TRANSPLANTATION OF THE LIPS OF THE BLASTOPORE IN RANA PALUSTRIS.

BY

WARREN HARMON LEWIS.

From the Anatomical Laboratory, Johns Hopkins University.

WITH 5 FIGURES.

Roux[1] first pointed out that the material which forms the embryo of the frog is laid down in the black-white ring around the equator of the egg, and that the embryo is formed by a process of concurrence in that this material grows over the white hemisphere. If this is prevented, as in his case of asyntaxia medullaris, a half embryo develops on either side of the equator of the egg. Roux's observations and experiments were confirmed by Morgan[2] in a somewhat similar series of experiments. Hertwig[3] was able to produce the bilateral half embryos by allowing the eggs to develop in salt solutions. I have seen many similar forms from eggs of rana sylvatica and rana palustris which were kept on ice for a prolonged period of time. In such abnormal forms the lips of the blastopore which fail to grow over the large yolk plug differentiate into these modified half embryos with a central nervous system, muscle, the chorda, and the roof of the archenteron.

More recently Morgan[4] has investigated the question of the location of the embryo-forming substances and concludes that the material is first in the upper hemisphere of the developing frog's egg and is later carried downward into the germ ring.

[1] Ueber die Lagerung des Materials des Medullarrohes in gefurchten Froschei. Anat. Anz., Vol. 3, 1888.

[2] The formation of the embryo of the frog. Anat. Anz., Vol. 9, 1894.

[3] Urmund und Spina bifida. Arch. f. mikr. Anat., Vol. 39, 1892.

[4] The relation between normal and abnormal development of the embryo of the frog, X. A re-examination of the early stages of the normal development from the point of view of the results of abnormal development. Arch. f. Entwickelungsmech. XIX, 1905.

The origin of the organ-forming materials in the frog's embryo. Biol. Bul., XI, 1906.

The following experiments were made, partly with the view of determining what organ-forming stuffs are present in the lips of the blastopore; but more especially to determine the extent of independent self-differentiation possessed by this structure when small pieces were isolated and transferred into strange environments. In the examples of asyntaxia medullaris there develops from the lips of the blastopore on either side a half embryo with spinal cord, notochord, and myotomes, so that evidently the fusion of the lips is not necessary for their differentiation, but it might be, of course, that some of the other relations are essential, such as the anterior, posterior, or the lateral connections with the embryo. But by cutting out and transplanting small sections of the lips these factors were eliminated.

The first series of experiments were made on rana palustris at a time when the dorsal and lateral lips are well marked, the ventral lip is just beginning to appear. Small blocks of tissue were cut from the dorsal and lateral lips (1, 2, 3, and 4, Fig. 1), so as to include the entire thickness of the lip with both ectoderm and endoderm. These pieces were transplanted beneath the ectoderm of older embryos of the same species in the region of the otic vesicle. The embryos into which they were transplanted show the beginnings of the tail bud. The pieces transplanted are soft and delicate, and of course very liable to injury during the operation by distortion, tearing, or loss of a portion.

In the first experiment (ta_1), piece (1), Fig. 1, was transplanted into an older embryo which was killed 7 days after the operation. The sections (Fig. 2) show in the region of the otic vesicle a large notochord, perfectly normal in its differentiation and extending for many sections in a direction parallel to the long axis of the central nervous system. Portions of the chorda are irregular in outline and it is not quite as large as the normal one in cross diameter. Near the chorda and ventral to the otic vesicle is a large mass of muscle, irregularly arranged, normal in its differentiation, showing striation and other characters of myotomic muscles. I am unable to find any nerves going to this muscle mass and it lies in a position where normally there is no muscle.

Ventral to the notochord and medial to the muscle mass is a piece of central nervous system having both white and gray substance and a central canal. In places the nerve cells seem to be degenerating. The central canal is closed at either end.

I am unable to determine if any of the transplanted tissue differentiated into endoderm, though some degenerating cells in this region may possibly be endoderm. It is evident from this experiment that tissue from the dorsal lip of the blastopore possesses great power of self-differentiation, is already predetermined, and does not need the usual normal relations with the rest of the embryo for its differentiation. At how early a period these tissues are capable of independent self-differentiation is, of course, only possible to determine by further experiments along perhaps somewhat similar lines of transplantation.

The transplanted piece in another similar experiment (ta_2) of this series, taken from the region (2) (see Fig. 1), differentiated into notochord, striated muscle, and nervous tissue. The latter, however, is only imperfectly differentiated and shows many degenerating cells. This embryo was killed 7 days after the operation.

In another experiment of the same series (ta_3), piece (3) (see Fig. 1), was transplanted and the embryo killed after 7 days. There differentiated from it a large mass of striated muscle without nervous connection and a small piece of the neural tube consisting of a single layer of cells (see Fig. 3). No traces of notochord were found so it seems probable that the cells of the transplanted piece destined to form chorda were lost during the operation. The more lateral position of piece (3) does not explain its absence as from piece (4) (see Fig. 1, experiment ta_4), a large normal appearing notochord and neural tube tissue differentiated, the latter contains many degenerating cells. No muscle tissue was found and its rudiment was probably lost in transplantation. Both of the above embryos were killed 7 days after the operation.

In another series (tb) four pieces from the dorsal lip of the blastopore of a gastrula, the same age as in the preceding series, were transplanted into the otic region of similar older embryos, with beginning tail buds. These embryos were killed 6 days after the operation. As in the preceding series muscle and chorda develop into quite normal tissue, the former without any nervous connection. The neural tube is always present but shows more or less extensive degenerative changes.

In one of these experiments (tb_3) the chorda is in contact with the ectoderm, the latter shows here a modification of its staining reaction with hæmatoxylin and Congo red. Here, also, the cells of the inner layer of the ectoderm are elongated in an axis perpendicular to the surface. These modifications were very likely brought about in some way through the contact influence of the notochord.

In another similar series (te) an embryo (te_2) was killed 9 days after the operation. The sections show ventro-lateral to the otic vesicle a well-formed chorda, a group of striated muscle fibers which seem to be spreading out in the form of a muscle (*m, m*, Fig. 5), and a small and imperfect neural tube. The other experiments in this series show that the dorsal lip has differentiated into chorda, muscle, and nervous system, but all three tissues are not always present, however, owing to loss, probably in transplantation, of their rudiments. The mutual relation of these three tissues varies in such a manner that there is evidently no interdependence, as regards differentiation. The muscle develops perfectly normally without any nervous connection for 9 days at least after the transplantation, as in none of the experiments can nerves be traced to the muscles, either from the host or the transplanted nervous tissue.

These tissues do not seem to influence the configuration or arrangement of the connective tissue about them in any especial manner nor with the exception of the one instance where the chorda has modified the ectoderm do they influence other tissues in the region in which they are developing. In one instance (te_7) the chorda rudiment was evidently transplanted near the normal chorda and has differentiated into a chorda, lying parallel to the normal one, both are encased in cartilage of this region, and in some places there seems to be a slight excess of cartilage about the abnormally placed chorda.

It is possible that by the transplantation of small pieces or even groups of cells from younger and younger embryos that the localization of the primary organ or tissue-forming substances can be traced back, step by step, to their more primitive locations in the egg. It may be possible, also, to determine in these early stages correlations necessary for the formation of secondary tissues or for the differentiation of these.

In almost all of these experiments the tissues which have developed from the transplanted piece are much greater in bulk, very much greater in the case of the chorda and muscle than such a piece would have produced in the same time had it remained in the normal position in the embryo from which it was taken. This is an indication of how the neighboring parts in a normal embryo must interact upon each other, regulating the size or extent of growth for each such part. It is possible that when such pieces are freed from this influence of the whole on the part, that cell division can take place more rapidly and so produce a larger piece from the same number of cells than under normal conditions.

Although at this early gastrula stage the dorsal and lateral lips of the blastopore are already determined as regards there subsequent differentiation to give rise to chorda, muscle, and nervous tissue, there is evidently considerable difference in the power of self-differentiation in that the chorda and muscle develop into much more normal-appearing tissue than the rudiment of the nervous system. The latter, though predetermined at this time, does not seem to be able to differentiate into perfectly normal tissue after its transplantation. It is thus probably dependent on certain, as yet unknown, relations in its normal environment for its more perfect differentiation. Just what these relational factors are or for how long a period they must act is not clear, but a somewhat later stage after the first faint indications of the medullary plate appear the nervous tissue or its rudiment possesses great power of self-differentiation.

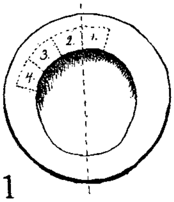
FIG. 1. Outline of blastopore region showing pieces transplanted from the dorsal and lateral lip.

FIG. 2. Experiment ta_1. Piece of the dorsal lip of the blastopore (see (1) Fig. 1) transplanted into the otic region of an older embryo, the tail of which is beginning to show. Embryo killed 7 days after the operation. Section through otic region showing piece of nervous system (*n*) with central canal and marginal layer, chorda (*c*) and large muscle fiber mass (*m*). × 100 diameters.

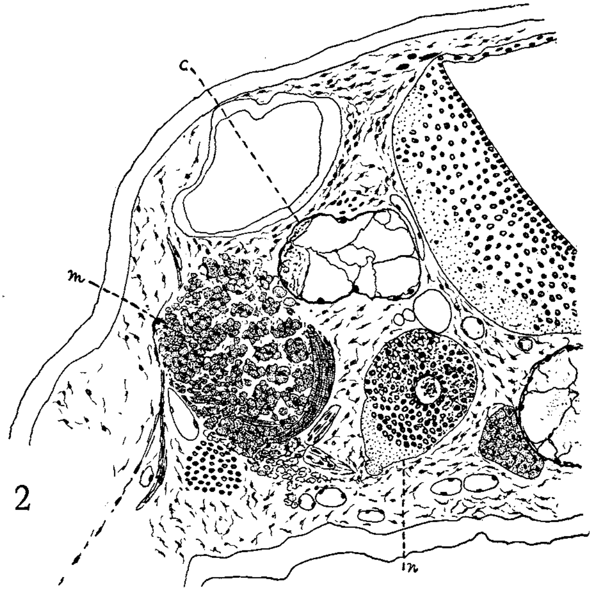
FIG. 3. Experiment ta_3. Section through abortive neural tube, from piece (3) Fig. 1. × 200 diameters.

FIG. 4. Experiment ta_4. Section through chorda and brain from transplanted piece (4) Fig. 1. × 100 diameters.

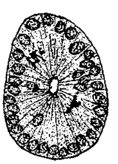
FIG. 5. Experiment te_2. *c*, chorda; *m*, *m*, muscle; *n*, neural tube. × 50 diameters.



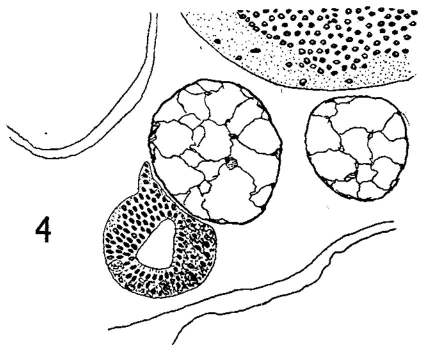
1



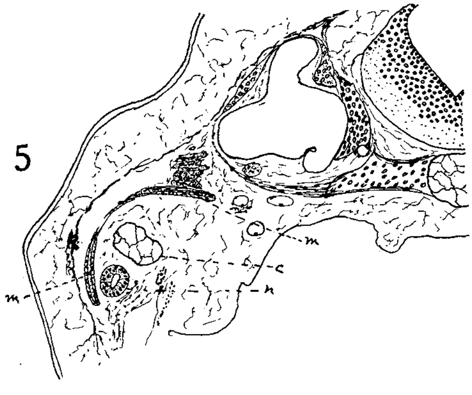
2



3



4



5