THE BLINDNESS OF THE CAVE FAUNA AND THE ARTIFICIAL PRODUCTION OF BLIND FISH EMBRYOS BY HETEROGENEOUS HYBRIDIZATION AND BY LOW TEMPERATURES. ¹

(WITH 13 FIGURES.)

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I.

While many of the animals inhabiting caves are blind or have degenerated eyes, the same phenomenon is rarely found among animals that live in the open. At first sight this seems to suggest that the disuse of the eyes in the complete darkness of the cave has gradually led to the degeneration of the eyes and this idea seems at one time to have been widely accepted. In forming a judgment of the connection between the darkness of the caves and the blindness of cave dwellers we must remember that some of the cave dwellers have perfectly normal eyes. Thus Eigenmann, to whom we owe the most thorough study of this subject, points out that of the four species of salamanders living habitually in North American caves two have apparently quite normal eyes. They are *Spelerpes maculicauda* and *Spelerpes stejnegeri*. Two others living in caves have quite degenerate eyes, *Typhlotriton spelaeus* and *Typhilomolge rathbuni*.² If disuse is the direct cause of blindness we must inquire why *Spelerpes* is not blind.

Another difficulty arises from the fact that a blind fish, *Typhlogobius*, is found in the open (on the coast of southern California) in shallow water, where it lives under rocks in holes occupied by shrimps. One must again ask the question: How can it happen that in spite of the exposure to light *Typhlogobius* is blind?

The most important fact is perhaps the one found by Eigen-

¹ From the Rockefeller Institute for Medical Research, New York.
mann in the fishes of the family of Amblyopsidæ. Six species
of this group live permanently in caves and are not found in the
open, while one lives permanently in the open and is never
found in caves and one comes from subterranean springs. The
one form which is only found in the open, Chologaster cornutus,
has a simplified retina aside from having a comparatively small
eye, in other words, its eye is not normal. This indicates the
possibility that the other representatives which are only found
in caves also might have abnormal eyes even if they never had
lived in caves.

Through these facts the old idea becomes doubtful, namely,
that the cave animals had originally been animals with normal
eyes in which the disuse had led to a gradual hereditary de-
generation of the eyes. Instead we must consider the possi-
bility that in the blind cave animals as well as in the blind animals
which live in the open the tendency towards blindness developed
independently of presence or absence of light. From this point
of view the tendency toward degeneration of the eye appears
as a hereditary mutation comparable to the inherited glaucoma
which is known in the human. Glaucoma is a form of blindness
caused by the atrophy of the optic nerve in consequence of an
increased intraocular pressure and this high pressure seems to be
duced by a certain disturbance in the circulation in the eye.
The fact that these patients are born with normal eyes and do
not become blind until later in life shows that lack of light has
no share in the development of this hereditary disease or muta-
tion. The question then remains, how can we account under
such an assumption for the fact that blind animals are so preva-
lent in caves and so rare in the open? We shall return to this
question later on.

II.

We will now review briefly the literature dealing with observa-
tions and experiments on the influence of light on the formation
of eyes. The fact that the eyes of mammals are formed in
complete darkness (in the uterus) may serve at the outset as a
warning against overestimating the effect of light on the forma-
tion of eyes. F. Payne¹ has raised sixty-nine generations of a fly

(Drosophila) in complete darkness without noticing any changes in their eyes or in their sensitiveness to light.

Recently Uhlenhuth\(^1\) has demonstrated in a very striking way that the development of the eye does not depend upon the influence of the light or upon its functioning. He transplanted the eyes of young salamanders into different parts of their bodies where they were no longer connected with the optic nerves. The eyes after transplantation underwent a degeneration which was followed by a complete regeneration. Uhlen-

\(^1\) Read at the meeting of Anatomists at St. Louis, December 1914.
apparently due to the interruption of the circulation in the eye and the regeneration probably set in with the reestablishment of the circulation in the transplanted organ.

The older literature had many observations which were assumed to prove an influence of light on development but the writer has shown in a former paper that these conclusions were not supported by the facts on which they rested.

The writer has for years paid special attention to the possibility that light might influence the formation of organs in animals, but he has succeeded in finding only one form in which such an influence can with certainty be demonstrated, namely the hydroid *Eudendrium*. In his experiments in Naples he had noticed that the polyps of this form regenerate better in the light than in the dark and in Woods Hole he could convince himself that the stems of *Eudendrium* cannot regenerate their polyps if kept permanently in the dark.²

Kammerer seems to be the only author who still takes it for granted that cave animals owe the degeneration of their eyes to lack of light, and his support for this view consists in the statement that five young cave salamanders (Proteus) developed larger eyes under certain (somewhat puzzling) conditions of illumination. In other cases the eyes of Proteus remained unaltered in the light. It would be advisable to make certain whether or not there are two varieties of Proteus and moreover it would be desirable to repeat these experiments on a larger scale.

The writer wishes to publish in this note the results of some experiments made in 1912, which prove that in the fish Fundulus it is comparatively easy to produce embryos with degenerated eyes by various means except by the one Kammerer holds responsible for this phenomenon, namely lack of light. The reader may in passing be reminded of the fact that in this form Stockard and later McClendon induced the formation of cyclo-

pean eyes by altering the constitution of the sea water (addition of magnesium salts or of alcohols and other narcotics.)

The writer's attention to an abnormal development of the eyes in this embryo was first attracted in his experiments on heterogeneous hybridization. He noticed that among the anomalies noticeable in heterogeneous fish hybrids the lack of circulation was the most common, but that incomplete or abnormal development of the eyes was not infrequent. Such embryos often give the impression that they have no eyes at all, though in reality a histological analysis would probably show that some of the organs of the eye are present. But it is safe to say that the condition of the eyes is such as to make them unfit to form a retina image and for this reason we may call these embryos with abnormal (or apparently lacking) eyes blind; in the same sense in which this term is used in the case of the blind salamanders or

fish found in caves which also have some of the elements of an eye but in a condition unfit for use. The term “blind fish” is therefore used in the following as synonym with imperfect or degenerated eyes.

III.

Blindness Produced through Heterogeneous Crossings.

Figs. 1 and 2 are camera drawings of hybrids between *Fundulus heteroclitus* ♀ and *Menidia* ♂. The first embryo is eight, the second fourteen days old. In neither of these two embryos are eyes noticeable, though of course a histological examination might have divulged rudimentary eyes. The embryos are in this respect to all appearances comparable to the blind cave fish or salamanders.

It is a known fact that certain blind animals have in an early stage a comparatively better developed eye than in later stages, *i.e.*, that the eye either stops developing early while the development of the rest of the body continues or that the eyes degenerate.
directly. The writer noticed not infrequently that the eyes of the cross between Fundulus and Menidia were normal in the beginning but appeared more abnormal the further the development progressed. We will illustrate this statement by two series of drawings. The first case is illustrated by Figs. 3 to 6. Fig. 3 shows the embryo four days old. The embryo has normally developed eyes with lenses. The embryo’s heart, which is visible in front, was not yet beating and no pigment was formed. The same embryo is shown two days later in Fig. 4. The eyes instead of developing any further are less distinct. Fig. 5 is the same embryo two days later (eight days old). The eyes are hardly recognizable, it is difficult to decide without sectioning whether the lens still exists. The internal ear is distinguishable. The heart is pulsating.

Fig. 6 shows the same embryo at the age of two weeks. Apparently the pigment is the only organ of the eye which is developed.
Figs. 7 and 8 illustrate the same fact, that a rather normal eye with a lens was formed while later on only a very imperfect eye remained. Fig. 7 shows the embryo when four days old with a lens and an apparently normal eye, while five days later (Fig. 8) no eye was perceptible.

IV.

BLIND FISH PRODUCED FROM PURE BREEDS OF FUNDULUS HETEROCLITUS.

The writer was under the impression that the abnormalities in the development of the heterogeneous hybrids in fish were due to the influence of the foreign sperm as in the similar case of the fertilization of the egg of the sea urchin with the sperm of the star fish, where the mortality is also enormous. In order to prove this assumption he tried to find a method which would allow him to produce embryos with abnormal eyes in the pure breeds of heteroclitus. He has already mentioned in a former publication that such abnormal embryos were obtained when
their development was retarded by putting them into sea water to which some KCN was added. Fig. 9 shows a blind embryo obtained in this way. The eggs were put immediately after fertilization into a weaker solution of KCN in sea water where they remained sixteen days. They developed slowly. The embryo was drawn two days after it had been put back into normal sea water. The heart-beat and circulation were established. This method of producing blind embryos is not reliable and need not be discussed any further.

A better method was found by exposing the newly fertilized eggs to a low temperature (from between 0 to 2°C) for some time. The writer found that the egg of Fundulus can be put for weeks into such a low temperature after the embryo is once formed, without any injury to the latter. As soon as it is put back to room temperature it continues to develop. This corresponds with the idea that the low temperature only retards the chemical reactions underlying development. If, however, the
egg of the same fish is exposed to such a low temperature immediately after fertilization or when the eggs are in the process of early segmentation they suffer severely.

Thus in one experiment the eggs of *Fundulus* (fertilized with sperm of their own species) were distributed into a number of Erlenmeyer flasks and put on ice immediately after fertilization and kept at a temperature of between 0 and 2° C. After four

Fig. 9.

hours, seven and a half hours, twenty-three hours, thirty-two hours, forty-eight hours, sixty-four, and ninety-six hours one lot was returned to room temperature. All the eggs taken out after forty-eight hours or later were dead; few of those taken out after thirty-two and twenty-three hours survived. A considerable percentage were abnormal and resembled the heterogeneous hybrids between *heteroclitus* ♀ and *Menidia* ♂. Most of those taken out after four and seven and a half hours survived, but about 20 per cent. to 30 per cent. were abnormal. Among the abnormal embryos a number of blind fish were found.
A second lot of eggs were put on ice four and a half hours after fertilization when they were in the 4-cell stage or beyond. These eggs were just as sensitive to the effect of the low temperature as those put on ice immediately after fertilization.

This experiment was repeated four times with the same result. In another experiment the eggs were allowed to develop to about the 128-cell stage at normal temperature before they were put into a temperature of 0° (about fifteen hours after fertilization). Eggs that were kept at that low temperature for two days were still able to develop into normal embryos but those that had been kept three days at the temperature of from 0 to 2° C. were practically all dead.

When the eggs were put into a temperature of from 0 to 2° C., after the embryo was formed and the circulation established, they could resist the low temperature for weeks. When put back to normal temperature they recovered and developed normally.

Further experiments are required to ascertain more accurately when the eggs become immune to the temperature of from 0° to 2°.
It is only this very low temperature at or very near the freezing point which is injurious for the newly fertilized eggs. If the temperature is a little higher, e.g., 7°C, the newly fertilized eggs can live for weeks in it without being injured. Thus in the experiment just mentioned some of the eggs were put immediately after fertilization into a temperature of about 7°C. These eggs developed very slowly but no abnormal embryos were observed although some of the eggs were kept at a temperature of 7°C for four weeks. Those that were kept still longer at that temperature suffered but probably not from the low temperature but from the fact that the flasks in which they were kept were closed.

These experiments thus establish the interesting fact that immediately after fertilization the eggs of Fundulus are rapidly injured or killed when exposed to a temperature of 0°C or a little above, while after the embryo is formed they can be exposed to
such a temperature for a month without suffering. The newly fertilized eggs can, however, be exposed without injury to a temperature of 7° for weeks and possibly longer.

As already stated, among those eggs which were put into a temperature of from 0 to 2° C. a certain number had defective eyes. Thus the egg Fig. 10 was put immediately after fertilization into a temperature of between 0 and 2° for twenty-four hours and then put back to room temperature. The egg developed like a typical heterogeneous hybrid. Fig. 10 represents the embryo when two weeks old; it had a beating heart but no circulation. No eyes are noticeable. Fig. 11 represents an embryo from another experiment; in this too the egg had been kept for four hours (immediately after fertilization) on ice (0 to 2°). Apparently no eyes are formed but the circulation is established.

Figs. 12 and 13 are added to show to what extent the abnormal embryos, produced by a short exposure of the egg to a very low temperature (0°) immediately after fertilization, resemble the monstrosities which are formed in the case of heterogeneous
hybridization. The embryo in Fig. 12 consisted practically only in a pulsating heart, what was left of the embryo was much less conspicuous than it is in the drawing. The embryo was ten days old. The egg had been put into the temperature of between 0 and 2° when it was in the 2-cell stage and had remained there for twenty-four hours.

The remnants of the embryo Fig. 13 had the same history. In this case, curiously enough, one eye with a lens and the tail was all that could be recognized. The monstrosity when drawn was eleven days old. An inexperienced observer might easily have concluded that in this case only the eye had been developed, while in reality the whole embryo had developed but the eye had survived while other parts perished.

The analogy with teratomata is obvious.
V.

The writer was anxious to know whether it was possible to produce deficiencies in the eyes of Fundulus by raising them in the dark. It was necessary to carry on such an experiment with a large number of embryos. Since it was possible that a short exposure of the unfertilized egg or of the sperm to the light might already have some effect, the females and males of Fundulus heteroclitus to be used for the experiment were put into an absolutely dark room where everything was prepared for the experiment. The females and males were stripped of their sexual cells in the dark and the jars containing both sperm and eggs were put into dark boxes and kept in the dark room for four weeks. Although many embryos had died, hundreds had survived. All had perfectly normal eyes. This experiment confirms similar experiments made by the writer in previous years.

All these experiments show that while it is comparatively easy to produce blind Fundulus embryos, or, more correctly speaking, fish with degenerated eyes, by heterogeneous hybridization or by low temperature or by lack of oxygen (or by an excess of magnesium salts or by alcohol, as shown by Stockard and McClendon) no such result can be produced by lack of light.

VI.

If we consider all the facts in the case there is nothing at present to warrant the assumption that the blind cave animals owe the deficient development of their eyes to the lack of light, since lack of light is according to our present knowledge a less efficient agency in the causation of an abnormal development of the eye than a number of other injurious influences. Under these conditions we must be prepared to consider the possibility that many if not all the blind species found in caves owe their blindness to other influences than those of the cave. Eigenmann states that no blood vessels enter the eye of the blind cave salamander Typhlotriton. Since the experiments of Uhlenhuth as well as those of Stockard and of the writer reported in this paper indicate the importance of the circulation and of chemical factors in the development of the eyes it is not impossible that in the blind fish (Amblyopsidæ) as well as in the blind sala-
manders a hereditary disturbance in the circulation or nutrition of the eye or its surroundings is the cause of the degeneration of the eyes. There remains then the one difficulty mentioned in the beginning, namely to account for the fact that the relative number of blind species is greater in caves than in the open.

Eigenmann has shown that all those forms which live in caves were adapted to life in the dark before they entered the cave. These animals are all negatively heliotropic and positively stereotropistic and with these tropisms they would be forced to enter the cave whenever they are put at the entrance to the cave. Even those among the Amblyopsidæ which live in the open have those tropisms of the cave dweller. This eliminates the idea that the cave adapted the animals for the life in the dark.

Eigenmann's observation makes it also clear that blind animals are comparatively rare in the open and that animals with normal eyes are not in the majority in the caves. Only those animals can thrive in the caves which for their feeding and mating do not depend upon visual mechanisms; and conversely animals which are not provided with visual mechanisms can only under exceptional conditions hold their own in the open where they meet the competition of animals which can see. This would account for the fact that in caves blind species are comparatively more prevalent than in the open.

In spite of all this, Eigenmann is inclined to assume that the darkness of the caves was a factor in promoting the blindness of the cave fauna. While those Amblyopsidæ which live in the open have already abnormal eyes those species of the family which are found in the caves have more degenerated eyes than those in the open and Eigenmann is inclined to ascribe this fact to an accumulated influence of the darkness. This would, however, compel us to account in a similar way for the incipient degeneracy of the eyes of those Amblyopsidæ that have never been found in caves and for the complete blindness of Typhlogobius; and would leave us at a loss to account for the presence of salamanders with perfectly normal eyes in the caves. It seems to the writer that consistency would demand to consider a common mode of origin of all these blind forms, namely as mutations, i. e., as the result of some factorial change in the germ the cause and nature of which we are not yet able to define. Among the
Amblyopsidae various mutations in the state of the eye may be constantly arising (as in the case of Drosophila); and some of these mutants, especially those that are perfectly blind, can not hold their own in the open while in the caves they can preserve and perpetuate themselves. This assumption would not exclude the possibility that Kammerer's observation may have been correctly interpreted by him, it would only provide for the possibility that his conclusion can not be generalized—a possibility which from the experiments mentioned in this paper must be seriously considered.

SUMMARY.

1. It is shown that blind embryos or more correctly embryos with degenerated eyes can be produced by heterogeneous hybridization in fish embryos (e.g., Fundulus heteroclitus ♀ and Menidia ♂). Since in these cases as a rule no circulation exists the inference is possible that the anomalous condition of the eye may be due to lack of circulation.

2. Blind embryos of the pure breed of Fundulus heteroclitus may be produced by the addition of KCN to the sea water.

3. It is shown that immediately after fertilization (by sperm of their own species) and during the early stages of segmentation the egg of Fundulus heteroclitus is rapidly killed or injured if it is exposed to a constant temperature of about 0° (or slightly above); while it may be exposed to a slightly higher temperature (e.g., 7° C.) for weeks without being injured. If the egg is exposed to the low temperature after the embryo is once formed it can resist the low temperature of from 0 to 2° C. for weeks without permanent injurious effects.

4. If eggs of Fundulus heteroclitus are fertilized with the sperm of the same species and exposed immediately after fertilization for a number of hours or a day to a temperature of between 0 and 2° C. abnormal embryos can be produced a certain percentage of which may show degenerated eyes.

5. Lack of light does not influence the development of the eyes of Fundulus.

6. It is pointed out that internal mutational changes and not lack of light may account for the blindness of certain cave fish and salamanders.

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DOI: https://doi.org/10.2307/1536260
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