Experimental Studies on the Eye of the Frog Embryo.

By

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With Plate VI.

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It was shown by Bonnet (3) in 1780, that if the greater portion of the eye of *Triton* is removed and only a small piece remains attached to the optic nerve, a new eye will regenerate in the course of a few months. This result was confirmed later by the experiments of Blumenbach (2), Philippeaux (13), and Colucci (4). At the present time, interest in experimental work on the eye of amphibians seems to be concerned principally with the origin and manner of formation of a new lens after complete or partial extirpation. Much careful work has already been done along this line, noticeably by Wolff (20—22) and by Fischel (5—7) both of whom have worked on the lens formation in the eye of *Triton* which seems to be a very favorable subject for such investigations.

The experiments recorded in the present paper were begun in the spring of 1900; but before any definite conclusions had been reached, Spemann (18) published the results of a small series of experiments on young embryos of *Rana fusca.* In these experiments Spemann destroyed one side of the head before the medullary folds had closed in order to ascertain whether the various processes in the development of the eye are correlated or not. From his results, Spemann concludes that an optic vesicle can change into an optic cup when the influence of the lens is barred out, and that the formation of a lens is dependent on contact between the epidermis and the optic cup: the optic cup, it appears, must reach a definite grade of development before a lens arises, and no lens forms before this time. The first of SPEMANN's conclusions, i. e. that the optic vesicle can change into an optic cup without the presence of a lens, I can fully confirm; but my results do not support SPEMANN's other conclusions as will be shown later.

I. Method.

Embryos of *Rana palustris* were used for all experiments, and, with the exception of those described in Section II, they were operated upon at the stage of development when the brain vesicles are well defined and the medullary folds are beginning to unite. In making the operation, the embryo was transferred to a piece of moist filter paper placed on the stage of a dissecting microscope, and then a puncture was made with a heated needle on one side of the head in the region of the fore-brain from which the optic vesicle would normally develop.

The operation is a severe one, and about one-half of the 800 tadpoles operated upon died within twenty-four hours as a direct result of the injury. Of the embryos that survived the operation, nearly one-fourth, when killed and sectioned, showed perfectly normal eyes on both sides of the head; in the remaining embryos either an eye was lacking on the injured side of the head or, if one formed, it was more or less abnormal in shape and position. This lack of uniformity in the results is due, in part at least, to the fact that it is impossible to heat the needle at uniformly the same temperature in all of the operations, and also to the fact that the puncture did not always injure exactly the same region of the fore-brain.

Removing the entire eye from an embryo in which the lens and cornea have already formed is a comparatively simple operation and one that is rarely followed by fatal results if sufficient care is taken during the operation not to injure the surrounding tissues. If a heated needle is inserted into the centre of the optic cup, the tissues adhere to its surface and the entire eye can be withdrawn at once. The tadpole very soon recovers from the shock of the operation, and the injured surface is entirely healed over in the course of two or three days.

II. The Effects of a Total Extirpation of the Eye of a Tadpole After the Formation of the Lens and the Cornea.

Experiments made by PHILIPPEAUX and by COLUCCI have shown conclusively that the adults of various species of amphibians are not able to regenerate an eye after the total extirpation of the old one. Amphibian embryos, however, have a much greater power of regeneration than have the adults, and it might be possible that the embryonic tissues of the head would possess the power to regenerate a new eye, although this power is totally lacking at a later period of development. To test this point the eye was removed from a number of tadpoles measuring about 12 mm. in length. Only seven out of total of 125 tadpoles that were operated upon died in the course of the week following the operation, and in these cases the brain had been injured when the eye was removed, as one side of the head was greatly distorted after the wound had healed. Beginning about a month after the operation and continuing for a number of weeks, a few of the embryos were killed at intervals of five days, although there was no external evidence of an eye on the injured side of the head in any case. Serial sections through the heads of these embryos failed to show, in a single instance, the slightest indication of a regeneration of the missing eye. The outer ectoderm, which had completely covered the injured region in the course of 2—3 days after the operation, appeared to be of the same thickness and the cells seemed to have the same general character as those on the uninjured side of the body. The region of the head from which the eye had been removed was always found to be filled with loose mesenchyme tissue. Occasionally a small amount of pigment was present. This pigment had either been introduced from the outer ectoderm when the operation was made, or, possibly, it was a part of the pigmented coat of the eye that had remained behind when the eye was removed. In most of these embryos the tissues of the head, even those which had been in close contact with the extirpated eye, showed not the slightest trace of the operation, although in a few cases the forebrain appeared somewhat distorted and its wall was thinner than normal on the injured side.

The results of this series of experiments on amphibian embryos are in accord with those obtained by other investigators on adult forms: in neither case is it possible to obtain the regeneration of an eye after total extirpation. As a complete optic cup and a lens can regenerate from a very small fragment of the eye, it is evident that the eye tissues are able to produce any part of the eye that may be lacking: the other tissues of the head, however, do not possess this power which is as completely lacking in the embryo as in the adult.

III. The Effects of Totally Destroying the Eye-forming Region of the Fore-brain before the Closure of the Medullary Folds.

If the portion of the fore-brain that normally produces an optic vesicle is totally destroyed before the medullary folds have closed, have the other parts of the brain or any other tissues of the head the power to produce an eye and thus restore the normal form of the embryo? This was one of the problems I had in mind when these experiments were begun.

Many of the young embryos in which the fore-brain was punctured before the closure of the medullary folds showed no traces of an eye on the injured side of the head, although, in some cases, they were kept alive for three weeks after the operation. In every instance there was a well developed optic cup on the uninjured side of the head, and a lens had formed or was in the process of formation from the ectoderm. In nearly all of these embryos the wall of the fore-brain was much thinner than normal on the injured side, in some instances being only one or two rows of cells in thickness. The ectoderm covering the wounded surface seemed perfectly normal in all cases and the region in which the eye would normally have developed was filled with a large number of mesenchyme cells. Usually some scattered pigment granules were also present as was found in the experiments in which an entire eye was removed from an older embryo (Section II).

It is evident that, in these cases, all of the eye-forming tissue on one side of the head was either destroyed by the operation or rendered incapable of development and that no other tissue of the head has the power to produce an eye.

IV. The Development of a Normal Eye After a Puncture of the Fore-brain.

A number of embryos operated upon before the closure of the medullary folds developed perfectly normal eyes on both sides of the head. In some cases the effects of the operation were to be seen in an unusual thinness of the wall of the fore-brain; in other cases the brain no longer occupied its normal position in the median plane of the body but was drawn over towards the injured side, doubtless as a result of the way in which the wounded surface healed.

It is probable that, in these embryos, the region of the brain affected by the operation lay in front of or at the side of the eye-forming region; therefore, as the eye-forming tissue was not injured, the development of the eye could proceed in a perfectly normal manner in spite of the injury. In all of these embryos the eyes maintained their normal relations with respect to the surrounding tissues, and both eyes of an embryo were usually in the same stage of development, although in some few instances the lens on the uninjured side of the body was in a slightly later stage of development than was the one on the side that had been punctured.

V. The Formation of an Optic Cup without the Formation of a Lens, or without any Connection between the Eye-forming Tissue and the Brain.

If only a portion of the brain tissue that normally forms the optic vesicle is destroyed by the heated needle, the remaining tissue continues its development, somewhat slowly, and eventually becomes a more or less normal optic cup regardless of its location or of its connection with the rest of the brain. Fig. 1 shows a very extreme case of this kind. The tadpole from which this drawing was made was operated upon the second of April, 1902, and was killed four days later. Three days after the operation this embryo attracted attention because it was the only one of the 27 embryos in the series that did not show an eye on either side of the head. It was somewhat less developed than the other tadpoles in the series at this time and it swam very feebly and in an irregular manner, resting on one side when it reached the bottom of the dish in which it was kept. A complete series of sections through the head of this embryo shows the presence of but a single eye (Fig. 1, *O.C.*) which was not visible through the deeply pigmented ectoderm of the body wall because it occupies a very abnormal position between the floor of the fore-brain (Fig. 1, *F.B.*) and the roof of the pharynx (Fig. 1, *Ph.*). This eye, which is fairly normal in shape, is in the optic cup stage of development, and it measures 0.3 mm. in diameter through the line *XY*, thus being approximately the same size as the normal eyes found in other embryos of the same age. No lens is present in any section of the eye of this embryo, and in no place is there a thickening of the inner layer of ectoderm that might possibly be considered an early stage in the formation of a lens. The eye is entirely surrounded by mesodermal tissue and there is no trace of an optic nerve. It is evident, therefore, that this eye has developed through the stage of the optic vesicle to that of the optic cup without connection with the brain and without the presence of a lens.

The very abnormal position of this optic cup can doubtless be attributed to the fact that in the operation a portion, or perhaps all, of the eye-forming tissue of the fore-brain was separated from the rest of the brain and yet not greatly injured. In the process of healing, this eye-forming tissue came to occupy a position between the brain and the pharynx and here it continued to develop as long as the embryo lived. It is obvious that an eye in this location can be of absolutely no use to the organism, no matter if is should continue it development and should become an entirely normal structure. The formation of such an eye seems inexplicable unless we believe with SPEMANN (18), »daß in der Medullarplatte die einzelnen Teile des Augenbechers schon bestimmt sind«, and, therefore, that the portion of the fore-brain that normally forms the eye is so specialized that it will develop into an eye so matter what the surrounding conditions may be. Apparently the power of self-differentiation must be conceded to eye-forming tissue as it is capable, under certain conditions, of developing into a fairly normal structure when entirely removed from its normal relations with the other structures of the head (Fig. 1).

Fig. 2 shows an outline drawing of a section through the eye region of an embryo killed four days after the puncture of the left side of the fore-brain. The brain itself shows no signs of the operation, and on the right side of the head there is a normal eye with a well developed lens. On the left of the fore-brain is an optic cup, absolutely normal in shape and in the structure of its cells, yet its greatest diameter through the line AB is but 0.20 mm., while that of the normal eye through the line CD is 0.35 mm. There is no sign of a lens in connection with this small optic cup, nor is there any thickening of the ectoderm in the vicinity of the eye that might be considered to be an early stage in the formation of a lens. This embryo shows the power of regulation possessed by the organism, as the portion of the eye-forming region of the fore-brain remaining after the operation did not produce a fragment of an optic cup of usual size but developed into a very small optic cup of normal shape. I can see no reason why this eye could not have become functional had a lens developed later in connection with it.

A portion of a section through the head region of an embryo operated upon the nineteenth of April, 1903, and killed six days later is shown in Fig. 3. On the left side of the head, which was uninjured, there is a normal eye with a lens already completely separated from the ectoderm. The right eye, which is shown in Fig. 3, comes directly from the fore-brain whose wall on this side is formed of only one to two layers of small cubical cells. This eye has presumably reached the stage of the optic cup although its shape is very unlike that of a normal eye at the same period of development. The upper edge of the optic cup is in direct contact with the inner layer of ectoderm; but in no section of the head is there any indication of a thickening of the ectoderm to form a lens. If, as maintained by some investigators, the formation of a lens is dependent on contact between the optic cup and the ectoderm, it is not clear why a lens has failed to form in this case as the normal eye on the opposite side of the head developed a lens at least three days before the embryo was killed. It is, of course, obvious that the right eye in its present condition is so distorted that a lens would be useless even if it had developed; but, as will be shown later, many eyes almost, if not quite, as abnormal as this one have a well developed lens and, therefore, the abnormality of the eye alone should not have prevented the formation of a lens. It may be possible that the embryo was killed before the eye on the injured side had reached the proper stage of development for it to be able to cause the formation of a lens from the ectoderm; but it is, of course, impossible to determine this point definitely.

In five other embryos I have found a well developed optic cup on the injured side of the head that had no connection whatever with any part of the brain. The conditions in these embryos, however, were not as abnormal as were those in the embryo shown in Fig. 1, as in each case there was a perfectly normal eye on the uninjured side of the head, and the eye that had formed on the punctured side, although it was entirely separated from the brain, was in its normal position in the head and had the opening of the cup turned towards the exterior of the body. In three embryos a lens had developed from the ectoderm and occupied its usual position in the eye; in the other two embryos no lens had developed on the injured side when the embryos were killed. The development of the eye on the injured side of the head always lagged behind that of the normal eye, although apparently taking place in the usual manner.

In one of his experiment on *Hyla viridis* SCHAPER (15) cut off the anterior part of the head of an embryo in such a way that the right eye was entirely removed while the left one remained in its normal position. When this embryo was killed, eleven days after the operation, it was found that the fore-brain was entirely lacking and that there had been no regeneration of the missing eye. The left eye was somewhat smaller than the eye of a normal embryo at the same stage of development, although it appeared in every other way to be perfectly normal, and it had a well developed lens. The interesting fact about this eye is that it had no optic nerve and, consequently, it had absolutely no connection with the portion of the brain remaining after the operation. In discussing the result of this experiment, SCHAPER states: »Wir können nicht umhin anzunehmen, dass während der vorliegenden Entwickelungsperiode funktionelle Reize auf Wachsthum und Differenzirung des erhaltenen Auges keinerlei Einfluss ausgeübt haben: seine Weiterentwickelung geschah lediglich nach den Principien der Selbstdifferenzirung.« This principle of »self-differentiation« is the only one apparently, that is adequate to explain the development of an optic cup in such cases as the one described by SCHAPER and in those of my own experiments in which an optic cup developed without any connection with the brain.

The results of the experiments in this series confirm the conclusion of SPEMANN and of RABL (14) that an optic cup can develop from an optic vesicle when there is no lens present. To this conclusion may, perhaps, be added that the change from an optic vesicle into an optic cup is likewise independent of any connection with the brain and that it is the direct result of a process of self-differentiation in the eye-forming tissue of the fore-brain.

VI. The Formation of a Lens from the Ectoderm in Connection with the Development of an Optic Cup.

An eye that develops in an embryo of *Rana palustris* after a puncture of the eye-forming region of the fore-brain usually possesses a lens which is more or less normal in structure. As presumably the operation destroyed that portion of the ectoderm that would normally produce a lens, the question arises as to whether there is a definite region of the ectoderm that alone is capable of producing a lens, or whether any portion of the ectoderm of the head has the power to form a lens provided it receives the proper stimulus. It is possible also that, under certain conditions, a lens can form from the edge of the optic cup, as in *Triton*, and thus enable an eye which can not obtain a lens from the ectoderm to become functional when it has reached the proper stage of development.

In making the operations for this series of experiments, the punctures were intentionally made in different regions of the fore-brain in order to produce as many kinds of abnormal eyes as possible. This was done in the hope that it might be possible to determine the extent of the power possessed by the optic cup as well as by the ectoderm of the different regions of the head to form a lens.

After operations of this character in which it is impossible to determine the extent of the injury until the embryos are killed and sectioned, the results are rarely found to be uniform and it is not possible to draw any general conclusions from a small number of experiments. The cases that are described in this sections were selected from over 150 embryos in which the eye on the injured side of the head was more or less abnormal. That so few of the embryos gave results from which conclusions could be drawn can be attributed either to the fact that a lens had not yet developed in connection with the eye on the injured side of the head when the tadpole was killed (although it had always formed in a perfectly normal manner on the uninjured side); or to the fact that a lens had already developed so far when the embryo was killed that it was not possible to determine its origin. The chances of being able to determine the origin of the lens on the injured side of the head are most favorable if the embryo is killed from three to four days after the operation.

Fig. 4 shows a portion of a section through the head of an embryo that was operated upon the thirty-first of March 1903, and killed four days later. The embryo, therefore, lived two days less than the one from which Fig. 3 was drawn and presumably its organs were correspondingly less developed. Sections through the head of the embryo showed, on the uninjured side, a normal eye with its lens entirely separated from the ectoderm; on the injured side of the head, as shown in Fig. 4, there projects from the base of the fore-brain a very abnormal optic cup. The inner layer of this eye is a nearly straight mass of cubical cells which curves but slightly at its upper edge where it joins the outer pigmented layer. Although this optic cup is fully as abnormal as the one shown in Fig. 3, yet it has a well formed lens (Fig. 4, *L*) entirely separated from the ectoderm and in contact with the upper edge of the cup. The lens, which was probably derived from the ectoderm in a normal manner, is very much smaller than the one on the opposite side of the head as it measures but 0.046 mm in diameter, while the lens of the normal eye measures 0.107 mm. in diameter. In this embryo it is not probable that the edge of the abnormal optic cup ever came in contact with the ectoderm, and yet a lens has developed from the ectoderm although the conditions for its formation were apparently not as favorable as they were in the case of the eye shown in Fig. 3.

An outline drawing of a section through the eye region of an embryo killed five days after the puncture of the fore-brain is shown in Fig. 5. On the right side of the head is a normal eye with its lens completely separated from the ectoderm. On the left side of the head, the fore-brain is connected through an optic stalk with a very abnormal eye whose structure in greater detail is shown in Fig. 6. This eye is exceedingly small, its diameter through the line *AB*, Fig. 5, being 0.116 mm., while that of the normal eye through the line *CD*, Fig. 5, is 0.334 mm. The outer edge of the optic cup is 0.071 mm. away from the inner layer of ectoderm, yet there projects from the latter, opposite the opening of the cup, a structure that unquestionably must be considered as an early stage in the formation of a lens. Instead of developing in a normal way, this lens, which measures 0,025 mm. through the line *XY*, Fig. 6, is a somewhat rounded mass of cubical cells attached by a thick stalk of similar cells to the inner layer of ectoderm, the whole structure extending 0,088 mm. from the ectoderm. Another section of this eye shows the lens lying very close to the upper edge of the optic cup and containing a central cavity. As the embryo was killed before the lens had broken away from its attachment to the ectoderm there can be no question of it its origin and manner of formation.

In all cases in which it is possible to determine definitely the origin of the lens found in connection with an abnormal eye, it is certain that the lens is derived from a thickening of the ectoderm. Very frequently the abnormal eye lies some distance behind the plane of the normal eye on the opposite side of the head and yet the ectoderm of this region of the head is as able to produce a lens as is that more anterior. In some cases, as in Fig. 9, a lens may form from the ectoderm near the upper edge of the optic cup; in other cases the lens may develop near the lower edge of the cup (Fig. 7); in still other embryos (Fig. 8) there may be a thickening of a considerable portion of the ectoderm in the vicinity of the optic cup. In the eye show in Fig. 8, the thickening for a lens occupies all of the region in front of the opening of the optic cup for several sections, and as the eye is much further back in the head than is the normal eye on the uninjured side, the ectoderm in front of it, could not possibly be that which would normally produce the lens.

The recent experiments of Lewis (10) have shown conclusively that not only is the possibility of lens formation present in all of the ectoderm of the head, but also that the ectoderm from other portions of the body, if grafted over an optic cup, will develop into a lens. The power to form a lens must, therefore, be conceded to all of the body ectoderm, and is not to be limited to the descendents of the cells of the sense plate as believed by Schaper (16).

It is stated by Rabl that »die Ursache für die Entstehung der Linse in einem formativen Reize zu suchen, der von dem freien Ende der Augenblase auf die Berührungsstelle mit dem Ektoderm ausgeübt wird«. This opinion is also held by Spemann (18) and by Lewis. The latter investigator states that »epithelial cells which normally give rise to a lens do not do so when the optic vesicle fails to come in contact with them for a sufficient length of time or at the proper stage«. From the results obtained in some of the above experiments it seems to me certain that contact between the optic cup and the ectoderm is not the only stimulus that is capable of causing the development of a lens. In the case of the eye shown in Fig. 6, the edge of the optic cup could never have come in contact with the ectoderm, and yet a lens is forming, unquestionably, from a thickening of the inner ectoderm opposite the eye. In some of his experiments, Lewis also obtained a lens from the ectoderm when the optic cup was imbedded some distance in the head and in such cases the lens always developed on a pedicle in a way similar to that shown in Fig. 6. Lewis explains these cases as follows: »A long epithelial process was probably formed at the time of the healing of the operation wound and thus gave the deeply placed eye a chance for contact with the ectoderm«. It seems to me rather remarkable that this long epithelial process, purely an accidental result of the operation, should invariably be formed directly opposite the opening of optic cup, and that it is never found in cases in which the optic cup is close to or in contact with the ectoderm. Such an explanation does not seem to me adequate in the case shown in Fig. 6, or in others of the same nature which I have obtained. In my experiments the character of the operation was such that the portion of the ectoderm in contact with the heated needle was usually destroyed and, even if it was shoved inside the head, it would have been rendered incapable of development. In all of the hundreds of tadpoles that I have sectioned after such operations I have never found a similar projection of ectoderm in the injured region of the head unless it was directly opposite an optic cup and was forming a lens. In some cases the pedicle of the lens was long, in others short, depending entirely on the distance between the optic cup and the ectoderm. Contact between the optic cup and the ectoderm, on the other hand, does not necessarily lead to the formation of a lens. In the case of the eye shown in Fig. 3, the upper edge of the optic cup is in direct contact with the inner ectoderm and yet there is no sign of lens although one has developed on the opposite side of the head. In some instances Lewis failed to obtain a lens when the optic cup was in contact with the ectoderm, and he considers this to be due either to the fact that the optic cup had not been in contact with the ectoderm for a sufficiently long period of time or that it had not reached the proper stage of development for it to be able to cause the formation of a lens. Contact between the optic cup and the ectoderm may, in some cases, furnish the stimulus that calls forth the formation of a lens; but that it is not the only stimulus that can produce this result is evident from such cases as that shown in Fig. 6. Whether the stimulus for lens production always comes from the optic cup, when one it present, or whether it may come from the nervous system or from some other source I have not been able to determine.

VII. The Formation of a Lens-like Structure from the Ectoderm without the Presence of an Optic Cup.

That a lens-like structure can develop from the inner ectoderm when an optic cup is totally lacking on the injured side of the head is shown by the two following cases selected from seven of the same general character that were obtained during the course of these experiments. Part of a section through the head of one of these embryos is shown in Fig. 9. On the left side of the head, which was uninjured, the eye is somewhat abnormal, and it has a lens which has not yet separated completely from the ectoderm. On the right side of the head there is absolutely no sign of an optic cup, and the wall of the fore-brain is much thinner than normal, thus showing the severity of the operation. On the injured side, directly opposite the place where the lens is forming for the normal eye, there is a pronounced thickening of the inner ectoderm (Fig. 9, *L*). This thickening of the ectoderm, which is shown more highly magnified in Fig. 10, extends over four sections of the head and is like the lens forming on the opposite side of the head except that it lacks a central cavity and is somewhat smaller, measuring but 0.034 mm. in diameter while the lens on the opposite side of the head measures 0.053 mm. in diameter.

Another case in which a lens-like structure formed without the presence of the optic cup is shown in Fig. 11. In this instance the structure is somewhat larger than that shown in Fig. 10, as it measures 0.075 mm. in diameter through the line *XY* Fig. 11, and is precisely similar in structure to the lens for the normal eye of an embryo at the same stage of development. In this case, also, an optic cup was entirely lacking on the injured side of the head, although a perfectly normal eye was present on the opposite side of the head and its lens was still connected with the ectoderm, being but a little more developed than the one shown in Fig. 9.

The other cases I have found in which an optic cup was lacking and yet a lens-like structure was developing from the ectoderm are very similar to those shown in Figs. 9—11. Unfortunately, I have no older stages that show whether, under these abnormal conditions, this structure would continue to develop and eventually separate from the ectoderm.

These results apparently furnish evidence in favor of the view that in a definite region of the ectoderm the cells are destined to form a lens and, therefore, that they will produce such a structure even if an optic cup is lacking. That a lens should develop under such abnormal conditions is not more surprising than is the fact that a portion of the eye-forming region of the fore-brain will continue its development and form a fairly normal optic cup (as shown in Fig. 1) even when it is removed from all of its normal relations, cut off entirely from all connection with the brain, and surrounded by mesenchyme tissue. It seems evident that the power of self-differentiation is possessed in a remarkable degree by those tissues in the head of the frog embryo which normally form the optic cup and the lens; and each of these structures can apparently develop, to a certain extent at least, absolutely independently of the other. The change of an optic vesicle into an optic cup and of the ectodermal cells into a lens, is due, apparently, not as much to correlative differentiation as to unknown causes inherent in the cells themselves.

The formation of a lens from the ectoderm without the presence of an optic cup has, as far as I am aware, been found as yet by but one investigator, MENCL (11), who has described such a case in a very abnormal *Salmo salar* with two heads. One head appeared perfectly normal; but the other showed no eyes externally. When the latter head was sectioned, there was found absolutely no sign of an optic cup on either side of the head, although there were lenses on both sides, the left one being more dorsal and situated somewhat further back than the one on the right. Both lenses were fairly normal in structure, although the left one was somewhat smaller than the right and was still connected with the ectoderm from which it originated without question. From this case MENCL states that we must admit that the lens of the eye can arise independently of the formation of the optic cup, and he adds further: »Die Hirnaugen mit epidermalen Linsen treten bei den Wirbelthieren als eine neue, ausschließlich ihnen gehörende Erwerbung auf, und sie bilden sich im Bereiche eines bestimmten Kopfsegmentes; die ganze diesem Segmente zugetheilte Epidermis ist Träger einer gewissen Tendenz, die darin besteht, im Verlaufe einer gewissen Entwickelungsstufe die Linse zu bilden. Wenn auch die Augenblasenbildung völlig ausbleibt, was in einzelnen anomalen Fällen zu Stande kommt, so werden doch die Augenlinsen, obzwar zwecklos, gebildet. Der diese zwecklose, wie durch Erinnerung der Epidermiszellen auftauchende Linsenbildung auslösende Faktor ist die Vererbung.«

In the figures given in MENCL's paper showing the location of the two lenses, there is very little space between the brain and the body ectoderm, and on one side the lens lies in a well defined depression of the brain wall. SPEMANN (19) has offered a very different explanation of the formation of these lenses from that given by MENCL. He believes that there is only an apparent lack of the optic cups, the wall of the brain near the lenses in his estimation »nichts anderes ist, als die nicht abgegliederte und außerdem nachträglich rückgebildete Retina«. There is absolutely nothing in the structure of the brain wall in the figures shown by MENCL that would indicate that it is a degenerate retina. If we accept SPEMANN's explanation, then MENCL's idea of the self-differentiation of the lens is, of course, valueless.

The results of the experiments on embryos of *Rana palustris* in which a lens-like structure developed without the presence of an optic cup appear to support MENCL's position. In the case shown in Fig. 9, the brain wall on the injured side, which is much thinner than normal, is 0.233 mm. away from the ectoderm in the region where the lens-like structure has formed, and all of the cells of which it is composed are apparently similar in structure. There is not the slightest reason, as far as I can see, for assuming that the wall of the brain opposite the place where this structure is forming represents the retina, and, even if it did, it is so far from the ectoderm that it would seem that it could have no influence whatever on the lens formation. It appears that the power of self-differentiation must be granted to lens-forming cells of the ectoderm in the embryo of *Rana palustris*, and it seem probable that a stimulus similar to that which is capable of causing the formation of lens-like structures in the ectoderm of the frog embryo when an optic cup its lacking might also have acted to produce the lens-like structures in the *Salmo salar* described by MENCL.

VIII. The Formation of a Lens from the Edge of the Optic Cup.

In all of the embryos so far described, and in a large number of others where abnormal eyes were formed, the lens, if it developed at all, was unquestionably derived from a thickening of the inner layer of ectoderm as is the case in normal development. In *Triton* and in others of the Urodela where the lens has been extirpated from the eye of a young or of an adult animal, a new lens arises in the course of a few weeks or months, not from the ectoderm, but from the iris. The manner of its formation in *Triton* according to WOLFF (20) is as follows: »Das innere epitheliale Blatt der Iris verliert sein Pigment, welches von massenhaft herbeieilenden Leukocyten fortgetragen wird, am Pupillarrande wuchert das Epithel, aus diesen Wucherungen entsteht am oberen Rand der Pupille ein Linsensäckchen, und aus diesem Linsensäckchen bildet sich — nunmehr nach vererbtem Typus — die vollständig normale Tritonenlinse.« It appears also, from the recent experiments of BARFURTH and DRAGENDORFF (1) that a new lens can be formed from the edge of the optic cup in the young chick embryo, although the power to produce a lens seems to be entirely lacking in the eye of the adult bird.

The experiments of MÜLLER (12) and also those of FISCHEL (5—7) have confirmed the earlier work of COLUCCI and of WOLFF regarding the origin of the lens from the edge of the iris in the eye of *Triton*. FISCHEL has also shown that if the iris is intentionally injured in several places more than one lens may be formed; and that not only any part of the iris is capable of forming a lens, but also that lens-like structures can be produced from the retina.

The eye of the Urodela is thus found to be capable of regenerating a lost lens, but instead of being produced from a thickening of the ectoderm as one might expect would be the case from the course of the normal development of the lens in ontogeny, the new lens arises from the iris of the eye and generally from its upper edge. The reason for the formation of the lens at the upper edge of the iris was first considered to be due to the action of gravity on the eye, but an ingenious experiment made by WOLFF (21) has shown that the new lens regenerates from the upper edge of the iris even when the animal is kept constantly upon its back and therefore gravity does not determine it location. According to WOLFF it is »nicht das Fehlen, sondern der Verlust der Linse, nicht die einzige auslösende Ursache, sondern eine wesentliche Vorbedingung für die Regeneration zu bilden scheint«. FISCHEL, on the other hand, believes that »die auslösende Ursache liegt vielmehr stets in der durch die Operation bewirkten Reizung der Iris«.

In explanation of the formation of the new lens in the eye of *Triton* from the iris rather than from the ectoderm, SCHIMKEWITSCH (17) has stated that: »Man darf nicht vergessen, dass die Linse der paarigen Augen eine spätere Bildung ist, und dass es sehr wahrscheinlich ist, dass ursprünglich die paarigen Augen eine ebensolche Linse besaßen, wie wir sie im unpaaren Auge der *Hatteria* sehen,

d. h. hervorgegangen aus der Wand der Augenblase selbst.« The formation of the lens from the edge of the iris, on this assumption, is merely a return to ancestral conditions and might, if true, very possibly be met with occasionally in the course of the normal development of an individual.

In his most recent paper, FISCHEL (7) has stated that the formation of a lens from the edge of the iris is easily explained if one but assume, »dass die Fähigkeit zur Linsenbildung nicht allein einem ganz bestimmten Gebiete des Ektoderms, sondern ursprünglich allen (mindestens in einem bestimmten Körperabschnitt) Zellen dieses Keimblattes zukommt. Es ist klar, dass diese Annahme auch durch die hier mitgetheilten Thatsachen eine gewisse Stütze erhält: entstammen doch die Zellen der Iris und Retina, deren Fähigkeit zur Linsenfaserdifferenzirung erwiesen wurde, gleichfalls dem Ektoderm. Fasst man diese Annahme in die Form, dass ursprünglich allen Zellen des Ektoderms die Fähigkeit zukommt, sich in Linsenfasern, bezw. in linsenfaserartige Elemente umzuwandeln, und dass diese Fähigkeit auch auf gewisse Derivate des Ektoderms — centrales Nervensystem und Augenbecher — übergeht, so wird man sowohl den Thatsachen der normalen Ontogenese (Entwickelung der Linse im Auge und im Parietalorgan), als auch denen der Regeneration (Neubildung der Linse von der Iris, Lentoide der Retina) gerecht«.

In the ontogeny of the Anura, as far as is known, the lens of the eye always arises as in the Urodela from a thickening of the inner layer of ectoderm. KOCHS (9) has shown that in the tadpole of *Rana fusca* a new lens regenerates after the extirpation of the old one, but he was not able to determine its origin. In the experiments made by SPEMANN and by LEWIS the new lens was invariably derived from the ectoderm of the body wall.

Among the embryos with abnormal eyes that were obtained in the course of the present experiments, there were two which seem to furnish evidence that in the Anura, as well as in the Urodela, a lens may occasionally develop from the edge of the optic cup. Fig. 12 is an outline drawing of a part of a section through the head of an embryo that was operated upon the nineteenth of April, 1902, and killed six days later. The embryo was sectioned somewhat obliquely and, therefore, but a very small, pigmented portion of the normal eye is shown on the right of the mid-brain (Fig. 12, *N.E.*); the rest of this eye with its well developed lens and optic nerve lies in sections anterior to the one drawn. The brain of the embryo appears normal and shows not the slightest trace of the operation. Interest in this tadpole centers in the eye on the left side of the body which lies quite close to the mid-brain and 0.316 mm. from the body wall. This optic cup, which is somewhat abnormal, is connected by an optic nerve with the brain in a more anterior section than that shown, the connection being slightly behind that of the normal eye. The structure of this eye is shown more in detail in Fig. 13. The inner layer of the optic cup is composed, as normally, of several rows of cubical cells and the outer layer is formed of a single row of pigmented cells. The lower portion of the cup, as is frequently the case in these abnormal eyes, is not much curved and lies nearly parallel with the wall of the mid-brain. At the upper edge of the cup, where the two layers unite, there is a knob-like structure that bears a striking resemblance to an early stage in the formation of a lens in the eye of *Triton* as figured by WOLFF and by FISCHEL. This rounded projection from the centre of the upper wall of the optic cup extends over four sections of the embryo, and on either side of it the two layers of the optic cup are joined in the normal manner. There is no central cavity in any section of this structure and no indication of a constriction between it and the two layers of the optic cup which run into it. The cells forming this structure are dividing very rapidly and each section shows a large number of karyokinetic figures, many more than I have found in any other part of the eye in any of the embryos that I have studied. In no section of the head of this embryo is there any indication of a thickening of the ectoderm to form a lens, and, judging from the results that have been obtained in the experiments on the eye of *Triton*, I can see no reason why this projection from the upper wall of the optic cup must not be considered as an early stage in the formation of a lens from the tissues of the eye itself.

Fig. 14 shows a portion of a section through the head of another embryo from the same series as that from which Fig. 12 was drawn. The position of the lens in this eye makes it appear highly probable that the lens developed from the edge of the optic cup, although, of course, this cannot be proven. The development of this eye was much more rapid than was that from which Fig. 12 was drawn, and the lens had already become a separate structure at the opening of the optic cup when the embryo was killed. In Fig. 14 the lens is seen to be sharply marked off from the edge of optic cup; but in other section of the eye the flattened surface of the lens lies against the somewhat flattened edge of the optic cup and it might very easily be supposed that the two were connected. Such close contact between the edge of the optic cup and the lens is not usual when the lens is derived from the ectoderm in the normal manner. In this embryo the optic cup lies 0.113 mm. beneath the ectoderm, and its upper edge is so greatly curved that it does not seem as if the lens could possibly have been derived from the ectoderm in the manner shown in Fig. 6. The normal eye on the opposite side of the head is not shown in the figure because the section is somewhat oblique and also because the eye on the injured side lies considerably behind that on the opposite side of the body. The effects of the operation are further shown in this embryo in the formation of the brain wall which is very much thinner than normal on the injured side. The structure of the abnormal eye is shown under higher magnification in Fig. 15. The cells of both layers of the optic cup appear normal and the shape of the cup itself is not as abnormal as is the one shown in Fig. 13. There can be no question but that the rounded structure in the opening of the optic cup is a lens which has a central cavity rather large in proportion to the size of the lens. The lens is somewhat smaller than the one connected with the normal eye on the uninjured side of the body as it measures but 0.060 mm. in diameter while the one connected with the normal eye measures 0.107 mm. It is much to be regretted that transitional stages between the lenses represented in Figs. 13 and 15 could not be obtained.

While it seems to me very probable, from the two cases described above, that the lens in the eye of the frog embryo is capable, under certain conditions, of developing from the edge of the optic cup, I do not wish to state positively that such is the case. It is conceivable, of course, that such a knob-like thickening at the upper edge of the optic cup as that shown in Fig. 12, would never have formed a normal lens, and also that the lens shown in Fig. 14 was derived from the ectoderm, although I can find no indication whatever that such was its origin. Taking the evidence from these two embryos alone it seems to me highly probable that, under certain conditions, the upper edge of the optic cup is capable of beginning the formation of a lens. Whether such a lens would continue to develop and eventually become functional remains to be determined.

IX. Summary.

1) If an entire eye is removed from an embryo of *Rana palustris*, the tissues of the head are incapable of regenerating a new one.

2) There is no development of an eye if all of the eye-forming region of the fore-brain is destroyed before the medullary folds have closed.

3) Destroying any part of the fore-brain except the eye-forming region does not interfer with the normal development of the eye.

4) The eye-forming tissue of the fore-brain appears to have the power of self-differentiation as it will develop into a more or less normal optic cup when removed from its connection with the rest of the brain and without the presence of a lens.

5) Any ectoderm of the head appears to have the power to produce a lens provided it receives the proper stimulus.

6) Contact between the optic cup and the ectoderm is not necessarily the stimulus that leads to the development of a lens, as a lens can be formed from the ectoderm when the optic cup is some distance beneath the surface of the body.

7) A lens-like structure may be formed from the ectoderm when there is no optic cup on the same side of the head. The process appears to be a self-differentiation of lens-forming tissue of the ectoderm.

8) It seems probably that, under certain conditions, a lens can develop from the upper edge of the optic cup as is the case in the regeneration of the lens in the eye of *Triton*.

Bryn Mawr College, Bryn Mawr, Pa., Nov. 11, 1904.

Zusammenfassung.

1) Beraubt man einen Embryo von *Rana palustris* eines ganzen Auges, so sind die Gewebe des Kopfes nicht imstande, ein neues zu bilden.

2) Es kommt nicht zur Entwicklung eines Auges, wenn der gesamte augenbildende Bezirk des Vorderhirns vor dem Schluß der Medullarwülste zertört wird.

3) Die Zerstörung des Vorderhirns bis auf den augenbildenden Bezirk hindert die normale Entwicklung des Auges nicht.

4) Das augenbildende Gewebe des Vorderhirns scheint die Fähigkeit der Selbstdifferenzierung zu besitzen, da es auch außer Zusammenhang mit dem übrigen Gehirn und ohne das Vorhandensein einer Linse sich zu einem mehr oder weniger normalen Augenbecher entwickeln kann.

5) Den spezifischen Reiz vorausgesetzt scheint jede Partie des Kopfectoderms die Fähigkeit der Linsenentwicklung zu besitzen.

6) Die Berührung des Augenbechers mit dem Ectoderm ist nicht unumgänglich notwendig als Reiz für die Linsenentwicklung, da eine Linse auch entstehen kann, wenn der Augenbecher noch in einiger Entfernung unter der Körperoberfläche liegt.

7) Eine linsenähnliche Struktur kann vom Ektoderm hervorgebracht werden, ohne daß ein Augenbecher auf derselben Seite existiert. Der Prozeß scheint eine Selbstdifferenzierung von linsenbildendem Gewebe aus dem Ectoderm zu sein.

8) Es erscheint möglich, daß sich unter gewissen Bedingungen eine Linse aus dem oberen Rande des Augenbechers zu entwickeln vermag, wie dies bei der Linsenregeneration von *Triton* der Fall ist.

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Explanation of Figures.

Plate VI.

All figures were outlined with the aid of a camera lucida. Figs. 1, 2, 5, 7, 9, 12, and 14 were drawn under a ZEISS obj. AA, oc. 4; the other figures were drawn under a ZEISS obj. D, oc. 4.

In lettering the figures the following abbreviations are used: *F.B.* fore-brain; *M.B.* mid-brain; *L.* lens; *N.E.* normal eye; *Ph.* pharynx; *S.* sucker; *O.C.* optic cup.

Fig. 1. Section through the head of an embryo killed four days after the puncture of one side of the fore-brain. An optic cup has developed between the brain and the upper wall of the pharynx.

Fig. 2. Part of a section through the head of an embryo killed four days after the operation. A small optic cup, but no lens, has developed on the injured side of the body.

Fig. 3. Part of a section through the head of an embryo killed six days after the operation. The optic cup that has formed on the injured side of the head is in direct contact with the inner ectoderm but no lens has developed.

Fig. 4. Part of a section showing the abnormal optic cup in an embryo killed five days after the puncture of the fore-brain.

Fig. 5. Outline drawing of a part of a section through the head of an embryo killed six days after the operation. The optic cup is some distance from the ectoderm, a lens is forming in an unusual manner.

Fig. 6. Enlarged drawing of the abnormal eye shown in Fig. 5.

Fig. 7. Section through the head of an embryo killed six after the puncture of the fore-brain. A lens is forming from the ectoderm opposite the lower edge of the optic cup.

Fig. 8. Part of a section through the head of an embryo belonging to the same series as that from which Fig. 7 was drawn. All of the inner layer of ectoderm opposite the optic cup is thickening to form a lens.

Fig. 9. Outline drawing of a part of a section through the head of an embryo killed three days after the puncture of the fore-brain. A lens-like structure is forming from the inner layer of ectoderm without the presence of an optic cup.

Fig. 10. Enlarged drawing of the lens-like structure shown in outline in Fig. 9.

Fig. 11. Enlarged drawing of another, slightly larger, lens-like structure that developed from the ectoderm without the presence of an optic cup.

Fig. 12. Part of a section of an embryo killed six days after the operation. There is a knob-like thickening at the upper edge of the optic cup which may be an early stage in the formation of a lens.

Fig. 13. Enlarged drawing of the optic cup shown in outline in Fig. 12.

Fig. 14. Part of a section through the head of an embryo belonging to the same series as that from which Fig. 12 was drawn. A lens is present which presumable developed from the edge of the optic cup.

Fig. 15. Enlarged drawing of the optic cup and lens shown in outline in Fig. 14.