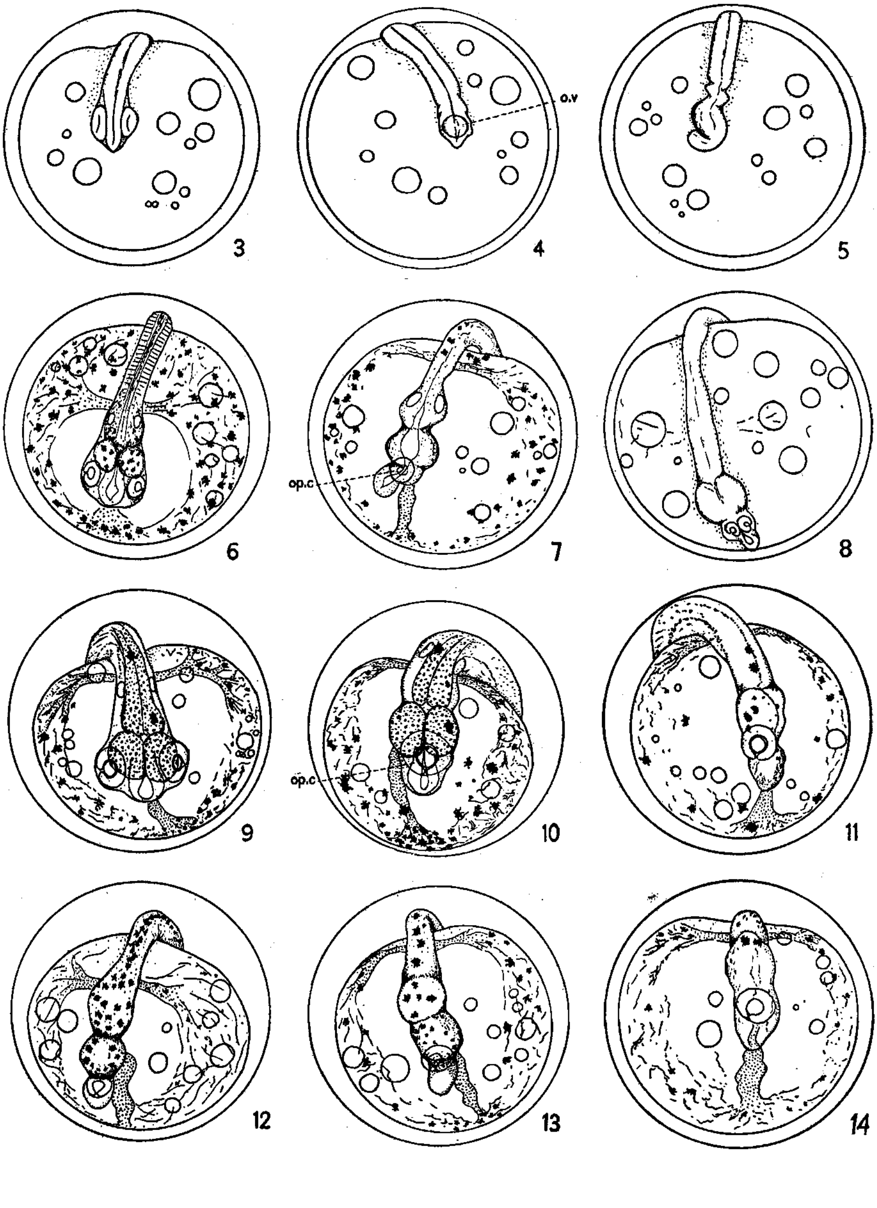
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Fig. 11, is evident. Fig. 12 shows a common type of cyclopia with the three primary brain regions separated by waist-like constrictions. Two other variations of the narrow tubular condition are found in Figs. 13 and 14. The embryos are five days old and no changes of importance occur from this time until the hatching period is reached, except the usual progressive development of the eye structures.

The normal embryos generally begin hatching when about twelve days old, one cyclopean monster hatched at this time but most such indviduals were much later than the normal in coming out. A twelve day cyclopean fish is seen in dorsal view in Fig. 15 and ventrally in Fig. 16. The large cyclopean eye projects forward and occupies the position usually taken by the mouth at this time. A slight indention along its mid dorsal line suggests its double nature, although the ventral view (Fig. 16) shows this same eye to possess only one pupil and lens. The brain of this specimen is practically normal. An embryo with the two eyes intimately approximated is shown in front view in Fig. 17. The eyes are joined and each looks forward in a direction slightly towards the side to which it belongs. A common variety of cyclopean fish is one in which the eye is unusually small and occupies an extremely anterior position; Fig. 18 shows such an embryo. This variety is usually unable to hatch, although a few were assisted in breaking through the membrane. They swam rather abnormally, owing to a twisted condition of the body. A dorsal and ventral view of a cyclopean fish is shown in Plate I, Figs. A and B. This indicates the striking appearance presented by these embryos.

b Free-Swimming Cyclopean Fish

Many embryos, showing the cyclopean defect in various degrees, hatched normally and were capable of swimming in a manner indistinguishable from ordinary two-eyed fish. These monsters gave many indications of ability to see. They went to the more brilliantly lighted side of the dish with the normal ones. They darted away in a normal fashion when any object was placed in front of the eye, while similar objects put at equal distances from their tails caused no excitement. In two instances they lived for ten days, which is about as long as the two-eyed embryos can survive without food. At this time the entire content of the yolk-sac has been absorbed. The embryos in nature doubtless begin feeding previous to this stage. The cyclopean individuals appear to be as active as the normal and their ability to live would seem to depend only upon the possibility of their obtaining food.

A normal fish eight days after hatching is illustrated by Fig. 23. The mouth projects forward beyond the dorsal tip of the head and the two eyes are lateral in position. A cyclopean embryo eight days after hatching is shown in Fig. 24. Here the two eyes are united and occupy the position which the mouth has in Fig. 23. In Fig. 25 a perfectly cyclopean eye is shown in dorsal view: the same individual is seen in lateral and ventral views in Figs. 26 and 27. This fish swam in a normal manner. In the lateral position the mouth is shown projecting ventrally as a proboscis-like structure. This condition is due to the fact that the single antero-median eye occupies the position normally assumed by the mouth and thus obstructs the usual forward growth of its structures. The mouth, therefore, remains ventro-posterior to the eye and grows downward, presenting the proboscis-like appearance.

Such a condition recalls in a striking way the nose of the mammalian cyclops. In mammals the cyclopean defect is accompanied by a proboscis-like nose situated in the forehead above the median eye. The nose in normal development grows downward to its facial position, but in cyclopia the median eye obstructs its path and forces the formation of the proboscis-like organ in the forehead. The same explanation holds for the fish's mouth where the eye prevents its forward growth, producing the proboscis-like organ.

It is interesting to find that the mouth in cyclopean fish stands in a position so as to fall in the gill series as number one, all the gills and the mouth have the same general direction. I have found that in Bdellostoma the mouth arises in a manner similar to the gills and actually at first arches dorsally and only secondarily arches ventrally. It may have originally been a member of the gill series, as Dohrn (1875) has long thought. It would be

Camera sketches of the living embryos in magnesium solutions

Fig. 15 Dorsal view of twelve day cyclopean monster, the antero-median eye with furrow indicating its double nature.

Fig. 16 Ventral view of the same individual, the eye possesses a single pupil and lens.

Fig. 17 A twelve day embryo, ventral view showing two eyes intimately approximated.

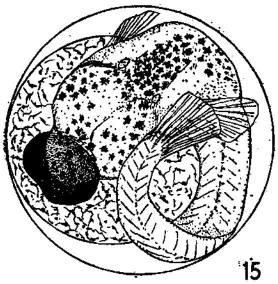
Fig. 18 Fourteen day embryo. Small extremely anterior cyclopean eye with protruding lens, extreme cyclopia.

Fig. 19 A five day Monstrum monophthalmicum asymmetricum; the left eye has no mate.

Fig. 20 A similar twelve day monster lacking its left eye.

Fig. 21 An incomplete diprosopus monster seventy-two hours old. Two brains, two normal lateral eyes and one perfect middle eye, the other middle eye indicated by the circular lens *L*.

Fig. 22 The same monster when eighteen days old, three perfect eyes. The embryo hatched three hours after this drawing was made and swam abnormally.



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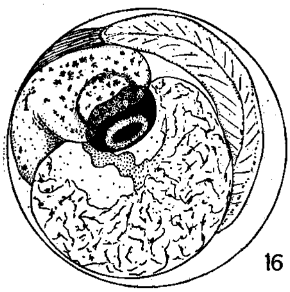

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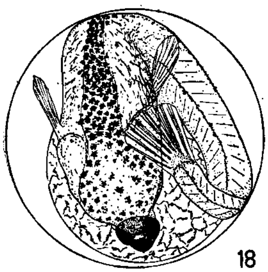

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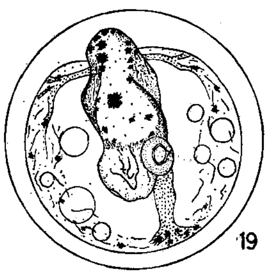

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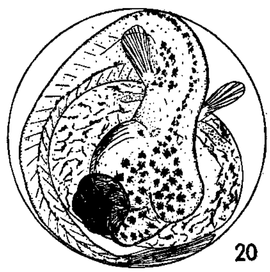

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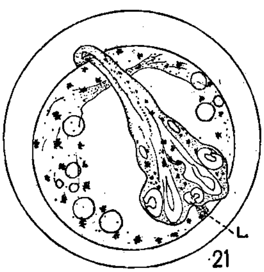

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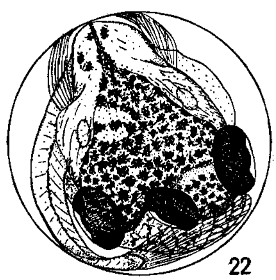

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Camera sketches of free-swimming fish

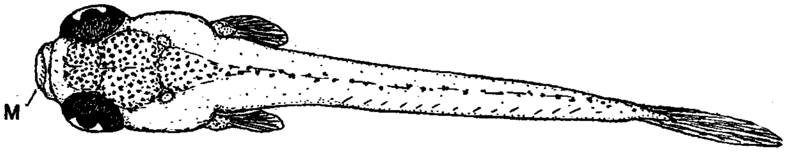
Fig. 23 Normal individual. *M*, its anteriorly placed mouth.

Fig. 24 Incomplete cyclops, two eyes joined and occupy the position usually taken by the mouth.

Fig. 25 Dorsal aspect of a perfect cyclops. Antero-median single eye.

Fig. 26 Lateral view of same. The mouth *M* is forced by the eye to remain in a ventral position and hangs down as a proboscis-like structure.

Fig. 27 Ventral view of same fish, note perfectly single eye, one lens and one pupil, *ys.*, yolk-sac.



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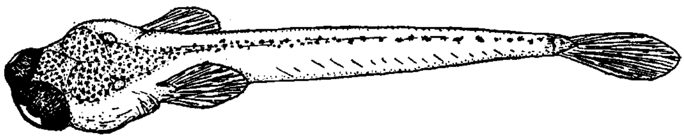

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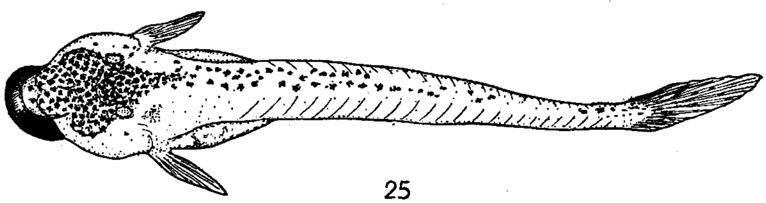

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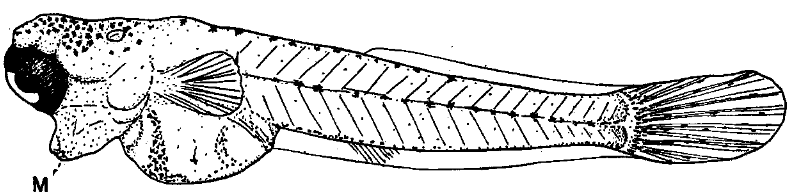

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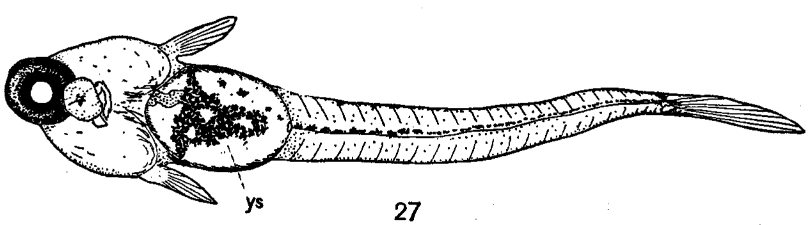

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interesting to know whether the "cyclopean mouth" is functional. The mouth does not possess a wide opening as it would normally although a small aperture is sometimes distinguishable near the end of the proboscis. No attempt was made to feed the embryos.

c Living Monstra Monophthalmica Asymmetrica

These asymmetrical one-eyed monsters may also be identified in early stages of their development. They have a single eye situated on one side of the head. Such an eye appears in some cases as though it were cyclopean and one might easily imagine the cyclopean eye becoming displaced from its usual median position to one side or the other of the head. Studying such eyes in section, however, clearly shows their single unmated origin and condition. An embryo of this kind is shown when five days old in Fig. 19. The brain is slightly abnormal and the pigmentation scarce for such a stage of development. The eye occupies the usual place of the paired eye of that side. A twelve day embryo shortly before hatching is illustrated by Fig. 20. The shape of the body and of the head is comparatively normal. The unpaired eye is slightly forward of its usual position.

Many of these embryos hatched. A few of them swam in circles, often whirling around with great rapidity, much as Japanese waltzing mice do. Others swam in irregular spirals and only progressed in a straight direction with difficulty. This peculiar one-sided manner of swimming is not due to asymmetrical vision, but results from a defective muscular arrangement, the animal's body being slightly bent or twisted so that it is unable to straighten perfectly. Some embryos with this eye on one side had normally straight bodies and these were capable of swimming in a direct course with apparently as much ease as a two-eyed fish or the symmetrical cyclopean embryos.

These monsters also lived, free-swimming, for some time. In a few cases their mouths were perfect, but in others the mouth parts were distorted or twisted by an asymmetrical condition of the ventral head region.

MORPHOLOGY OF CYCLOCEPHALIA

It was mentioned above that the optic outpushings became visible on the sides of the brain at about thirty hours after fertilization. At this time the brain of Fundulus is a solid mass of cells without a central ventricle. The optic bodies are not hollow at first, but are solid outpushings which later develop central cavities. The cavity generally forms in the optic outpushings while the brain is yet solid. Dareste ('91) has advanced hypothetically the idea that if the anterior vesicles of the brain did not develop, a contact would be maintained between the "parties retiniennes" up to a certain time and consequently they would unite to give a median cyclopean eye. If this were in reality the cause of cyclopia we might expect all Teleosts like Fundulus to be normally cyclopean since in them the eyes arise while the brain is without a ventricle. Spemann ('04) finds in cyclopean Triton embryos that although the tube is hollow, the eye anlagen are defective from the beginning. The matter of a closed brain would then seem to be unimportant in a consideration of the causes of cyclopia.

a Earliest Indication, Exact Position and Condition of the Eye

When forty-one hours old, the brain as shown in trans-section by Fig. 28, is still a solid mass. The two normal optic outpushings have developed small cavities but no indication of invagination of the vesicles or ectodermic lens structures are seen.

A section through the optic region of an apparently one-eyed monster when forty-one hours old, is shown by Fig. 29. The sections of this series show only one ventro-lateral eye vesicle. The vesicle is large and distinctly optic in nature, while on the opposite side is shown a thick cellular wall from which the brain is becoming separated. Such an individual resembles more a *Monstrum monophthalmicum asymmetricum* than it does the cyclopean type.

Fig. 30 shows a transverse section through the eye of a forty-nine hour embryo which exhibits a perfectly clear case of cyclopia. Here the brain is beginning to form a cavity and the optic vesicle with a well defined central cavity is just invaginating to form the optic cup. This eye occupies an almost ventro-median position and is united to the brain by a solid cellular stalk. Its contact with ectoderm from which the lens will arise is not established as the head-fold does not yet extend back to this point. An eye in such a ventral position will oftentimes come in contact with the ectoderm at a later stage than would a normal lateral eye. Ordinary two-eyed individuals at this age (forty-nine hours) were, like this cyclops, just beginning to form the optic cups and the lateral ectoderm over the incipient cups showed a slight thickening, the earliest indication of a lens. As a rule the cyclopean eye is somewhat slower than the normal in its rate of development and may generally be compared with the eyes of slightly younger two-eyed individuals.

Several embryos at this age lack eyes entirely and belong to the blind variety presently to be described.

Two-eyed embryos when fifty-four hours old possess well-formed optic cups and lenses still connected with the ectoderm, although projecting into the cavity of the cup. The nasal pits are clearly marked ectodermal invaginations in an anterior and slightly median position relative to the eyes. The brain possesses a well developed central cavity. A cyclopean eye of a distinctly double composition from a fifty-four hour embryo is shown in cross-section by Fig. 31. The optic cup is bilateral and two lens anlagen are indicated by the thickened ventral ectoderm. The section of the brain dorsal to this eye is small and hollow. It is a portion of the diencephalon which is between a larger telencephalon and a much larger mid-brain. This eye would finally have produced a large median cyclopean organ of the double type with two retinal areas and two lenses. Its connection with the brain is through two closely approximated stalks and two optic nerves would probably have formed later. Comparing such an eye with that of Fig. 32, of like age we find that here the optic cup is single and one lens is forming. Both sections show the eye in practically similar positions. The embryo from which Fig. 32 was taken possesses a well formed telencephalon and two lateral nasal-pit thickenings of the anterior ectoderm.

A horizontal section of a fifty-four hour double-eyed cyclops

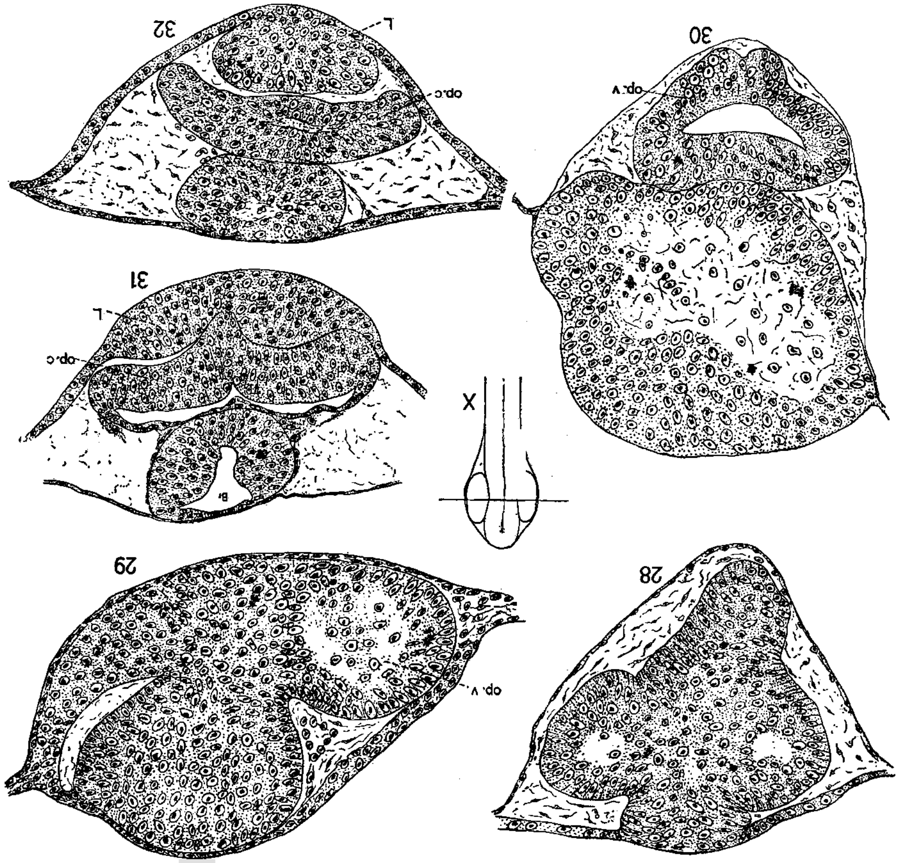

Fig. 28 A trans-section through the optic outpushings of a normal forty-one hour embryo. The brain is solid and cavities are just forming in the optic outpushings.

Fig. 29 Trans-section through the single optic vesicle (*op.v.*) of a forty-one hour embryo from $\frac{19}{60}$ M $MgCl_2$. The optic process is situated laterally and no indication of a like process exists on the other side.

Fig. 30 A slightly oblique section through the cyclopean optic vesicle of a forty-nine hour embryo [illegible]

Fig. 31. Cross section through double cyclopean eye of fifty-four hour embryo from $\frac{17}{60}$ M $MgCl_2$, *op.c.* optic cup; *L*, lens thickening of ectoderm; *Br*, normal bilateral brain.

Fig. 32 Section of single cyclopean eye in similar embryo. *L*, lens; *op.c.* optic cup, small solid diencephalon above; *X*, guide figure indicating the plane of the sections.

is given in Fig. 33. Such a section is most instructive. The condition of the eye is much the same as that shown by the transverse section, Fig. 31. The cup is double and two ventral lenses are present. The section passes below (ventral) the diencephalon so that no part of it shows; the telencephalon is indicated in front of the eyes and a thickening of the forward ectoderm shows the nasal plate, posteriorly or behind the eyes the mid-brain is cut in horizontal section.

A sagittal section of a typical cyclopean embryo is shown by Fig. 34. Here we see the eye and the brain in the third dimension. The telencephalon in front, the diencephalon above the eye, and behind this the large mid-brain with a spacious median cavity. In front of the eye is also shown a median ectodermal thickening, the double nasal pit. The eye is single and exactly ventromedian in its position and connects in a more lateral section with the brain at about the point where the telencephalon and diencephalon join. The lens and retina are differentiating into their typical structures. One may obtain a clear mental reconstruction of the cyclops monster at this age by comparing Figs. 31, 32, 33 and 34, the transverse, horizontal and sagittal mid-planes of the cyclopean eye.

The early stages just described illustrate the cyclopean defect in its various degrees, and the eye throughout its development retains the original condition of singleness or doubleness. No evidence whatever can be found of subsequent fusions during development. Two clearly approximated eyes arise in that condition and remain so without fusing to give a double cyclopean eye, and a double eye never attains to the single condition by a more intimate union of its parts. The statement made in my (1907a) former paper, p. 257, that "the fusion of the two components may take place at different periods within a certain limit" is incorrect, as I (1908) have pointed out in a short note on the subject. This statement was one of interpretation and was based on a comparison of late embryos which showed different degrees of cyclopia. It seemed from such an incomplete study that the eyes were more or less double or compound, depending upon the stage in development at which they had become approxi-

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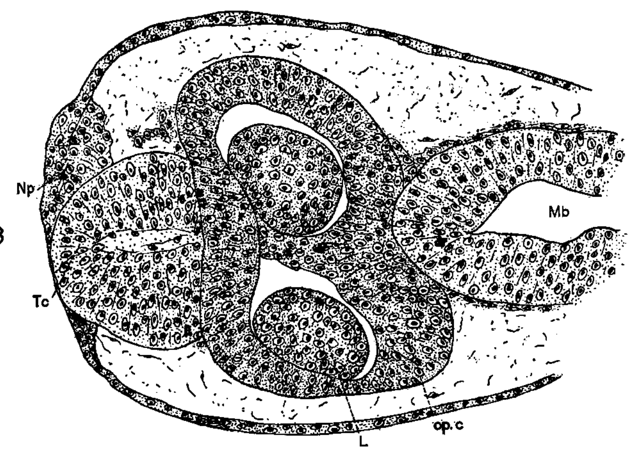

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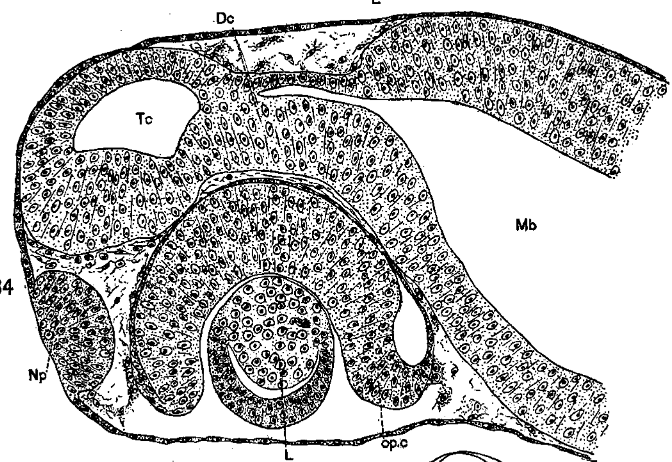


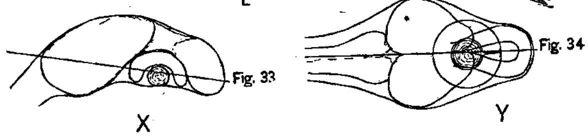


Fig. 33 Almost horizontal section through a double cyclopean eye of a fifty-four hour embryo in $\frac{17}{60}$ M $MgCl_2$. See guide figure *X* for plane of section. *Np.*, nasal plate; *L*, lens; *op.c.*, optic cup; *Tc.*, telencephalon; *Mb.*, mid-brain.

Fig. 34 Sagittal section (guide figure *Y*) through typical single cyclopean eye showing its ventral position below the diencephalon *Dc.* The nasal pit, *Np.* is median; *L*, lens; *op.c.* optic cup; *Tc.*, telencephalon; *Mb.*, mid-brain.

mated. The point is one which can only be proven by a number of direct observations on all ages of cyclopean embryos and careful study of sections; such a study has convinced me that no fusion of the eyes takes place after they are once clearly given out from the brain.

It seems advisable for later stages to consider groups of embryos showing various degrees of the cyclopean defect.

b Incomplete Cyclopia; Double Eyes

Under the term incomplete cyclopia may be considered individuals with eyes abnormally close together although separate Among Fundulus embryos such individuals exist and a series of stages connect these embryos with those in which the two eyes are intimately connected or joined together. An individual of this kind when sectioned will show the eyes as in Fig. 35. This section is from a four day embryo, the two eyes are united in the median line of the head and both are perfect eyes with a lens, single retina and one optic nerve. The choroid coat as indicated by the heavy line is just beginning to form. Fig. 36 shows a section of two eyes which are more intimately united. This case is the common "hour-glass" eye of cyclopia. The two eyes are independent, except for their waist-like connection and each has its lens, single pupil, retina and distinct opticus. The optic nerve of the right component is seen entering the optic cross at the base of the brain. The brain in this embryo is remarkably perfect, as it is in many cyclopean monsters, and I see no reason whatever for attributing the defect to a "single brain" or any other gross malformation of the cephalic region. Many embryos with deformed brains possessed two normal eyes and the converse is true, many normal brains were accompanied by cyclopean eyes.

Leaving the "hour-glass" eye, we find the double-eye shown in Fig. 37, having a common optic chamber each half of which is supplied by one component. Two lenses and two pupils are present and generally two optic nerves, although they may run so nearly parallel that the two are difficult to distinguish. A single nasal pit is present in the embryo from which Fig. 37 is a section. All of the cyclopean monsters possess two distinct auditory vesicles.

Fig. 38 is a section through a unique double eye; no other such case was found. The two retinal components are connected along their median dorsal line within the brain and extend down facing one another. They are like the two sides of a leguminous pod; between the two a single lens is placed suggesting the seed in the pod. Enclosing the ventral part of the retinal components is a choroid coat shown in heavy black. This choroidal coat does not fully encompass the retinal areas, a part of which extends dorsally far up into the brain. The anterior end of the eye is V-shaped in section. The optic cup anlagen in this case must have been closely united from their first origin in the brain, since portions of the retinal region are still contained within the brain itself, yet during development they did not fuse into a single eye. A single nasal pit is present and the mouth is ventral and proboscis-like.

An almost single eye is indicated in section, Fig. 39. The choroid coat surrounds the retina, the latter showing slight traces of its compound nature. Two lightly staining regions of nerve tissue are seen and the entire eye is unusually wide laterally. The single lens is normal. The brain here is also normal and the eye occupies a ventro-median position. A further union of the eyes gives the

c Perfect Single Cyclopean Eye and Normal Brain

The cyclopean eyes are in many cases perfectly single, resembling in all respects, except their position, one eye of a normal pair. They are placed immediately ventral and their antero-posterior mid-plane is in the median line of the embryo. The brain in such a cyclops is often normal in all general respects. Figs. 40 and 41 represent horizontal sections through the brain regions of such a cyclopean fish when seventy-seven hours old. Fig. 40, the more dorsal section, passes through the mid-brain and shows the two lateral, hemisphere-like bodies (corpora bigemina) with well formed cavities. Behind these the section cuts the floor of the hind-brain for some distance and finally crosses it where the head bends. Passing ventrally through a number of sections, we find the one shown in Fig. 41. Here only a small ventral

Transverse sections of different degrees of double cyclopean eyes

Fig. 35 Section of eyes in four day embryo, the two eyes united. Choroid coat beginning.

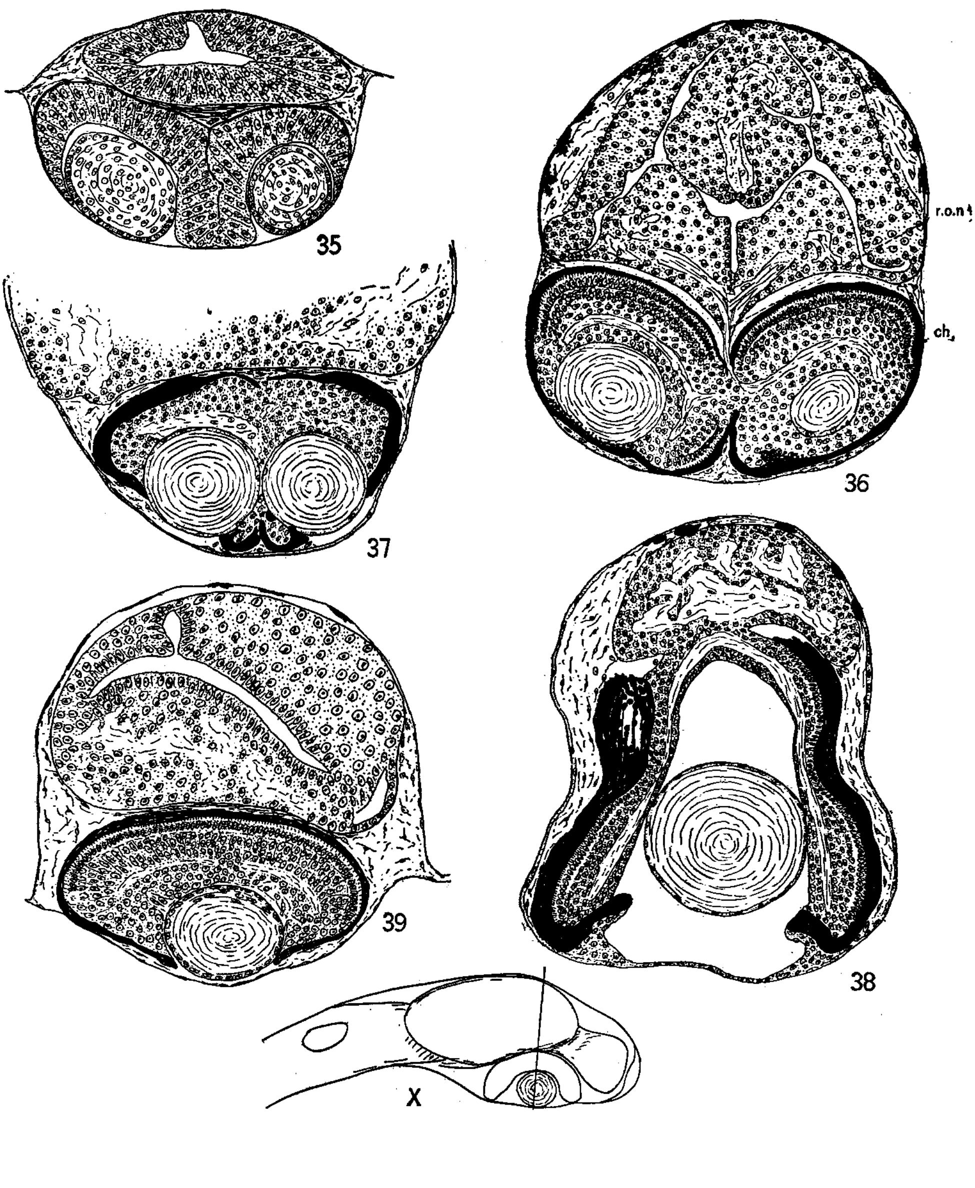
Fig. 36 Section of "hour-glass" eyes, the optic nerve of the right component entering the normally bilateral brain. From a sixteen day embryo, the retinæ and lenses differentiated. *r.o.n.*, right optic nerve; *Ch.*, choroid coat.

Fig. 37 Section of eye in hatched embryo. Double-eye with two pupils and two lenses. Retina undifferentiated.

Fig. 38 Hatched cyclops, section through the peculiar eye with two components facing and lens between them (see text).

Fig. 39 Section through almost single cyclopean eye, only indication of its compound nature paired retinal arrangement. Brain normal.

Guide figure *X* indicates the plane of all sections and the eye position in the several specimens.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r.o.n.
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part of one of the corpora bigemina is cut and the completely single eye with its lens is found lying ventrally and in a median position. The double olfactory pit is seen in front of the eye and somewhat to one side of the head. The posterior part of the section runs below the hind-brain and finally cuts it as the head bends just in the middle region of the well formed auditory vesicles. The section thus presents the three sense organs, the single cyclopean eye, the nasal pits united into a double pit; the paired ear vesicles alone are in their usual positions.

A transverse section through the eye of a four day embryo is illustrated in Fig. 42. The retina is unusually wide laterally but no other indication of doubleness is shown. The choroid coat is beginning to form and the eye is connected with the floor of the brain by a single cellular stalk. The retina at this age is only slightly differentiated and there is no arrangement into layers. This embryo has two distinct nasal plates. Several of the cyclopean fish show the nasal plates separate, although they are usually represented by an anterior double plate near the middle line.

A nine day embryo of which Fig. 43 represents a section through the eye has a finely developed brain, well expanded laterally and perfect in general shape and structure. The eye is completely single and the retina is partially formed into layers; the lens is almost transparent and the vitreous humor is being formed about it. The eye has all structures closely similar to those in a paired eye of this age and would doubtless have functioned had the embryo hatched. This specimen has a single nasal pit.

Another cyclops of perfect structure when studied in sections at thirteen days old showed the mouth posterior to the eye, hanging as a ventral proboscis-like mass. Two nasal plates were present and the eye was single. This eye, Fig. 44, was unusually far forward and although the retina was well differentiated into layers the humor had not perfectly formed behind the lens. The small section of the brain is shown in Fig. 44 to be bilateral and not unusual in appearance. Passing forward through the series of sections to a place where the anterior end of the cyclopean eyeball stops, a minute lens is found lying in a ventro-median position,

Fig. 45. This lens, although only nine micromillimeters in diameter, has differentiated and shows perfect lens fibers arranged in the usual concentric fashion. It has no connection whatever with the eye, nor with any part of the central nervous system. The small lens doubtless originated and differentiated its tissue in an independent manner. The independent origin and self-differentiation of lenses will be clearly shown in a following section of this paper. Fig. 45 also illustrates the two lateral nasal plates in section.

The cyclopean eye is thus seen to be at times single in nature, showing no trace of a double composition. This may be considered the climax or perfection of cyclopia, if such an expression is permissible. Eyes not completely united, or double-eyes, are the incomplete or imperfect cyclopean condition, while the single condition reduced or distorted may be termed extreme cyclopia.

d Extreme Cyclopia: From the Abnormally Small Anterior Cyclopean Eye to Entire Absence of Eyes

Many cases are found representing the condition of extreme cyclopia. They may be considered in order, beginning with the least modified. In discussing the living embryo mention was made of those with a small cyclopean eye placed far forward (Fig. 18). Sections of such eyes show them to be of a more or less imperfect nature and sometimes deeply buried in the tissues of the head. Fig. 46 shows a section through the small eye of a hatched embryo eighteen days after fertilization. This eye is placed in the extreme anterior tip of the head and the section shows on the right side pigment spots which lie on the front end of the forehead. The eye is unusually small and the living embryo was abnormal, being unable to swim directly forward. The nasal pits are united in the anterior eye region and a proboscis-like mouth is situated ventrally.

Two still more abnormal cyclopean eyes are shown in transverse section by Figs. 47 and 48, both from thirteen day embryos. In Fig. 47 the eye is close to the single olfactory pit, the retina is differentiated into layers, but the lens is larger than the optic cup so that it cannot fit completely into it. The brain of this individ-

Sections of perfectly single cyclopean eyes

Fig. 40 Horizontal section through mid-brain showing its corpora bigemina, *Cb*, and floor of hind brain, *Hb*, in seventy-seven hour cyclops.

Fig. 41 A more ventral section of same series, *E*, the Cyclopean eye; *ol.p.*, olfactory pits united. *Hb*, hind-brain and *Av.*, auditory vesicle; *Cb*, floor of one mid-brain lobe. Guide figure *X* gives plane of each section.

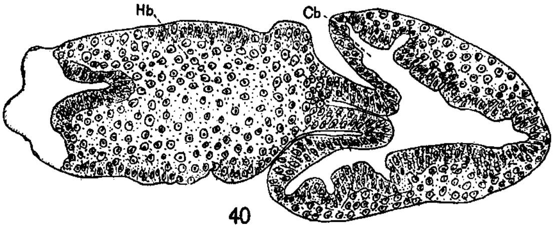
Fig. 42 Trans-section of a four day single cyclopean eye in exact ventro-median position. *ch*, choroid coat; *p*, pigment spot.

Fig. 43 Similar section of nine day eye. Humor cavity behind the lens. Note perfectly bilateral brain. *p*, pigment spot.

Fig. 44 Section of single median eye below perfectly bilateral brain, thirteen days old.

Fig. 45 A more anterior section in same series as Fig. 44. The forward tip of the eye *ch* is seen. A small lens *L* lies free near the ventral ectoderm; *ol.p.*, olfactory pit; *p*, pigment spots on anterior end of brain.

Guide figure *X* indicates plane of all sections.



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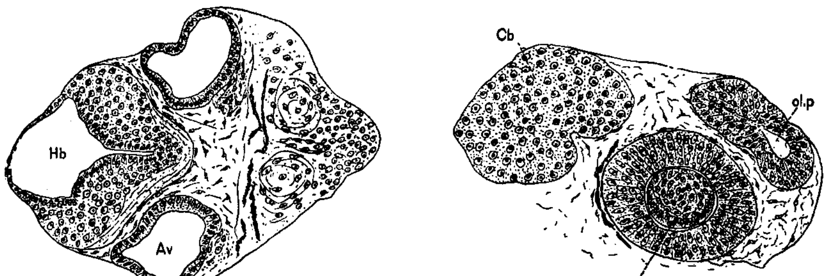

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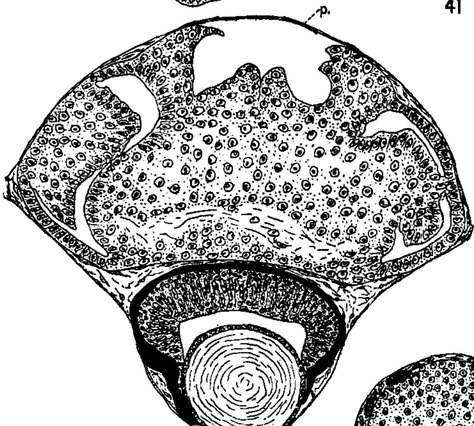

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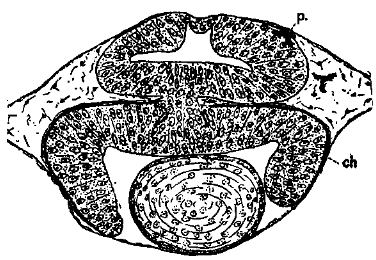

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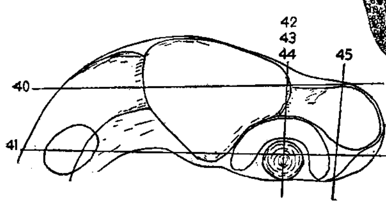

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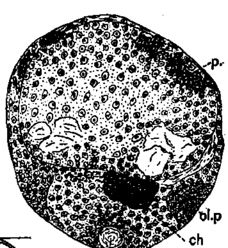

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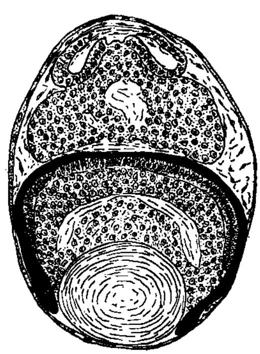

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ual is abnormal and the eye is out of the median line. The embryo of Fig. 48 was abnormal with the brain distorted so that the cyclopean eye was slightly to one side and far out beyond the head. The retina differentiates into layers but the lens lies out of the central position, and would be unable to function efficiently.

A peculiar condition is found in the embryo from which sections shown in Figs. 49, 50 and 51 were taken. This very small eye was again in an extremely anterior position, though almost in the median line. The lens is as large as the optic cup and protrudes far out beyond its edge. Fig. 49, the most anterior section of the three, passes through the great circle of the spherical lens and shows it entirely outside the optic cup. On passing back in the series to where the lens is less in size, we reach the anterior edge of the optic cup and choroid coat, Fig. 50. Continuing back in the series of sections, the lens disappears and the optic cup alone is shown in Fig. 51. The lens in this eye is clearly too large for the accompanying cup as was also the case with the two eyes just described. The size of these lenses is, therefore, independent of the size of the optic cup. Lewis' ('04) idea that the cup regulates the size of the lens does not apply to these embryos, nor does the rule for the amphibian that the origin of the lens is dependent upon the influence of the cup.

A step beyond this condition of a small anterior eye with its ill-fitting lens may be illustrated by an embryo in which the eye is a minute choroidal sphere buried in mesenchyme below the brain and in the median line. In life this specimen seemed entirely eyeless, but sections showed this small eye-like structure (Fig. 52) in the position typically taken by a cyclopean eye. Such cases as this emphasize the necessity of sections in order to correctly interpret the conditions of cyclopia and conclusions based only on superficial studies are necessarily unreliable. The nasal pits were in the normal lateral position. Passing back in the sections to the region usually occupied by the two eyes, it will be seen that on one side a typical lens occurs (Fig. 53). The lens is well differentiated and completely isolated from all connections with either nervous or eye tissue. A band of muscle is seen in the figure to touch the inner edge of the lens.

The occurrence of this lens recalls at once Herbst's ('01) argument regarding the independent origin of the lens. He held that "if the lens really developed independently of the optic cup, then in the case of median cyclopia the two lateral lenses should arise in their usual positions; but they do not, and furthermore, the cyclopean cup gets a lens from ectoderm out of the usual lens-forming region." The Fundulus embryos show lenses arising at times in their usual places and often in other places, independently of the optic cup. We may suppose that in these embryos certain areas of the ectoderm are at times out of their normal positions, and thus explain the promiscuous distribution of independent lenses.

Finally, embryos exist in which no indication of the optic cup can be found, these may be said to have passed beyond the extreme cyclopean condition. They are not ordinary individuals that are merely blind, since the mouth is usually distorted and sometimes the snout-like structure which accompanies cyclopia is present. This suggests the possibility that the "proboscis-mouth" is not entirely due to its normal position having been usurped by the cyclopean eye. Some of these embryos have free lenses and others no optic parts at all. Figs. 54 and 55 are two transverse sections from the same embryo, the anterior one shows a lens lying against the olfactory pit but free from all connection with the central nervous system. Fig. 55 shows a second lens lying close against the brain tissue. This embryo has no indication whatever of optic cups, and seemed eyeless in life. Other individuals when carefully examined in section had neither an optic cup nor any lens-like structures.

We have thus reviewed a series of forms beginning with the usual two-eyed embryos and passing through all degrees of double eyes to single cyclopean eyes, to extremely small cyclopean eyes, to individuals finally with only lenses present and no optic cups and others with neither lens nor cup.

The extreme cyclopean condition

Fig. 46 Cross-section of hatched embryo, small cyclopean eye located in anterior tip of head. The nose is anterior to this section. *p*, pigment on "forehead" of embryo.

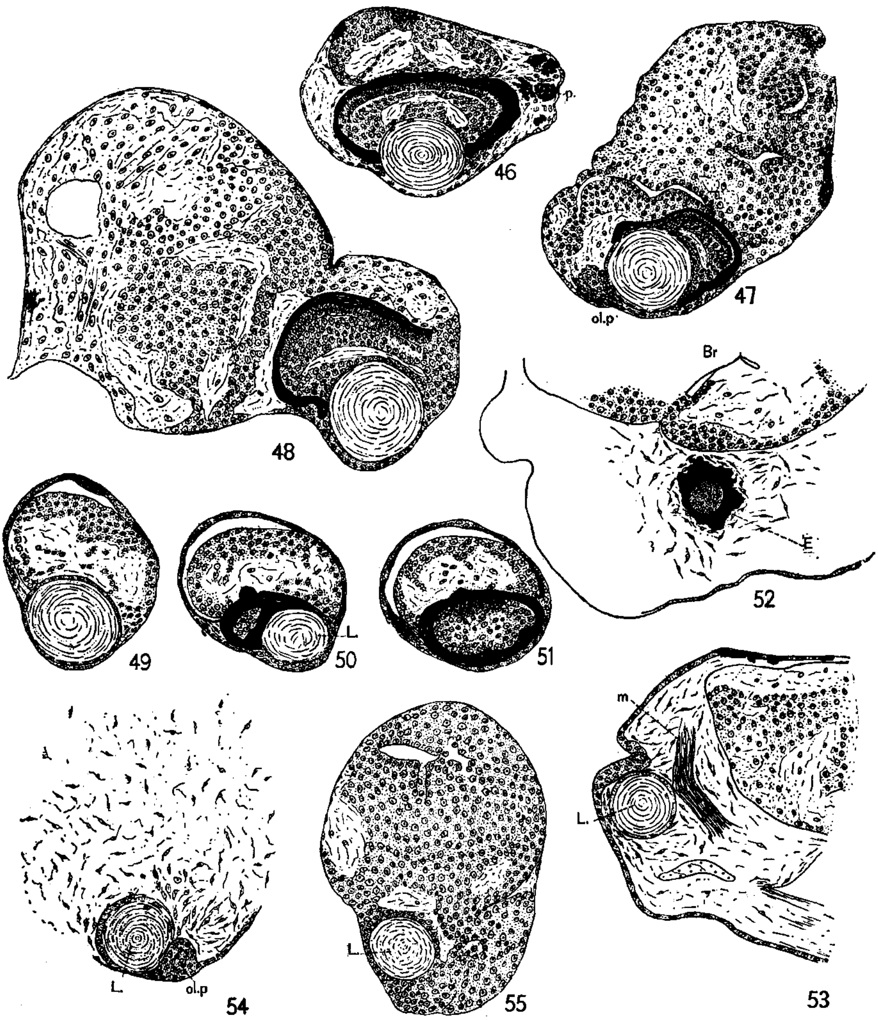
Fig. 47 Section of thirteen day embryo. Small cyclops eye with large lens, differentiated retina and abnormal brain partly surrounding the eye; *ol.p.*, nasal pit.

Fig. 48 Section of thirteen day cyclops with eye far forward and out of median line beneath an abnormal mass of the brain.

Figs. 49, 50 and 51 Sections of a small anterior cyclopean eye with large lens projecting out of optic cup. The first section Fig. 49, is most anterior, the great-circle of the spherical lens, Fig. 50, tip of lens in the edge of optic cup, and Fig. 51, center of optic cup behind the lens.

Figs. 52 and 53 Sections of thirty day embryo which seemed eyeless in life. Brain abnormal. Fig. 52, the cyclopean eye is represented by a choroid vesicle, *E*. The more posterior section, Fig. 53, shows a perfect lens *L*, in the usual lateral position, but no optic cup exists. A band of muscle *m* is between the lens and brain.

Figs. 54 and 55 Sections of two lenses *L*, one forward by the olfactory pit, *ol.p.*, the other more posterior and surrounded by brain tissue. No optic cup present in this nine day embryo.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INCOMPLETE DIPROSOPUS WITH THREE EYES AND ONE ADDITIONAL LENS

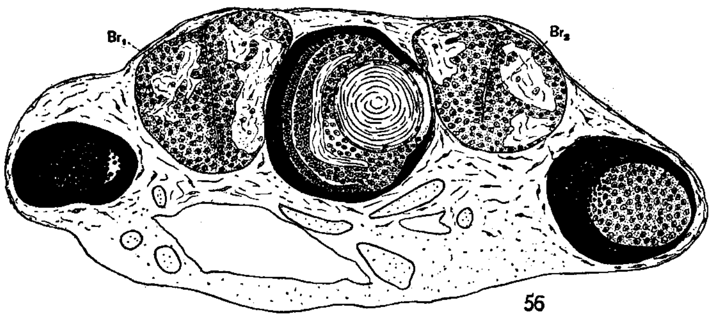
A most valuable object for study was an incomplete diprosopus monster which appeared in my solutions. This individual had two heads separated as far as the lateral eye region. It appeared as indicated by Fig. 21 when seventy-two hours old. The two brains are separate, almost back to the auditory vesicles. Two normal eyes are shown in outer lateral positions while between the heads one eye, perfect in shape, is mated with the outer eye of the left head and a circular body occupies the usual position of left eye on the right head. The embryo seemed normal in other respects and was in a vigorous condition.

The monster when eighteen days old had developed to the usual size and was still hardy. At this time it presented a striking appearance as indicated imperfectly by Fig. 22. Three large eyes normal in form and capable of movement looked out from the double head. All visible evidence of the circular body shown near the middle eye when seventy-two hours old had disappeared. The middle eye was clearly paired with the left eye of the left head component and the right eye of the right head seemed mateless. A single pair of auditory vesicles were present. The young fish respired and twisted vigorously within the membrane. Three hours after this drawing was made, the embryo hatched and swam about in a circular fashion, the body not straightening perfectly. The free living animal was kept for five days and then preserved for sectioning.

The sections show the presence of two brains, one spinal cord and one normal mouth leading into a pharynx with its series of gills, while a second short throat is present in the right head. There are two notochords back to the middle of the yolk-sac and one from there on. The rear end of the medulla becomes single and only one pair of ear vesicles are present. There are two olfactory pits anterior and median to the lateral eyes.

Three perfectly normal eyes exist. They possess clearly differentiated retinæ, irides, humor chambers and lenses. Two of these eyes are connected in the usual way with the brain of the left head and one with the brain of the right. Fig. 56 is a section showing the middle eye somewhat back of its center so as to bring the edges of the other eyes into the figure. The middle eye is more anterior in position than the two lateral ones, owing to the slight obliquity of the left head. A distinct lens is shown in the cup in Fig. 56. On going backwards in the series we reach a section passing through the middle of the two lateral eyes and the posterior end of the middle eye (Fig. 57). The section shows dorsally the huge double brain and ventrally a central throat and most interesting of all a fourth lens. This lens lies against the outside choroid coat of the middle eye and is in just the position (recognizing a displacement due to development of the middle eye) to be the lens of the left eye of the right head, if such an eye were present. We thus have in this double head three typical eyes and the fourth represented by a free lens. It was impossible to detect the clear lens in the living embryo which emphasizes again the necessity of sections for a definite interpretation of the conditions existing in these monsters. Conclusions drawn from observations on the living eggs without the comparison of sections may be incomplete. The sections further make clear the nature of the circular outline shown against the middle eye of the seventy-two hour embryo (Figs. 21 and 57). Comparing the figures of sections and those of the whole embryos, it will be remembered that the sides of the sections are transposed, since the drawings of the total embryos are made from a simple microscope and the sections from a compound microscope which inverts the image.

This incomplete diprosopus monster increases the series of eye monstrosities so that it passes through the cyclopean group to beyond the normal. The diagram (Fig. 58) illustrates in a simple way the various conditions we have considered and emphasizes the continuous nature of the series. Beginning at one end with eyeless individuals, we pass gradually through a series with small buried cyclopean eyes (which may be indicated in the diagram by a palpebral opening, such as similar mammalian cyclops would show), to the perfectly single cyclopean eye, to the double eye with one lens and pupil, to the hour-glass eye with two lenses and two pupils, to two independent but closely approximated eyes, next to



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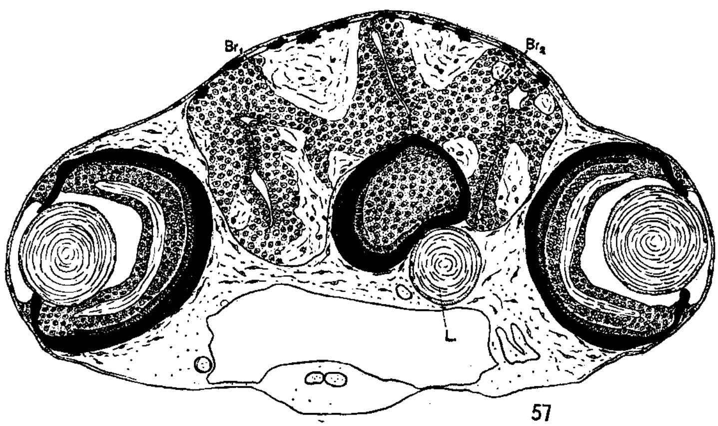

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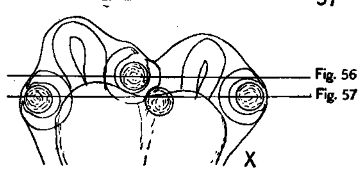


Fig. 56 Section through anterior median eye and edges of lateral eyes of hatched incomplete diprosopus; Br_1 Br_2, the two brains .

Fig. 57 More posterior section through middle of lateral eyes, posterior part of middle eye, and an additional fourth lens *L*.

Guide figure *X* makes both sections clear.

the normal condition and finally beyond to the incomplete diprosopus with three eyes and a fourth lens. The idea of arranging monsters in such a series including the normal is due to Prof. H. H. Wilder.

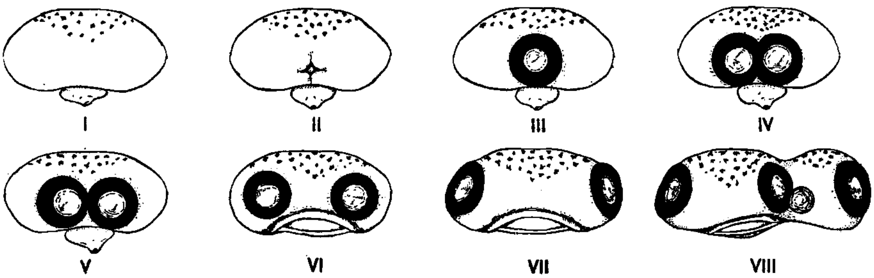


Fig. 58 Diagram of the various conditions shown by the "magnesium embryos" from entire absence of the cyclopean eye *I*, to deeply buried eye *II*, perfect single cyclopean eye *III*, double-eye, *IV*, two approximated eyes *V*, eyes unusually close together *VI*, normal *VII*, three eyes and fourth lens *VIII*.

The normal is a mean from which different degrees of abnormalities are but greater or less deviations. It is possible to arrange almost any type of abnormality in such a series. Supernumerary arms or legs on one side might exist in various individuals in different numbers down to the single normal one; other specimens could be found showing degenerate or small arms and finally armless or legless individuals are known.

MORPHOLOGY OF MONSTRA MONOPHTHALMICA ASYMMETRICA

A brief description of the asymmetrical monophthalmica monsters in life has been given above, but their true nature and structural conditions are impossible to detect without sections. It is found that here again a continuous series exists, beginning with the ordinary two-eyed individual through all gradations to the complete disappearance of one eye.

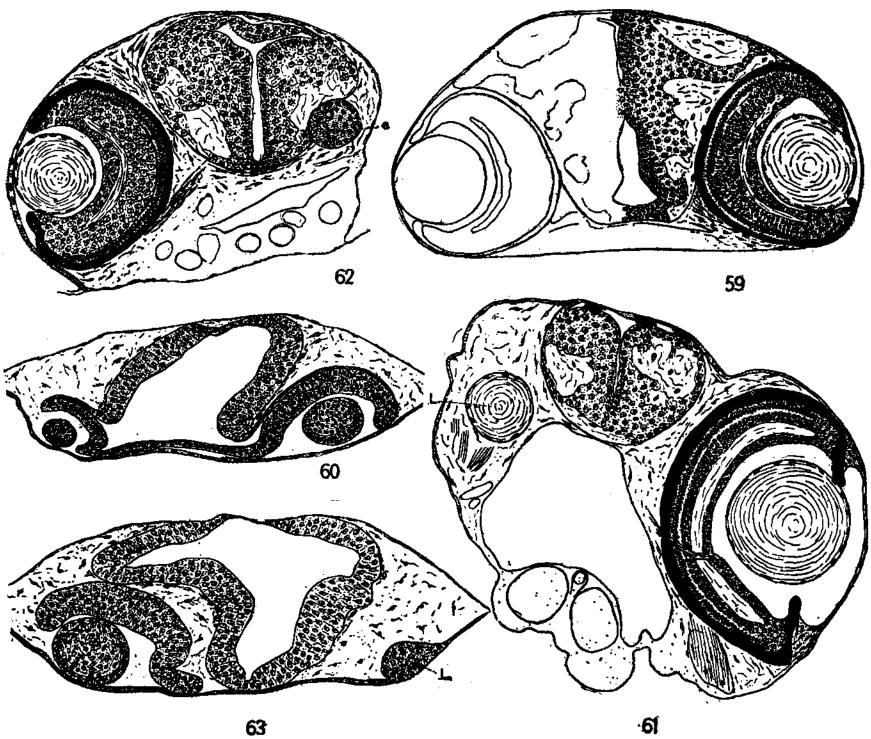
The section through the middle of the eyes in a normal embryo of thirteen days old is illustrated in Fig. 59. The eyes, of course, are equal in size and alike differentiated structurally. In the salt solutions, however, many embryos occur with one eye perceptibly smaller than its mate. A section through the eyes of an embryo of this kind when seventy-six hours old is shown in Fig. 60. The left eye is decidedly smaller than the right and possesses a correspondingly small lens. From the comparative study of a number of individuals it may be safely stated that this difference in size between the two eyes will not be overcome later, nor on the other hand will the small eye degenerate or disappear. The embryo will hatch with its eyes in dissimilar conditions comparable to the state of things shown by this seventy-six hour stage. The brain is normal and two nasal plates are present.

An embryo closely similar to the one just described was sectioned after hatching. Its large eye appears as in Fig. 61. More anterior sections show a small eye looking forward with a somewhat protruding lens in its cup. Behind this small eye is another lens lying free in the ectoderm (shown in Fig. 61). This lens is perfectly differentiated and appears to have arisen independently.

A further reduction of the eye is shown by Fig. 62. In this thirteen day embryo the left eye is perfect and the right is represented by a small cellular mass lying close against the brain. The lens of the right side is entirely wanting. In life the head was slightly one-sided, obviously on account of the asymmetrical eye development; no indication of the cellular mass could be detected and the embryo seemed truly one-eyed.

A section of another seventy-six hour individual which in life also seemed to be one-eyed is illustrated by Fig. 63. The brain is normal and almost bilaterally symmetrical, an ordinary left eye exists but there is not the trace of an indication of the right optic cup. An ectodermal thickening represents the right lens in process of formation in the position that it would typically occupy. This lens anlage must have arisen independently of a stimulus from an optic cup and is well removed from the brain, so that no direct stimulus from that source can be responsible for its appearance.

Other one-eyed individuals showed complete absence of all parts of the second eye, the lens as well as the optic cup failing to arise. The occurrence in the Mg solutions of these one-eyed embryos as well as the cyclopean embryos suggests that the chem-



Monstra monophthalmica asymmetrica

Fig. 59 Section through eyes of normal thirteen day embryo.

Fig. 60 Section of seventy-six hour embryo with one normal and one small eye and perfect brain.

Fig. 61 Section of the normal eye of a hatched embryo; a small eye with a lens is situated more anteriorly on the other side and behind this is a third lens, *L*, shown in the figure on the left side.

Fig. 62 Section of normal eye in thirteen day embryo, the other eye is represented by the cellular mass, *e*, close against the brain.

Fig. 63 Section of normal eye in seventy-six hour embryo, the brain is bilateral and perfect, but no indication exists of the right optic cup although the ectoderm of that side has formed a lens thickening *L*.

ical influence exerts a peculiar inhibition of that process of outpushing or separation by which the optic vesicles arise. Such an idea will be more fully considered in the general discussion given below.

The unequal eyes may possibly result from an unequal allotment of eye material to one side or the other. A major portion might go to the right side and a minor part to the left, or the entire eye anlagen might by chance occur on one side. This in a sense would be lateral cyclopia. Such reasoning is of course purely hypothetical.

INDEPENDENT ORIGIN AND SELF-DIFFERENTATION OF THE CRYSTALLINE LENS

Spemann ('01), Lewis ('04), and others have concluded from experiments on amphibian embryos that there is no localization of lens-forming material in any given area of the ectoderm. They further held that the formation of a lens is dependent upon a stimulation of the ectoderm through contact with the optic-vesicle or cup. Spemann ('05) in discussing the question of the self-differentiating power of the lens concluded from a consideration of Schaper's ('04) experiments on the frog that the lens is not capable of self-differentiation, but that a continued influence or contact of the optic-cup is necessary to cause the lens-plate or lens-bud to develop into a typical lens. LeCron ('07) has recently shown that the lens in Amblystoma is not self-differentiating. I ('07d) found in embryos of the blind Myxinoid, Bdellostoma stouti, that a lens-thickening formed in early stages while the optic-vesicles were near the ectoderm. During development the optic cup becomes distantly removed from the ectoderm and the lens-plate disappears as if it were unable to continue development independently of the optic cup contact.

On the other hand Mencl ('03) has claimed that the lens in Salmo salar is at times formed independently of the optic cup influence and Spemann ('07) has recently modified his attitude. Spemann finds that in a certain species of frog, Rana esculenta, the lens may arise independently of the optic cup. This lens also continues to develop and differentiates typical fibers. Most conclusive evidence favoring the independent origin and self-differentiation of the lens is furnished by the Fundulus embryos now under consideration.

Attention has been called repeatedly to the occurrence of lenses having no connection with other optical parts. It may be well at this time to summarize these cases which clearly show that in Fundulus the lens may arise independently and continue its development and differentiation.

Fig. 63 illustrates the budding off of the lens from ectoderm on the side of the head which lacks entirely an optic cup. Fig. 61 shows a lens fully differentiated though lying freely in the mesenchyme of the head. It will be recalled that this is a supernumerary lens; the large and small eyes of the embryo both possess lenses. An optic cup can not be responsible for this third lens. Fig. 57 of the incomplete diprosopus shows the fourth lens of the double head entirely outside the optic cup of the third eye which possesses a lens. Figs. 54 and 55 show two lenses in an embryo that possessed no trace of an optic cup. Fig. 53 indicates a lens in its usual position but no optic cup is present. In Fig. 45 a tiny lens is found in front of a cyclopean eye which possesses its own lens. Many other similar illustrations could be given.

No one could hold that this indiscriminate collection of lenses, all of which are entirely isolated from any connection with optic cups or other eye parts, as well as in nearly all cases from the brain itself has arisen through direct stimuli derived from the optic cups. It is also evident that the lens after its formation continues to self-differentiate.

It seems to me that in Fundulus the case is clearly proven that lens formation does not depend upon a direct stimulus from the optic cup. Such a dependence as advanced by Lewis ('04) for the frog is not, therefore, of universal application, nor is the view tenable that the differentiation of the lens depends upon a continued stimulus from the optic cup.

DISCUSSION AND CONCLUSIONS

The foregoing facts furnish important information as to the cause and manner of development of cyclopia, and the facts bear directly on previous ideas concerning this subject.

By treating the fish eggs with magnesium solutions, it is conclusively shown that the experimenter has the power without mechanically injuring the egg or embryo to cause what would have been a two-eyed individual to become a cyclopean monster. This undoubtedly is a case of the occurrence of cyclopia through the action of external influences on the developing egg. I conclude, then, that cyclopia does not necessarily result from germinal variations, but I make no claim that it may never arise in such a way. On the contrary, there is no reason why cyclopia should not occur through germinal variations as readily as does any other new feature. The fact that mammalian cyclopean monsters do not survive, or even if it be proven that the free-swimming cyclopean fish are incapable of living or reproducing, does not argue against the possibility that cyclopia may in cases be due to germinal variation. Such a statement is emphasized by a case I ('07c) recently recorded. In a flock of sheep in North Carolina two entirely legless lambs appeared in the spring of 1907. Again in 1908 two other similar lambs have occurred, one being the offspring of a mother which had previously borne a legless individual. These lambs were unable to feed without assistance and in nature would doubtless have died shortly after birth, but their peculiar occurrence in this flock is very probably due to germinal variations, either within the mother or father, or both. Students of inheritance consider sports to be due to germinal variations and the ability of such sports to survive depends merely on their adaptations to the surroundings and not in the least on their manner of origin. No reason can be given why a cyclopean individual might not occur as a sport due to sudden germinal variations. From the experiments contained in the present paper, however, it may be emphatically affirmed that cyclopia is *not always* due to germinal origin.

Spemann ('04) through an ingenious method of experiment, produced double-headed Triton embryos which exhibited various degrees of cyclopia. The eggs of this salamander when constricted about the periphery of the first plane of cleavage with a fiber-like ligature gave monsters with two equal heads. When the ligature was oblique with reference to this plane one of the heads was cyclopean to a greater or less degree. Spemann thought the defective head due to the loss of the anlagen of certain parts, consequently these parts never began development and organs situated lateral to them developed in contact from the start. In other words parts between the eye anlagen fail to form and thus the anlagen come in contact and so develop from the beginning. This explanation is of course entirely speculative, but it is supported in a manner by experiments which according to Mall ('08) Lewis has performed on the fish embryo. Mall states that Lewis found by pricking the extreme anterior end of the embryonic shield in Fundulus eggs that many of the eggs develop into cyclops embryos. It was found in some that the prick had destroyed the "nose" only. "This experiment shows conclusively that it is the absence of tissues between the eye ar lagen that allows them to come together and unite."

The above explanation no doubt holds for some cases of cyclopia produced by cutting or pricking; there it is evident that tissue is destroyed and the destruction of median tissue may cause the regions containing the eye anlagen to unite. It is difficult to apply this explanation to all cases. In the "Magnesium embryos," why should tissue between the eyes fail to form and not other tissues; why are the nasal pits united in some cyclops and separate in others? A close microscopic examination of the brain floors in cyclopean and two-eyed embryos shows no absence of recognizable parts in the former. The monstra monophthalmica asymmetrica are also to be explained; here one eye in some cases fails to come off from the brain. Is this due to the absence of its early anlage? The very small cyclopean eye sometimes buried deeply in the head, and the eye shown in Fig. 38 which is partly inclosed within the brain, as well as the entire absence of an eye, suggest another explanation that may apply to all cases in the magnesium solutions.

The small eyes close together, cyclopia in various degrees, the imperfect formation or absence of one eye and entire absence of eyes are all conditions common to the magnesium solutions and very rare or never occurring in other solutions, nor in the hundreds of eggs observed developing in sea-water. The conditions are, therefore, probably due to a common cause, and I suggest hypothetically that this cause is an inhibitory or anæsthetic effect of the magnesium on the process of outpushing and separation of the optic vesicles. Magnesium exerts a decidedly anæsthetic effect upon both vertebrate and invertebrate animals and is an inhibitor of muscular activity. It might possibly inhibit the giving off of the optic vesicles or prevent their separation in the brain, so that both might come off together as in cyclopia, and it might have caused the eye in Fig. 38 to be arrested when only halfway separated from the brain; the absence of one eye and complete absence of eyes would be perfect inhibition. It is necessary to find a definite point in the strength of the solutions in order to obtain the proper amount of inhibition for many weaker eggs are killed during early stages.

The strongest argument against such an hypothesis is the fact that Mg in distilled water solutions fails to cause cyclopia, whereas its anæsthetic or inhibiting powers should be most active in such a solution.

Dareste's ('91) idea that cyclopia is caused by a closed brain or the failure of the anterior vesicle to develop is unsupported, since in Triton with the hollow-brain tube present Spemann finds that the defect occurs. In Fundulus the optic outpushings are normally given off while the brain is yet solid, so that according to Dareste all of these fish would be cyclopean in nature.

Schwalbe ('06) in his Morphologie der Missbildungen des Menchen und der Tiere, considers cyclopia to result from unusual pressure exerted during early stages of development which does not cause the lateral parts to grow together but prevents them from developing at all. This position is somewhat in accord with the hypothesis suggested above. If pressure prevents the growing apart laterally of the anlagen which normally require energy to accomplish their separation, then by anæsthetizing a part, one accomplishes practically the same thing as by applying pressure.

The part in anæsthesia lacks energy to grow out laterally, thus the two eye anlagen remain together in the floor of the brain and come off as one median vesicle either double or single, depending upon the extent of separation possible under the given degree of pressure or anæsthesia.

Mall ('08), in his recent memoir on the causes underlying the origin of human monsters, gives an excellent survey and discussion of the evidence furnished by experimental teratology. In the body of the paper is presented a strong case in favor of external influence during development as the chief cause of many monstrosities. Here we may consider only the discussion of cyclopia. The idea of fusion of the two eye vesicles during their development is advocated, but the present evidence is against this position and is in accord with Spemann's ('04) view of an early defective anlage. Mall also inclines toward the idea of the single brain as being primarily responsible for cyclopia, but it is shown by embryos considered here that cyclopia often accompanies perfectly bilateral and bilobed brains, neither does a retarded growth of the frontal process necessarily follow in cases of cyclopia.

Experiments uphold the statement "that every egg has in it the power to develop cyclops monsters." The germinal theories of cyclopia are shown by the experiments to be unnecessary as explanations of its cause. The possibility of its occurrence through germinal variations, though to my mind extremely slight, is not entirely excluded by experiments. The experiments conclusively show the origin of cyclopia through external influences.

Much could be said pro and con regarding the significant nature of the cyclopean fish embryos as a specific response to a definite chemical environment. The suggestion is evident, though highly hypothetical, that cyclopia in man and mammals might be due to a similar chemical cause, an excess of Mg salts in either the mother's blood or the amniotic fluid surrounding the developing embryo.

The Magnesium embryo is as typical of these Mg solutions as is the now classic lithium larva of the sea urchin produced by Herbst ('92, '93) in his Li solutions, or Morgan's ('04) lithium frog embryos produced in a similar way. They all tend to show that different chemical conditions may each induce by their actions a specific type of larva from a given variety of egg.

SUMMARY

1 The eggs of the fish, Fundulus heteroclitus, give rise to a large percentage of cyclopean embryos when subjected during their development to solutions of magnesium salts in sea-water. Similar results follow if the eggs are placed in the solutions either before cleavage or when in the two or early four-cell stages, later stages were not tried. This is the first instance of repeatedly causing, by the use of chemical substances, vertebrate monstrosities such as are known in nature.

2 The peculiar embryos with the median cyclopean eye are able to hatch. Many of them swim about in a perfectly normal manner, darting back and forth to avoid objects placed in their field of vision as readily as do two-eyed individuals.

3 The cyclopean fish is exactly comparable to the monstrous cyclops of man and other mammals. Both have a median eye either double or single in its structure. The nose in the mammalian cyclops is a single proboscis-like mass above the eye. The nasal pits in the "Magnesium embryos" are sometimes united and sometimes separate, but the mouth hangs ventrally as a proboscis-like organ strikingly suggesting in form the nose in mammalian cyclopia. The mouth of Fundulus normally occupies an extremely anterior position but in the cyclopean fish the eye has usurped this place, thus preventing the usual forward growth of the mouth elements and forcing them to remain ventrally as the proboscis-like mass. (See Figs. 25, 26, 27.) In cyclopean mammals a similar mechanical explanation accounts for the condition of the nose. The median eye obstructs the path of down-growth which passes normally between the eyes, and forces the nose to form above the eye as a proboscis on the forehead.

4 A study of more than 275 living cyclops monsters and of many of these in section shows all degrees in the defect. Eyes unusually close together, intimately approximated eyes, the double

eye in a median position, the single cyclopean eye, an extremely small anterior eye, a deeply buried ill-formed cyclopean eye, and finally an entire absence of the eye. The embryos exhibit these various degrees of the cyclopean defect from the earliest appearance of the optic outpushings, and in no case was cyclopia due to a union or fusion of the two eye components after they had originated distinctly.

5 A second type of monster designated as Monstrum monophthalmicum asymmetricum, the monster with one asymmetrical eye, was also common in the magnesium solutions. These individuals have one perfect eye of the normal pair but the other is either small, poorly represented or entirely absent. This condition is also present from the first appearance of eye structures and is not due to degeneration or arrest of development.

6 Both types of monsters often form lenses independently of the optic cup stimulus. These self-originating lenses are also capable of perfect self-differentiation, forming lens fibers and appearing as transparent crystalline bodies. Such facts oppose the idea that the lens during its origin and development is in a dependent relationship with the optic cup, and show this view not to be of universal application.

7 The experiments conclusively prove that eggs may be induced to develop into cyclopean monsters by external influences. These influences do not mechanically injure or destroy certain eye regions as does cutting or pricking. It follows, therefore, that cyclopean monsters appearing in nature are not necessarily due to germinal variations, but are far more likely the result of some unusual external influence during development.

8 The occurrence of the various eye monstrosities shown by embryos which develop in magnesium solutions are all probably due to a common cause and I suggest the following hypothetically: Magnesium which possesses a decidedly anæsthetic effect on most animals and is inhibitory in its influences on muscular activity may retard through degrees of anæsthesia the optic outpushings in Fundulus embryos and thus account for the total absence of eyes, small eyes, eyes which failed to develop energy necessary for their normal separation and the other unusual conditions which

have been considered in detail in the present article. This view, of course, is hypothetical and objections to it are recognized.

Cornell University Medical College
New York City, October 1, 1908

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EXPLANATION OF PLATE I.

Fig. A Dorsal view of a cyclopean embryo in almost natural colors. The large antero-ventral eye shows a slight furrow indicating its double nature.

Fig. B The same embryo when the egg is rolled back towards the top of the page. A somewhat ventral view showing the single pupil and lens, the double condition of the eye is only indicated from above.

PLATE I

A.

B.

RICHEOLI, *del*