

EXPERIMENTS ON LOCALIZATION AND REGENERATION IN THE EMBRYONIC SHIELD AND GERM RING OF A TELEOST FISH (*FUNDULUS HETEROCLITUS*)¹

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SEVENTEEN FIGURES

From experimental studies² on a teleost egg (*Fundulus heteroclitus*) we came to the conclusion that there was no definite localization of organ forming substances in either the germ disc or the blastodisc. Normal but usually smaller embryos develop from the germ disc or blastodisc after the extirpation of various portions of the protoplasm. With the formation of the germ ring and the embryonic shield from the blastodisc a very pronounced change occurs and we find by a very similar method of experimentation that there is a very definite localization and almost complete lack of regenerative power in the embryonic shield. This changed condition appears with the formation of the axis of the embryonic shield, and the contrast with the previous condition is very remarkable. It is evidently associated in some manner with the development of the axial keel or central nervous system. The axial keel when first recognizable under the binocular microscope appears as a highly refractive point in the median line of the early embryonic shield. It elongates with the elongation of the shield, always extending the entire length of the median line of the shield. At or near the posterior end is located the growing point or node of the axis. The extirpation of the entire keel at any stage of the embryonic shield prevents any farther development of the embryo.

¹ These experiments were done at the Marine Biological Laboratory, Woods Hole, Mass.

² W. H. Lewis, Experiments on localization in the eggs of a teleost fish (*Fundulus heteroclitus*). *Anat. Rec.*, vol. 6, no. 1, 1912.

The method used was similar to that employed in the experimenting on the germdisc and blastodisc. The fish were stripped, the eggs fertilized and kept in sea water at room temperature. In experimenting the egg is held by a fine pair of forceps with sufficient pressure to prevent the yolk from turning within the vitelline membrane and then a fine needle is thrust through the vitelline membrane into the embryonic shield at the point desired and as the needle is withdrawn, pressure with the forceps sends out a stream of cells from the injured region leaving a wound, the size and position of which can be readily seen with the binocular microscope. The eggs thus operated upon were kept for days (eight to twenty-four) in small dishes, some hatching, others unable to do so.

Many experiments were done on the embryonic shields of various stages and but a small fraction of them are given in this paper.

Operations on the early embryonic shield

If the central or axial portion of the shield is removed, as shown in figure 1, development stops and the embryo dies. The material in the lateral portions of the embryonic shield and in the germring is apparently incapable of replacing that in and near the median line. At this stage the axial keel makes its first appearance and it is the removal of this structure which determines the result. Unless the axial material is entirely removed, abnormal embryos may result in which various parts are wanting. Sumner³ did a somewhat similar series of operations with the electric cautery on 96 eggs, 78 died and 10 showed, thirty-three hours afterwards, normal embryonic shields. He says "The entire embryonic region (so far as visible) of the early blastoderm may be destroyed by the cautery and an apparently normal embryonic shield may arise by a process of regeneration." In describing these experiments Sumner also states that at this stage he had great difficulty in distinguishing just where the embryonic region of the germring was situated, so he was evidently operating upon an earlier stage than that dealt with in series 1 of my experiments. I have already pointed out that various parts of the blastoderm may be removed without interference with the development of normal but smaller embryos.

³ Arch. f. Entwickl. Mech., Bd. 17, pp. 42-149, 1904.

Sumner's operations were probably on stages where the transition from a totipotence to an axial localization was in progress and it is not surprising that 10 out of 96 eggs operated upon should not have differentiated beyond the totipotent stage of the blastodisc, consequently normal embryos are to be expected.

If the posterior end of the embryonic shield is taken out, as shown in figure 2, the head end of the embryo will develop in a normal manner while the posterior portion of the embryo including the body and tail may be quite rudimentary or entirely wanting. The continued growth of the germring over the yolk and the closure of the blastopore takes place but the germring is apparently unable to contribute, after the operation, to the formation of a body or tail. The head end which develops is fairly normal in size and shape, its size depending upon the amount of tissue in the antero-posterior axis of the shield that was not removed during the operation. Sumner likewise found that the destruction of the region near the posterior end of the embryonic shield prevented the development of the posterior part of the embryo.

In another series of experiments the anterior end of of the early embryonic shields was removed (fig. 3), with the resulting failure of development of the head end of the embryo while the body and tail developed normally up to about the time of hatching. The size of the body and tail likewise varies with the amount of tissue of the axis not removed during the operation.

In still another series of experiments a small area from the middle of the axis of the embryonic shield was removed, as shown in figure 4, with the resulting absence or rudimentary development of the posterior part of the head and cervical regions. The extent of the non-development in this region seems to vary directly with the amount and position of the tissue removed from the axis. The head and tail ends of the embryo seem to go on developing in a normal manner or nearly so though they may be separated from each other by a considerable distance. Such embryos may live for many days until after the time for the normal ones to hatch, though they themselves do not hatch.

If material is removed from the lateral region of such early embryonic shields, that is, to one side of the axial keel (fig. 5), there

is neither interference with normal development nor absence of any part of the embryo.

In still another series of experiments at this early stage of the embryonic shield, the germring was removed just lateral to the shield (fig. 6). In some of the experiments as much as one-fifth or one-fourth of the ring was removed on one side or the other. In a few minutes the gap closes and the yolk becomes more or less constricted along the region of the germring as the two ends of the wound come together, the amount of constriction varying with the amount of germring removed. The smaller germring evidently exerts considerable tension on the yolk. The loss of substance from the germring does not interfere with the formation of perfectly normal embryos as regards size and form. Morgan cut the germring and likewise got perfectly normal embryos. The removal of such large sections of the germring with the subsequent formation of a normal embryo would seem to indicate that the germring neither contained any especial organ forming substances nor is essential to the formation of the embryo.

Not only may a section of the germring be removed from one side of the embryonic shield but segments of the germring from both sides of the same shield may be removed without interference with the development of a normal embryo (fig. 7).

These various experiments show that concrescence plays very little or no part in the formation of the teleost embryo. The material which forms or directs the formation of the embryo is early concentrated along the axis of the embryonic shield, while the germring merely gives rise to the cells which form the covering to the yolk. This early concentration of the material for the embryo may perhaps be some sort of an abbreviated form of concrescence.

Sumner's⁴ experiments 12, 22, 29, so far as indicating that the germring passes continuously into the embryo, would seem to me to indicate just the opposite, namely, that the germring probably does not contribute to the formation of the embryo body. The fact that the needle and the axis of the body approach each other is just exactly what one would expect to find taking place as the germring

⁴ Op. cit.

decreases in circumference and the blastopore becomes smaller and smaller. The material in the germring is utilized in covering the yolk but otherwise there is no proof whatever of its contributing to the embryo. We are as fully justified, and perhaps more so, in claiming that the embryonic shield gives off cells to the germring as that the germring supplies material to the embryonic shield. The growing point of the embryo is at or near the posterior end of the axis of the embryonic shield and the material for the extension of the embryo is elaborated here in the primitive streak.

Operations on older stages of the embryonic shield

Operations on the older stages of the shield give results similar to those on the earlier stages. Removal of tissue from the anterior end of the shield produces various malformations of the head and often complete absence of that region of the embryo, (fig. 8, 8a, 9, 9a). The removal of small amounts of tissue at the tip of the shield often results in cyclopean monsters with varying degrees of fusion of the two eye rudiments, or the partial absence of one eye or of both eyes.⁵ Likewise removal of tissue from the caudal end of the shield results in varying degrees of non-development of the caudal end of the embryo (figs. 10, 10a, 11, 11a).

As the embryonic shield elongates the extirpations of about the same amount of material from the posterior end of the axis gives rise to smaller defects in that part of the resulting embryo. This is exactly what would be expected if at or near the posterior end of the axis is located the growing point or node. There is of course continued differentiation and expansion along the entire axis of the shield as shown by the actual size of the extirpated area as compared with the much larger defects in the resulting abnormal embryos.

Just how far and how minutely predetermination and localization exist in the embryonic shield can only be determined by a more elaborate series of experiments. In experiment 15 (figs. 15, 15a) for example, the extirpation of a small amount of tissue at one side and extending into but not across the axis, resulted in producing

⁵ W. H. Lewis, The experimental production of cyclopia in the fish embryo (*Fundulus heteroclitus*). *Anat. Rec.*, vol. 3, no. 4, 1909.

an embryo which hatched and was able to swim abnormally. The brain on the side of the extirpated tissue was defective and the otocyst on that side was completely absent. Scarcely any two of the abnormal embryos are alike, they all show varying degrees of defectiveness. This is exactly what one would expect if there exists along the axis a definite localization, since it is almost impossible to plunge the needle into exactly the same point each time or to press out exactly the same amount of tissue even if the needle were plunged into the same point in a series of experiments.

In the older stages likewise, the removal of small areas along the median line of the shield gives rise to various kinds of defective embryos. If only a little of the superficial tissue is removed the resulting defect in the embryo is not very deep (figs. 12, 12a, 13, 13a, 15, 15a). After deeper and more extensive extirpation, the anterior and caudal portions of the embryo may be completely separated (figs. 14, 14a) yet they continue to develop in a fairly normal manner. As in operations on the anterior or posterior ends of the embryonic shield, the size of the defect varies with the amount of material extirpated and inversely with the length of the embryo. One may remove two or more such areas across the axis with the resulting development of three or four quite separate or partially separated segments of the embryo (figs. 16, 16a, 13, 13a). There is apparently no regeneration at all of the axial region of the embryonic shield and each individual region possesses great power of independent self-differentiation and this quite independently of the circulation, for in those experiments where the head end is separated from the caudal end of the embryo there is usually no circulation in the latter.

Removal of portions of the germring during any stage of the development of the embryonic shield apparently has no effect on the growth and development of the embryo.

Although the entire rudiment of the heart and head may be removed as in such experiments as 8, 9 (figs. 8, 9, 8a, 9a) the blood vessels in the posterior portion of the body and even in the tail, develop and fill with red blood corpuscles. The capillaries likewise often develop over the yolk sac. There is a striking difference in the pattern which such capillaries form on the yolk sac in the

embryos without circulation as compared with the normal pattern. Although in the former the capillaries contain red blood corpuscles the capillary net work is very irregular and the capillaries themselves differ greatly in size. In the normal the mesh work is elongated and the capillaries are of about uniform size (figs. 17, 17a) The pigment cells over the yolk do not present the characteristic normal pattern in the abnormal embryos without circulation but are scattered irregularly like those observed by Loeb.

Many experiments were made on still older embryos after closure of the germring during the early stages in the segmentation of the mesoderm. As in the preceding experiments on the embryonic shield there was no regeneration of the parts removed and one is able with ease to produce headless or tailless monsters or embryos with head or tail ends separated by wide gaps (figs. 16, 16a).

CONCLUSIONS

Previous experiments indicate that there is no very definite localization either in the germdisc or blastodisc until about the time of the formation of the germring and embryonic shield.

With the formation of the embryonic shield very definite localization takes place along the axis of the shield coincident with the formation of the medullary cord. Removal of any portion of the axis of the embryonic shield results in very definite defects in corresponding parts of the embryo.

The embryonic shield does not possess the power of regeneration of the parts removed from the axis. Whether before this period of development the germdisc or blastodisc possess the power of regeneration is doubtful as there are no indications of any definite localization.

These experiments also show that the germring takes no part in the formation of the embryonic shield but merely serves for the formation of the covering of the yolk. The embryo then is not formed by concrescence in the ordinary sense.

This method of experimentation affords an opportunity for experimental work on the central nervous system by the elimination of various portions at various stages. The development of the vascular system can also be studied experimentally.

PLATE 1

EXPLANATION OF FIGURES

1, 2, 3, 4, 5 Diagrams of early embryonic shields, 24 to 30 hours after fertilization; cross lines show the areas extirpated.

6, 7 Diagrams of embryonic shields, 30 to 36 hours after fertilization, to show extirpated portion of the germring.

8, 9 Diagrams of later stages of embryonic shields, 40 to 44 hours after fertilization; cross lines indicate amount extirpated.

8a Resulting abnormal embryo from experiment 8, 16 days after fertilization.

9a, 9b Abnormal embryos from experiment 9, 17 days after fertilization.

10, 10a Extirpation of posterior end of embryonic shield 36 to 40 hours after fertilization, and resulting abnormal embryo, 12 days after fertilization.

11, 11a Somewhat similar experiment to 10, 10a.

12, 12a Removal of small superficial area from the axis of an embryonic shield 46 hours after fertilization with resulting embryo, 15 days after fertilization.

13, 13a Removal of two superficial areas from the axis of an embryonic shield 46 hours after fertilization with resulting embryo, 8 days after fertilization.

14, 14a Removal of a large deep area from the axis a shield 42 hours after fertilization with the resulting abnormal embryo 14 days after fertilization. The head and tail are completely separated by a wide area.

15, 15a Removal of a small lateral deep area from the axis of an embryonic shield 48 hours after fertilization with resulting defect of brain and absence of otocyst, 24 days after fertilization.

16, 16a Removal of two areas from the axis of an older embryo 70 hours after fertilization and resulting defects, 15 days after the fertilization.

17 Capillary plexus from yolk sac of embryo without circulation.

17a Normal capillary plexus from yolk sac.

