# THE INFLUENCE OF ALCOHOL AND OTHER ANÆSTHETICS ON EMBRYONIC DEVELOPMENT

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WITH TWENTY TEXT FIGURES

The adult nervous system is peculiarly sensitive in its responses to the influence of alcohol and other anæsthetics. The writer has found it to be equally true that alcohol and anæsthetics exert a most striking influence over the development of the central nervous system and the organs of special sense. There is considerable variation in the way in which the several anæsthetics act on the developing animal; some of them, such as ether and chloretone, producing effects of a general nature, while alcohol and magnesium are more localized or specific in their action. A similar statement is true for the actions of different anæsthetics on the adult body.

In attempting to explain the occurrence of asymmetrically monophthalmic, cyclopean and blind individuals among fish that had been developed in solutions containing magnesium, the writer advanced the hypothesis that the anæsthetic property of Mg was the causal factor. Many reasons for such a view were put forward in a paper on the artificial production of these monsters (1909). To experimentally test this hypothesis various other anæsthetic agents have been used and all of them to a higher or lower degree inhibit the development of the optic vesicles in fish embryos, and thus give rise to various ophthalmic defects. Alcohol is most decided in its action, causing in some experiments as high as 90 to 98 per cent of abnormal eyes, generally cyclopean, which far surpasses the highest results obtained with Mg.

The effect of alcohol on the general development of the nervous system is more pronounced than that of Mg, and only a few of the alcoholic specimens ever develop sufficiently to hatch and swim about as do the Mg embryos. An explanation for this may be that Mg exerts an influence to inhibit dynamic processes, such as the out-pushing of the optic vesicles, while alcohol acts more especially on the nervous tissues. Mayer (1908) has shown that Mg inhibits muscular contractility without affecting in any way the nervous impulse or nervous rhythm.

The eye defects, it must be remembered, have only been obtained in solutions of one or another anæsthetic; the many other salt and sugar solutions which have been experimented with during four years ('06 and '07) have failed entirely to produce similar results.

The most important outcome of these experiments has been to prove conclusively that many monsters which occur in nature may be artificially produced by changing the environment of the normally developing eggs. The present experiments will demonstrate that this may be done even after development has proceeded for some time. These anomalous structures being the results of external influences and not germinal variations are to some extent within scientific control. A promising field is thus opened in the devising of means to control or regulate the development of the embryo and possibly to obviate certain monstrous conditions at least. Such possibilities were of course beyond our reach if defective germ cells were actually the cause of these monsters.

Mall ('08) has brought forward evidence to show that improper placentation or unfavorable developmental environment is responsible for most human monstrosities, many of which are aborted before reaching term. There is evidently much need of investigation aiming toward the control and regulation of the developmental environment of mammals.

## METHOD AND MATERIAL

The eggs of the fish, Fundulus heteroclitus, were used in all of the experiments. The method of treatment varies somewhat for the different solutions employed, so that it is best to describe each separately.

Alcohol solutions were prepared in sea-water on the percentage basis. The strength used being 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18 and 20 per cent, 60 cc. of each solution was poured into finger bowls and from 60 to 100 eggs, in the early cleavage stages, four and eight cells, were placed in each bowl. The stronger solutions killed all of the eggs, and those from 3 to 9 per cent gave the best results. In the 3 per cent alcohol solutions at times as many as 90 in every 100 embryos showed abnormal conditions of the eyes, being either eyeless, asymetrically monophthalmic or cyclopean, while in one experiment in a 5 per cent solution of alcohol in sea-water, there were 146 ophthalmic monsters against only 3 individuals with two separate eyes.

Chloretone of 0.1 per cent and 0.066 per cent in sea-water caused abnormalities similar to those produced by other anæsthetics. This substance is more general in its anæsthetic action than either alcohol or Mg, as will be seen in the discussion to follow.

Ether and chloroform also produce rather general effects on the developing embryo, yet a small percentage of cyclopean monsters occur among the embryos which are treated with 60 cc. of sea-water to which 2,2.5, and 3 cc. of ether has been added. In solutions of chloroform of about the same proportions and slightly weaker, a few monsters occurred of the type common to the other anæsthetics. Chloroform is rather toxic in its action on these eggs, large numbers dying in the weaker solutions, while others are so inhibited in their development, that various abnormal conditions follow.

Eggs were not exposed to any of the above anæsthetics for more than twenty-four or thirty-six hours. They were then placed in pure sea-water and continued development showing the abnormal conditions of the eyes and central nervous system that had been induced by their sojourn in the unusual environment.

Similar Mg solutions to those formerly employed were again used. A gram-molecular solution of $MgCl_2$ in distilled water was titrated and kept, to be diluted with sea-water to the proper strength just before the eggs were placed in it. The most favorable results were obtained in solutions of 16, 17, 18, 19, 20, 21 and 22 cc. of molecular $MgCl_2$ made up to 60 cc. by the addition of a sufficient amount of pure sea-water; e.g., 44 cc. of sea-water was added to the 16 cc. of molecular $MgCl_2$ and 43 cc. of sea-water to the 17 cc. of $MgCl_2$. The solutions are, therefore, $\frac{16}{60}$, $\frac{17}{60}$, $\frac{18}{60}$, etc., parts $MgCl_2$ to sea-water. In the $\frac{21}{60}$ solution 66 per cent of the embryos were cyclopean in one of the experiments. Eggs were exposed to the action of Mg shortly after fertilization and at various other times until they reached an early periblast stage, or were fourteen hours old, all with similar results. Although the most favorable time for introducing the eggs into Mg solutions is during the eight-celled stage. The developing embryos were returned to pure sea-water after the third day. The Mg is so slightly toxic that eggs may be kept in it and will continue to develop; the embryos actually hatch and swim about in the solution, being, however, slightly slower in their developmental rate and not so hardy as the specimens which are returned to the sea-water.

## The Action of Alcohol on Development

Weak solutions of alcohol exert a most decided effect on the developing fish embryos, causing deformities of the central nervous system, the eyes, and ears in a very large percentage of the specimens.

### *a. Defects of the eyes*

Typical cases of cyclopia showing in the different specimens all gradations, from merely closely approximated eyes, hour-glass eyes with two pupils and two lenses, oval eyes having the two component intimately associated, typical median cyclopean eyes with scarcely an indication of their double nature and extremely small ill-formed cyclopean eyes, were present in the weak alcohol solutions. All of these have been fully described in a former paper ('09) on the artificial production of cyclopea as a result of the action of Mg. The alcohol monsters in some cases also present various degrees of the monophalmicum asymmetricum defect which was common in the Mg experiments. Individuals may have one normal eye and the other eye in different conditions of arrested development from slightly small and defective to entirely absent, see figs. 1 and 2. An important point that was brought out by the alcohol monsters which was not noticed in the magnesium specimens is the fact that some of the embryos have both eyes equally small and defective, figs. 3, 8, 9, 10, and 11. The two eyes are symmetrically defective and the head appears to have small eye-spots instead of normal eyes; compare figs. 3, and 7, 9, 10 and 11 and 12. Finally, as in Mg solutions so also in the alcohol, many eyeless individuals are present.

The eye in some of the alcohol monsters is rather different from that found in the Mg embryos, and may possibly serve to indicate something of the condition of the eye anlagen in the brain. Many embryos possess optic cups with their concave surfaces facing almost directly toward the median sagittal plane of the head. In life the eye presents a heavily pigmented solid convex surface to the side of the head instead of the usual open pupil through which the lens may be seen within the cavity of the optic cup. Fig. 5 and 6 show front and lateral views of such an embryo; the side view indicates the peculiarly solid convex object seen when looking towards the lateral eye. Fig. 13 represents a section through this eye. The choroid coat of the eye-ball is pressed close against the body wall on the sides of the head and the concave retinal surfaces which should face outward are turned directly towards one another; a lens lies between the two eye components which are really separate except along their dorsal borders. Fig. 4 shows a somewhat similar specimen in which the eyes are entirely separate yet they have an arrangement almost identical to that just described. The eyes face the mid-plane of the head and turn their convex choroid coats out against the body wall. The only place at which the inner face of the eyes touches the ectoderm is the ventral body wall and from this a lens has arisen and lies between the two eyes. Fig. 14 illustrates a section through these eyes, in which a most peculiar arrangement exists. Optic fibers probably arising from the ganglionic layer of the retina, (although this can not be positively demonstrated in the sections), Camera Drawings of Living Fundulus Embryos

Fig. 1. An embryo twenty days old which was treated with 5 per cent alcohol. Only one eye is formed and it faces in a ventro-median direction, instead of towards the lateral wall of the head.

Fig. 2. An asymmetricum nonophthalmicum monster with one almost typical eye while the other eye is small and poorly formed, from a 4 per cent solution of alcohol in sea-water.

Fig. 3. An embryo with both eyes small and closely approximated; the eyes face ventrally. This type is common in all of the anæsthetic solutions.

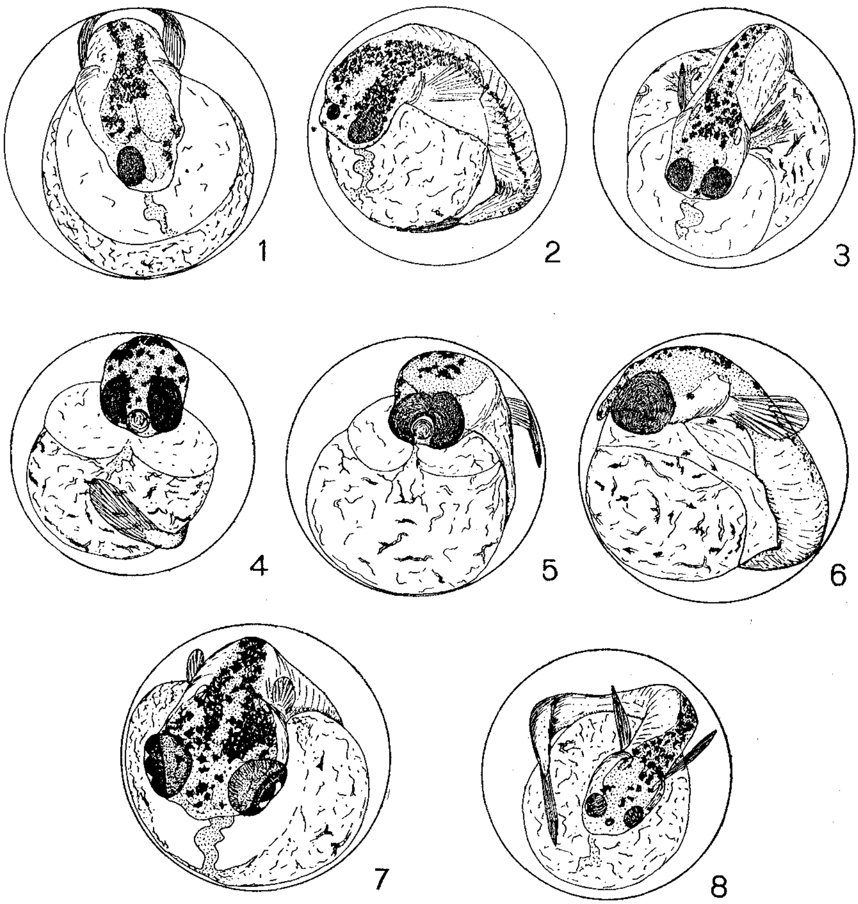
Fig. 4. A nineteen day embryo from 5 per cent alcohol. The two eyes are not connected yet their convex surfaces are turned out against the lateral walls of the head and the pupils face the median plane. A single lens lies between the two eyes. Fig. 14 shows a section of these eyes.

Fig. 5. A nineteen day embryo from 5 per cent alcohol. This front view shows the two eyes joined dorsally and facing one another with the lens between them.

Fig. 6. shows a lateral view of the same head with the convex choroid surface of the eye-ball close against the side of the head. Fig. 13 illustrates a section through these eyes.

Fig. 7. A normal Fundulus embryo when eight days old drawn to the same scale as the monsters.

Fig. 8. A twenty day embryo from 5 per cent alcohol; the eyes are small and defective as shown in the section fig. 10.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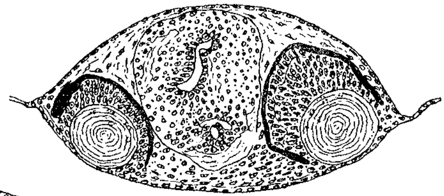
are collected into optic nerves which pursue extraordinary courses; instead of passing through the outer retinal layers and the choroid coat they take an almost directly opposite course and run across what should be the optic cup cavity (the humor cavity in these specimens is filled with loose cellular tissue) and out through the wide-open "pupil," forming a perfect cross and then passing into the base of the brain to end in the optic lobes.

The position of the lens must not be supposed to determine the actual pupil region of these eyes, as the lens clearly lies between them; see fig. 4 of the living embryo. The wide open pupils of the two eyes face or lead directly into one another. The position might be taken that the entire arrangement represents one large eye; this is not true however since the eyes are entirely separate in all of the sections and the optic cross could scarcely be expected to exist within the base of the eye itself as would be the case if this were one huge eye. The eyes really hang down from the brain as two large retinal disks. Fig. 11 represents a similar case with the eyes rather more flattened out laterally, and the existence of all gradations between the two conditions substantiates the above statement.

The retinal layers of these eyes which are nineteen days old, are poorly differentiated, the inner layer consisting merely of indefinitely arranged cells; a better differentiation is usually attained in normal specimens by the sixth or seventh day. A comparison of Figs. 13 and 14 with Fig. 12 illustrates in a way the more definite orientation of the inner retinal cells in the normal individual when compared with the defective eyes. It will also be noticed that the lenses in the two-eyed specimen are surrounded by clear humor spaces while the lens in the defective eyes lies buried in loosely arranged cellular tissue.

The right retina of the monster faces in the same direction as the left retina of the ordinary individual yet there is no indication of a reversal of the layers which might possibly be imagined in such a case.

The optic stalk was scarcely formed in these eyes, which is not infrequently true in cyclopia. The path of the optic nerve is, therefore, evidently not that usually taken along the



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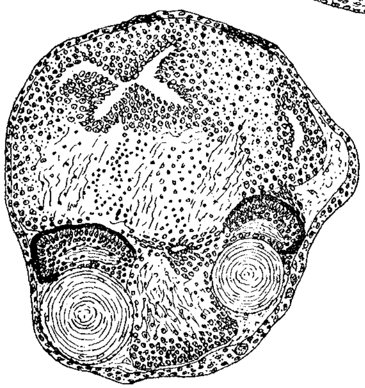

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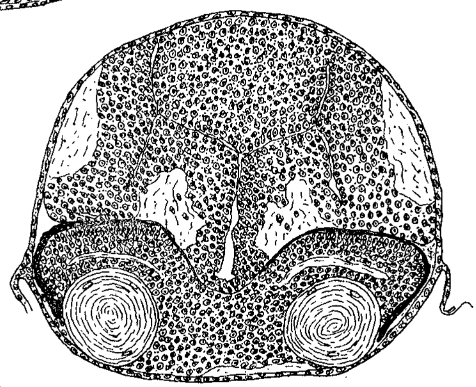

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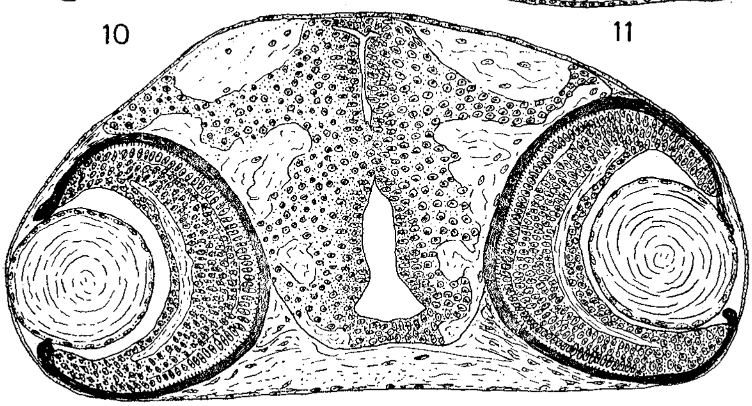

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FIG. 9. A section through the small defective eyes of an eight day embryo, after treatment with a 4 per cent solution of ether in sea-water. The brain is narrow and poorly developed.

FIG. 10. A section through the head of the embryo shown in fig. 8. Both of the eyes are small and defective with ill-fitting lenses and face in a ventral direction.

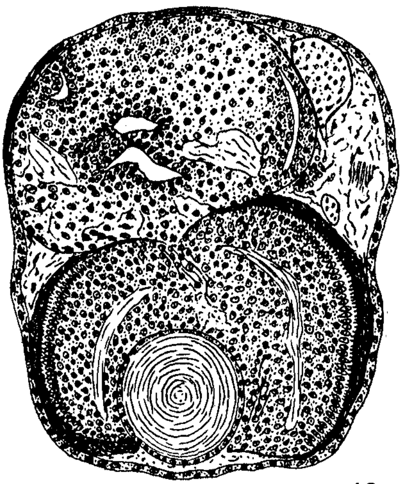
FIG. 11. A section through the eyes of a monster commonly found in the alcohol solutions. The eyes are joined beneath the bilateral brain and face ventrally.

FIG. 12. A section through the eyes of a normal thirteen day embryo.

optic stalk, but the optic nerve fibers grow directly into the brain from the region of the eye cavity itself.

It is interesting to find in this connection that Lewis ('70) describes in his experiments on tadpoles a strikingly similar course pursued by the nerves arising from some of the optic cups he had transplanted to various positions along the hind brain region of the embryos. He states that

In a few of these somewhat irregular transplanted eyes the optic nerve takes a very curious course, passing across the cup cavity from the ganglionic layer, through the pupil and then into the mesenchyme, ending there. In both of these experiments a small bundle of optic nerve fibers pierces the retina as far as the pigment layer. In transplanting these eyes the ganglionic layer was probably injured in such a way as to interfere with the normal path of the nerve fibers, and so they have probably followed the path of least resistance through the pupil and out into the mesenchyme.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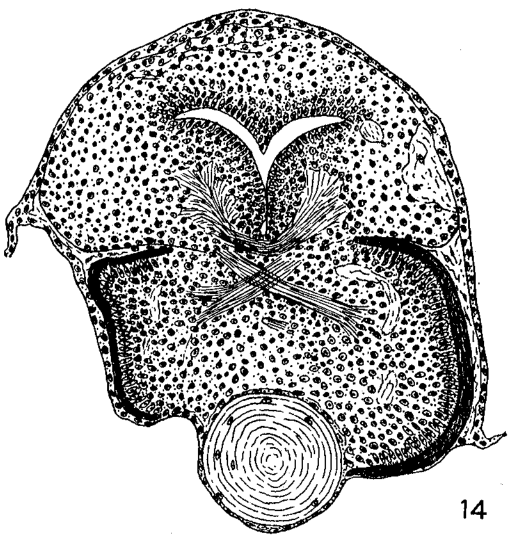
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FIG. 13. A section of the embryo shown in figs. 5 and 6. The optic cups are joined dorsally and face the median plane of the head; a lens lies between them and is surrounded by loose cellular tissue instead of by the humor.

FIG. 14. Section of the eyes in the embryo shown in fig. 4. The optic cups are not joined yet they face towards one another with their convex choroid surfaces pressed close against the head wall. The optic nerves run across what should be the humor chamber of the eyes and out through the wide pupils to form a perfect cross; the optic fibers then enter the brain floor. A lens lies between the eyes.

Lewis' illustrations are similar to Fig. 14 in so far as the course of the optic nerve is concerned. These experiments would seem to indicate that the direction taken by the optic nerve fibers is not firmly fixed but that they may pursue an almost reverse direction from that generally followed. Many of the eyes in the writer's specimens show various conditions of this kind and he must agree with Lewis in the conclusion that

> It would seem to me impossible to explain these various conditions of the optic nerve on any other basis than that they are outgrowths of nerve cells of the ganglionic layer of the retina.

Direct evidence is thus furnished for the outgrowth theory of the nerve fiber which has been so ably supported in the last few years by Harrison's ('08) experiments.

The present experiments warrant the following explanation for incomplete cyclopean eyes, or double eyes, when compared with the usual condition.

In normal development the eye anlagen push out from the ventro-lateral borders of the brain and turn dorsally as indicated in the diagram, fig. 15 A. The abnormal individuals with two eyes facing the median plane also have them more ventrally situated in relation to the brain, and it may be supposed that when the eyes arose from the brain their formation was directed ventrally instead of dorsally, fig. 15 B. This causes the eyes to hang below the brain and face one another as already shown in figs. 4, 5, 6, 13, and 14, instead of turning dorsally and facing outward as in figs, 11 and 12.

Similar conditions are also found in the development of a single eye. Fig. 1 shows an embryo with an eye on the right side only, yet this eye faces the median plane and is unusually ventral in position; it probably arose as indicated in the diagram fig. 15 D, where as other single-eyed individuals, the commoner type, have an eye looking out from the usual lateral position, fig. 15 C.

From these conditions we may determine whether cyclopia is brought about by a failure of certain central tissues of the brain to develop, thus allowing the eye anlagen to come together as Lewis ('09) has suggested, or whether through a lack of developmental energy necessary for the optic cups to grow dorsally and outward to meet the ectoderm as the writer ('09) has supposed. Considering the case of the single eye it might be held that the failure of certain central tissues of the brain to develop would cause the eye to arise too near the median line, but this lack of central tissue does not explain why the eye faces the median plane instead of the lateral head wall, and it is much less able to account for the absence of the eye on the opposite side of the head. On the other hand, if the conditions are due to a lack of the necessary developmental energy or an anæsthesia produced by the experiment, then it is evident that although one eye does succeed in pushing out from the brain it might not have sufficient developmental energy to grow dorsally and outward to the lateral body wall, but droops, as it were, into a more ventro-median position and faces in toward the median plane. Thus one-half of an incomplete cyclopean eye is formed. The other eye was entirely suppressed, lacking the energy necessary to push itself out from the brain. This inequality in the developmental powers of the two eyes is indicated by their frequent asymmetrical condition.

The two eye components do not always face the median plane and in such cases the eyes merely fail to grow out laterally. They come off ventrally from the brain and either face in a ventral direction or grow so as to face outward.

The experiments fail to give any definite clue as to where the optic anlagen are located in the brain before they become visible, although Lewis' operations on the embryonic shields of older embryos would seem to indicate that at that time the anlagen occupied somewhat lateral positions.

It is clear from the foregoing consideration that alcohol has the power to induce the same typical ophthalmic defects that were formerly described in the embryos from the Mg solutions. The property common to both Mg and alcohol is their anæsthetic effect on animals. The writer concludes that cyclopia, monophthalmicum asymmetricum and entire absence of eyes, all of which are more or less arrested or inhibited condition of development, result from anæsthesia during certain embryonic stages. Of course this may not be the sole cause of such defects; on the contrary the fact that they are produced in this way would indicate

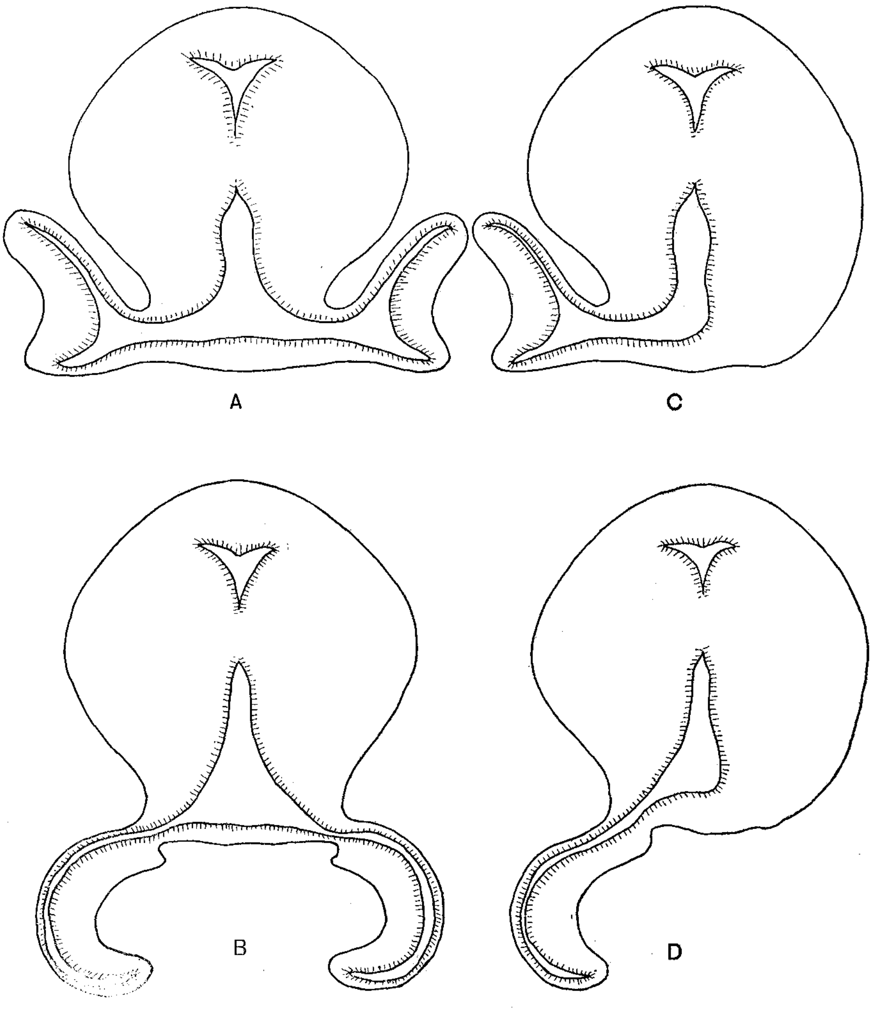


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FIG. 15. A diagram illustrating several positions taken by the optic cups in development. A, the usual case in which both cups push out from the brain and turn dorsally so as to face the lateral head wall with their convex choroid surfaces towards the brain; see fig. 12. *B*, the optic cups push out from the brain but instead of growing dorsally, they hang ventrally and face in towards the median plane, with their convex choroid surfaces against the outer head wall; see figs. 13 and 14. *C* and *D*, the same conditions are often realized when only one optic vesicle arises from the brain.

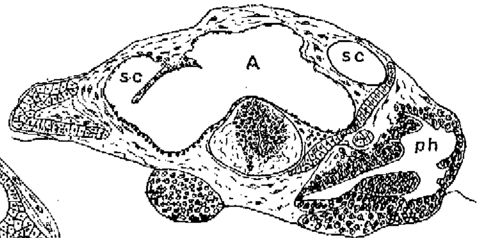
that any factor which might come in during early development to lower the developmental energy could possibly induce similar defects. In mammals such monsters probably arise as the result of some weakening or debilitating influence of the environment during early developmental stages, which need only have acted for a short space of time.

### *b. Defects of the auditory organs*

A very pronounced suppression in the development of the auditory apparatus is often noticed in the embryos which have been treated with weak solutions of alcohol. In many individuals only one ear exists. When this condition is found in an embryo with only one eye, two unequally developed eyes or a cyclopean eye with asymmetrical components, it is of interest to find that in all cases observed, the ear is on the same side of the head as the better formed eye. In rare cases both ears are absent, and again it often happens that the ears are apparently normal while the eyes are deformed. Fig. 16, a horizontal section through the head, shows two small abnormal eyes with a lens between them and two perfect ears with cartilaginous capsules, near the hind brain. Fig. 18, which is a section through the ear region of the embryo shown in fig. 4, illustrates two poorly formed ears; on the right side the ear is small and two semicircular canals are represented only by their ampullæ, the epithelial lining of which forms papillæ of cells with long hair-like processes growing from them as is indicated in the drawing. The left ear is almost entirely absent, its median section showing only the small cavity and ampullary papilla seen in the figure. Both ears, however, are surrounded by well formed cartilaginous capsules.

A remarkably abnormal ear is seen in fig. 17. The auditory vesicles have united so as to occupy a dorsal position above the posterior end of the brain. Only two semicircular canals are developed on each side. The cartilaginous capsules in this case seem unable to meet the situation and extend for only a portion of the way around the huge auditory cavity. This union of the lateral auditory vesicles, although formed by an entirely different principle, suggests the large double cyclopean eye.

The final persistence of the ampulla-like cavities seems to be the rule, these structures being present even when all other portions of the internal ear are absent. The ampullæ of the canals are perhaps particularly useful as organs of equilibration in these animals, and their stuborn persistence may be indicative of an ancient origin and suggests the primary function of the ear as an organ of equilibrium.

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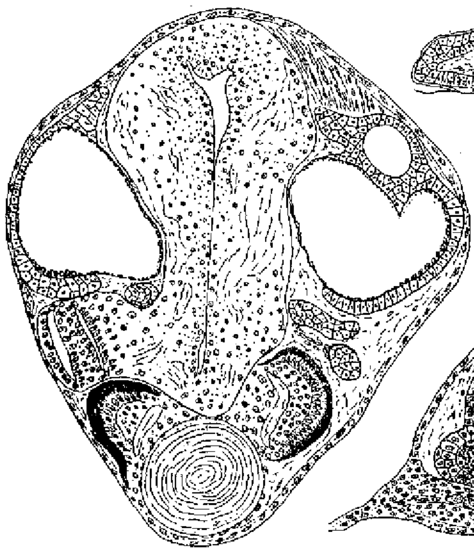

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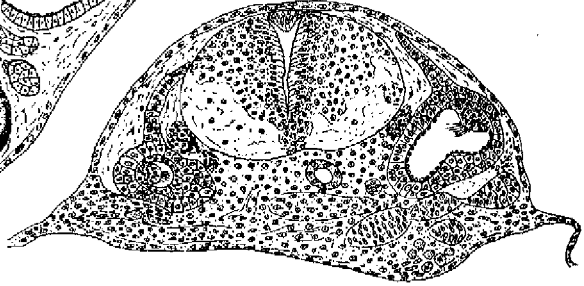

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FIG. 16. A horizontal section through the head of a thirteen day embryo from a 5 per cent alcohol solution. Two very small and defective eyes have alens between them, while more posteriorly two perfectly formed auditory vesicles are seen with cartilaginous capsules surrounding them.

FIG. 17. A section through the auditory region of an eight day embryo treated with a 4 per cent ether solution. The two auditory vesicles unite dorsally to form a huge cavity above the medulla and spinal cord. This embryo also shows an incomplete cyclopean eye and spina-befida. sc, semicircular canal; a, auditory vesicle, ; ph, pharynx.

FIG. 18. A section through the auditory vesicle of the embryo shown in fig. 4 and section fig. 14. The entire auditory vesicle is suppressed except the ampullæ of the semicircular canals. The right ampulla is larger and shows a papilla with hair-like fibers, while the left ampulla is almost completely closed, yet, it too, shows the papilla with projecting hairs. The cartilaginous capsules are small and thus adjusted to the tiny ear parts.

Although all parts of the ear are absent the cartilaginous capsules are present. The shape and size of the capsules, however, seem to be adjusted to that of the auditory vesicle when any part of it exists, as is indicated on the two sides of fig. 18.

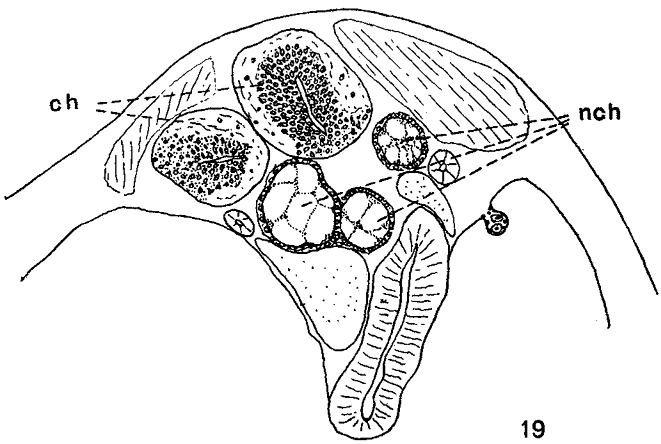
### *c. Defects of the central nervous system*

The abnormalities of the brain shown by the specimens treated with alcohol might easily form the subject of an extensive monograph so various and numerous are they. Only a few of them will be briefly mentioned.

In rare cases the brain is almost normal; the fore brain, however, is usually very narrow and gives to the head a characteristically pointed appearance. Dorsal herniæ at times occur in the region of the optic lobes and the hind brain. The histological structure of the brain is often peculiarly abnormal in both the arrangement and the appearance of the cells. The cells may be hyaline and in the region of the central cavity fail to take the stains. They may even be diffusely scattered in peculiarly defective specimens.

The spinal cord in some individuals also shows the hyaline appearance about its central canal, and spina-bifida in not uncommon. The latter condition no doubt results from the general inhibition in rate of development which is constantly true for the specimens in the alcohol solutions. The germ ring is slow in surrounding the yolk and consequently the trunk region of the early embryo is abbreviated. This condition interferes with the median cell proliferation forming the spinal cord so that a split or divided cord results and may extend for various distances in the trunk region. Fig. 19 shows a section through a trunk region with a double cord, *ch*, the notocord, *nch*, is also divided. Fig. 20 is a more posterior section of the same embryo and shows the cord and notocord again single as they are in more anterior region than that shown by fig. 19.

Many of the defects of the central nervous system are of a general nature and almost any substance that inhibits or interferes with the normal developmental rate may cause them. The writer does not intend to convey the idea that these are characteris-

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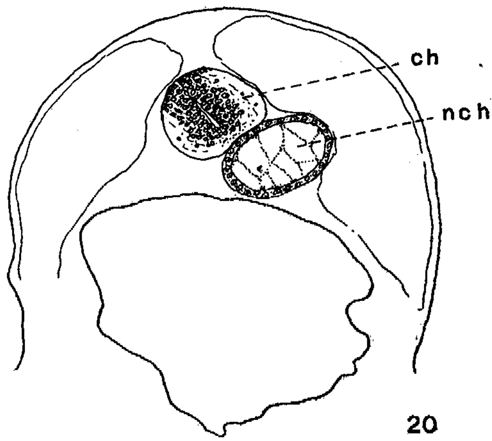


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FIG. 19. A section through the trunk region of an eight day embryo from a 4 per cent ether solution. The spinal cord, **ch**, is double (spina-bifida) and the notochord, **nch**, is divided into three parts.

FIG. 20. A more posterior section of the same embryo, showing the spinal cord and notocord again single as they are in regions more anterior than fig. 19.

tic anæsthetic effects, but they are strikingly common in the alcohol solutions. On the other hand, similar abnormalities of the nervous system are really infrequent in the weaker Mg solutions.

The slight effects of Mg on the development of the central nervous system are interesting when compared with the marked effects of other anæsthetics on these tissues. In this regard it is important to remember that in physiological experiments on nerve-muscle preparations Mayer ('08) has found that Mg acts directly as an inhibitor of muscular activity, exerting little if any effect on the activity of the nervous parts. The action of Mg in these experiments is not particularly on the nervous system but more largely on the dynamic processes concerned in the outpushing of the eyes.

The occurrence of the several ophthalmic anomalies is common to all of the anæsthetic solutions and similar conditions have not been found in embryos treated with various salts and sugars ('06, '07) which may inhibit general development and induce many other abnormalities.

## The Effects of Chloretone, Ether and Chloroform on Development

Chloretone, ether and chloroform when employed in weak solutions influence developing eggs in a way somewhat similar to that described for alcohol. The action of these substances is not so pronounced and may be described more as an inhibition of the general developmental processes. The embryos are usually small and recover very slowly from the inhibiting effects after being returned to sea-water. A few of the individuals in all these solutions exhibit the cyclopean defect in its various degrees just as it has been described in the specimens treated with alcohol and Mg.

The ophthalmic defects, cyclopia, monophthalmicum asymmetricum and entire absence of optic vesicles, are all conditions of arrested or inhibited development and are prevalent among embryos treated with solutions having anæsthetic properties.

The writer is led to conclude, therefore, that his former hypothetical explanation of why cyclopia occurred in embryos treated with Mg solutions was correct. The evidence strongly indicates that the ophthalmic abnormalities produced in these experiments are the result of an anæsthetic action during the early developmental stages.

## THE PERIOD OF DEVELOPMENT AT WHICH CYCLOPIA MAY BE INDUCED BY CHEMICAL AGENTS

The Mg experiments were repeated mainly to ascertain at how late a period in development eggs could be subjected to the solutions and subsequently develop into cyclopean monsters. The original experiments seemed to demonstrate the fact that cyclopia was due to external influences acting on the development of the optic vesicles, and not in any sense to a germinal variation. Nevertheless, H. H. Wilder ('08) in the face of these results, advanced a germinal theory to account for the origin of cyclopia and attempted to explain away the obstacle offered in the experiments referred to by claiming that the eggs were subjected to the solutions at so early a stage in development that germinal variations might still have been induced. This is impossible, as the writer has pointed out elsewhere ('09b), since germinal variations may only be induced before embryonic development has begun. After the two-or four-cell stage (the time at which the eggs were subjected to Mg) is reached any thing done to the egg has its effect on the developing embryo to cause this or that abnormal condition. The only germinal variation possible at such a period would be in the primordial germ cells of the developing individual, a variation which would not manifest itself until the next generation of individuals.

The following experiments prove beyond doubt that cyclopia may be produced by the action of environmental influences.

When eggs in the eight-cell stage, or three and one-half hours after fertilization, are subjected to the action of Mg solutions many of the resulting embryos will show the cyclopean defect.

If eggs be placed in Mg solutions five hours after fertilization, when in the sixteen or thirty-two-cell stage, an almost equally large number of cyclopean individuals will occur. The same is true when they are subjected to the solutions after having developed in pure sea-water for seven and one quarter hours and reached the sixty-four or higher cell stages.

Eggs that have developed eleven hours in pure sea-water and are in the early periblast stage with a somewhat flattened blastodermic cap, may be put into Mg solutions and caused to form cyclopean monsters. The percentage of cyclopean embryos arising from eggs treated at this late period is small, yet even after developing for fifteen hours in pure sea-water some eggs may be induced to form cyclopean monsters by treatment with $MgCl_2$ in sea-water solutions.

Eggs that were older than this before being introduced into the solutions failed to respond, all developing into ordinary two-eyed individuals. This is due to the fact that a considerable amount of time is necessary for the Mg to act upon the body substances of the early embryo and prevent normal eye development. The optic vesicles begin to push out from the brain before the thirtieth hour in development. Thus, after the first six or seven hours, the longer the eggs have been allowed to develop naturally the smaller the proportion of cyclopean individuals that may be artificially induced. After fifteen hours no embryo will be so affected, since an insufficient amount of time exists for the Mg to act on the eye anlagen.

The solutions are effective up to a stage in development preceding the formation of the germ ring and embryonic shield, and the action of the Mg on the eye anlagen probably takes place while the embryonic shield and outline of the embryo are forming.

There can be no further doubt that cyclopean monsters are caused by the action of a strange environment on the developing fish embryos. With such evidence at hand it is also highly probable that mammalian cyclops are due to the action of external influences on the embryo and not to an abnormal germinal tendency.

OTHER CASES OF CYCLOPIA IN FISH

The Italian observer, Paolucci ('74), has described a most remarkable cyclopean ray. The monster was almost adult in size, probably two years old, and measured 47cm. across the pectorial fins and 20cm. in length not including the long whip-like tail. Paolucci states that this cyclopean monster was captured in the Adriatic Sea near the shore and had evidently been able to cope with its surroundings and grow into a vigorous ray. So far as the writer knows this case of a cyclopean monster in nature being able to sustain itself and reach the adult stage, is unique.

Paolucci's specimen proves the correctness of the writer's statement ('09) that the cyclopean eye is not necessarily associated with a single instead of a double brain, or with any other serious defect in the brain region. This fact was clearly shown in the brain structure of many of the cyclopean embryos studied, as well as by their apparently normal behavior after hatching from the egg.

The cyclopean Funduli have been kept living for more than one month, which is as long as the experiment was tried. They would doubtless have lived much longer, as they were hardy and able to obtain an abundance of food from the vegetable particles in the sea-water. Paolucci's observation would indicate that the Fundulus monsters might be reared to maturity and possibly interbreed.

Gemmel ('06) has described four cases of cyclopia in newly hatched trout collected from a fish-hatchery in England. The conditions of the eyes and brains in these monsters are exactly similar to those in the artificially produced Fundulus monsters.

The developing trout's egg demands water of such high purity that trouble is often experienced in the hatcheries, and monstrous embryos commonly occur. These may result from weakened developmental forces due to an insufficient oxygen supply or to the accumulation of injurious chemicals about the eggs.

Gemmel ('06b) in describing cases of supernumerary eyes in the trout embryos records that in one case of an aborted twin head the lens alone of all the eye structures was present. Free lenses were also described and figured in other individuals. Free lenses occur very commonly in heads showing various eye abnormalites; a full consideration of these cases is recorded elsewhere.

## SUMMARY

1. When the eggs of the fish, Fundulus heteroclitus, are subjected during early stages of development to the action of weak solutions of alcohol the resulting embryos show marked abnormalities in the structure of their central nervous system and organs of special sense.

The eyes in such individuals are either both small with poorly differentiated retinæ, cyclopean, asymmetrically monophthalmic or entirely absent. These ophthalmic defects sometimes occur in as many as 98 per cent of the specimens. Such anomalies are closely similar to those previously induced with Mg, and in both cases are probably due to the anæsthetic property of the substances acting upon the eggs.

Alcohol tends to suppress the development and differentiation of the auditory vesicles. A few specimens are entirely without ears, others have one ear more or less perfectly developed while the opposite ear is scarcely formed at all and still other individuals have both ears extremely defective. In all cases examined the better ear is invariably on the side with the better developed eye if the eyes are also asymmetrically formed. The most persistent portion of the internal ear, or that part which exists when all other parts are wanting, is a cavity with an epithelial lining resembling closely in structure an ampulla of the semicircular canals. This fact may be interpreted to mean that the ampulla is one of the most ancient or fundamental parts of the ear, and it might further be considered indicative of the archaic function of the ear as an organ of equilibrium since this is the chief function of the ampullæ.

The brain is usually narrow and pointed in embryos that have been treated with alcohol. It occasionally has a dorsal hernia and shows regions of poor differentiation. The cell arrangement in the spinal cord are abnormal in many cases and spina-bifida

is not infrequent. These conditions of the central nervous system might result from any cause that tends to retard development and are not particularly characteristic of anæsthetic solutions as the eye anomalies are; yet the defects of the central nervous system are commoner in these anæsthetics than in any other solutions with which the embryos have been treated.

2. Chloretone, chloroform and ether induce much the same structural deformities in these embryos as does alcohol. They act, however, as more general anæsthetics, causing a retardation in development. The characteristic eye and ear defects are not nearly so common, though they do occur as a result of treatment with these three substances.

3. The effects of Mg on the developing fish's egg have been previously considered. This substance is even more local in its action than alcohol, the principal defects resulting from its use being various anomalous conditions of the eyes, whereas the nervous system generally may be in many cases structurally normal. The embryos on hatching from the egg are able to swim in the usual manner and live for more than one month in aquaria, which is as long as any effort was made to keep them. The latter fact would seem to indicate that the nervous system also functionates normally.

Magnesium was used to test at how late a period in develop ment the eggs might be introduced into the solutions with the subsequent development of the cyclopean condition. It was found that after normal development had proceeded for two, four, six, eight, ten, eleven, twelve or even fifteen hours, if the eggs were then placed in $MgCl_2$, solutions, many of the resulting embryos showed the cyclopean defect. At fifteen hours the eggs have reached the periblast stage and the blastodem is flattening down upon the yolk. The germ ring arises shortly after this time and begins its downward growth over the yolk mass.

Whenever eggs are allowed to develop beyond the fifteen hour period before being introduced into the solutions of $MgCl_2$ they invariably give rise to normal two-eyed individuals. The occurrence of cyclopia is less frequent when eggs are subjected at later stages than when introduced into the $MgCl_2$ solutions during the four or eight-cell stage. This is doubtless due to the fact that a considerable period of time is necessary for the Mg to act upon the substances of the embryo and influence the origin of the optic vesicles. When an insufficient time intervenes between the period at which the eggs are subjected to the action of the solution and that at which the optic vesicles are given off from the brain the Mg is unable to influence the tissues so as to induce the cyclopean condition.

The production of cyclopia by the action of Mg at such late stages in development proves beyond doubt that this deformity is due to the action of external or environmental conditions on the developing animal. Any explanation of cyclopia based on germinal hypothyses such as that recently advanced by H. H. Wilder must be reconstructed so as to conform to these facts.

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BIBLIOGRAPHY

GEMMEL, J. F. On cyclopia in osseous fishes. Proc. London Zoöl. Society, i, pp.
1906 443–449.
1906b Notes on supernumerary eyes, and local deficiency and reduplication of the notocord in trout embryos. Proc. London Zoöl. Society, i, pp. 449–452.
HARRISON, R. G. Embryonic transplantation and the development of the ner-
1908 vous system. Anat. Record, ii, pp. 385–410.
LEWIS, W. H. Experimental evidence in support of the theory of outgrowth
1907 of the axis cylinder. Am. Jour. Anat. vi, pp. 461–472.
1909 The experimental production of cyclopia in the fish embryo (Fundulus heteroclitus). Anat. Record, iii, pp. 175–181.
MALL, F. P. A study of the causes underlying the origin of human monsters.
1908 Jour. Morph. xix, pp. 1–361.
MAYER, A. G. Rhythmical pulsation in scyphomedusae. Carnegie Institute of
1908 Washington, Pub. No. 102, pp. 113-131.
PAOLUCCI, L. Sopra una Forma Mostruosa della Myliobatis Noctula. Atti della
1874 società Italiana di Sc. Naturali. xvii, pp. 60–63.
STOCKARD, C. R. The development of Fundulus heteroclitus in solutions of lith-
1906 ium chlorid, with appendix on its development in fresh water. Jour. Exp. Zoöl., iii, pp. 99–120.
1907 The influence of external factors, chemical and physical, on the development of Fundulus heteroclitus. Jour. Exp. Zoöl., iv, pp. 165–201.
1909 The development of artificially produced cyclopean fish, "the magnesium embryo." Jour. Exp. Zoöl., vi, pp. 285–338.
1909b The origin of certain types of monsters. Am. Jour. Obstetrics. lix, no. 4.
WILDER, H. H. The morphology of cosmobia. Am. Jour. Anat., viii.pp. 355-
1908 440.