

are not enough to establish the complete indifference of this condition for the present result, but it is apparent that no very great effect is felt.

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¹ "The Rate of Establishment of a Discrimination," *J. Gen. Psychol.*, **9**, 302-350 (1933).

² "A Discrimination Based upon a Change in the Properties of a Stimulus," *Ibid.* (in press).

³ "On the Rate of Formation of a Conditioned Reflex," *Ibid.*, **7**, 274-286 (1932); and "Resistance to Extinction' in the Process of Conditioning," *Ibid.*, **9**, 420-429 (1933).

EXPERIMENTS ON EARLY DEVELOPING STAGES OF FUNDULUS

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Hitherto the ultimate fate of the cells in *Fundulus* was thought to be established during gastrulation (Lewis,^{1,2} Hoadley³). The present investigation presents results obtained from explantation and transplantation experiments which give evidence that differentiation is controlled in part by inductive processes. Blastoderms separated from the yolk were cultivated to study the movements and the differentiation of the cells when freed from the mechanical influences afforded by the yolk. Transplantations of materials from the dorsal lip of the blastopore and from the embryonic shield were made to embryonic and extra-embryonic regions of shield stages; the donors of grafts were studied for defective parts. Experiments on Perch eggs (Oppenheimer⁴) have shown that dorsal lip transplants induce embryo-formation, and that grafts of cells from gastrulating stages continue to differentiate when implanted upon the yolk-sac epithelium or in the embryonic body of host embryos.

The explanted blastoderms were separated from the yolk and periblast between the one and the one hundred and twenty-eight cell stage. Morgan⁵ secured perfect embryos after one-half to two-thirds of the yolk was removed, but failed to obtain further development if the yolk was reduced to the same size as the blastoderm. The isolated blastoderms in my experiments form spheres; cleavage proceeds at the normal rate but the cleavage planes are in different relationship to each other than those of the blastoderm cleaving on the yolk. After about eighteen hours a large central cavity develops within the round mass of cells. Slightly later a thin-walled vesicle with an epithelium one cell in thickness arises at one pole; at the other pole the remaining cells form a solid mass.

When the cells composing the generalized cell mass have migrated and are aggregated into smaller groups, further cellular differentiation frequently occurs. Chromatophores appear and embryonic structures are recognizable. In some cases well-formed embryos develop. One had well-differentiated fore-, mid- and hindbrain, eyes and ears, a row of seven somites, chromatophores and a beating heart. In this embryo the thin-walled vesicle described above occupied the position normally filled by the yolk. The yolk-sac epithelium is a self-differentiating structure. Histological study of the explants where embryo-formation is imperfect will be necessary to show what tissues and organs have differentiated and to what extent the process is effected by interactions of the materials from different germ-layers.

The differentiation to embryos and embryonic structures with the exception of the yolk-sac epithelium occurs in blastoderms isolated at the thirty-two cell stage and later. The blastoderms isolated at younger stages differentiate the hollow vesicle only. In the early blastoderms where no membrane separates cells from yolk a considerable amount of protoplasm exudes from the cells when first removed from the yolk. The loss is less significant in thirty-two celled and older blastoderms; the central cells have then separated from the periblast and only the marginal cells are in continuity with it. The close correlation of the incomplete development of blastoderms with the greater loss of protoplasm suggests that materials necessary for the subsequent completion of differentiation are located ventrally in the cleaving blastoderm. At present an alternative explanation must be recognized, since such materials may be added to the blastoderm from the periblast before the thirty-two cell stage.

Dorsal lip grafts induce embryo-formation when transplanted into either embryonic or extra-embryonic regions. When the embryos are induced from embryonic regions there is a close but not infallible correspondence in level of primary and secondary embryonic structures. The correspondence is less marked in the case of embryos induced by transplants to the germ-ring in extra-embryonic regions. In no case was the induced embryo complete. The induced embryos form as do the normals from embryonic shields. Stained grafts show that self-differentiation of grafts has not been misinterpreted as induction.

When materials from the shield are implanted in embryonic regions the grafts are not absorbed into the host tissue and then re-sorted, but may remain discrete from the host structures. In some cases they are influenced by the host: in one case a graft to the head-region formed an apparent third optic lobe; in another (Perch) a graft in the somite region became contractile striated muscle. Histological study will be required to ascertain to what degree the grafts are affected during differentiation by their relationships to other structures within the embryo.

The materials transplanted from the shield to extra-embryonic regions undergo self-differentiation, cf. Mangold.⁶ In at least one case the graft produced more kinds of structure than were lacking in the donor; the donor showed defects in the mesencephalon; the graft differentiated nervous system, heart and striated muscle. Thus the differentiation of cells already in place in the shield is dependent not only on inherent capacities and on the actions of gastrulation, but also upon the developmental inhibitions exerted by surrounding tissues after gastrulation.

Differentiation in *Fundulus* is thus seen to be affected by influences exerted during and after gastrulation. The study of predetermination in the cells of the blastula is at present incomplete. In most cases defects of the blastula are regulated (cf. Lewis, Hoadley, loc. cit.). In transplantation experiments, when cells are transplanted from blastula to blastula there are no extra structures in the embryo if the grafts have become mechanically homogeneous with the host tissue. Occasionally, however, they maintain their cellular individuality and appear as masses in the embryo. The early appearance of yolk-sac epithelium in the explanted blastoderms, and the difference in behavior of blastoderms isolated early and late in cleavage, clearly show that processes occurring before gastrulation have a decided influence in the control of differentiation.

¹ Lewis, W. H., *Anat. Rec.*, **6**, 1-6 (1912).

² Lewis, W. H., *Ibid.*, **6**, 325-333 (1912).

³ Hoadley, L., *J. Exp. Zool.*, **52**, 7-44 (1928).

⁴ Oppenheimer, J. M., *Proc. Soc. Exp. Biol. and Med.*, **31**, 1123-1124 (1934).

⁵ Morgan, T. H., *Anat. Anz.*, **8**, 803-814 (1893).

⁶ Mangold, O., *Naturwiss.*, **19**, 475-476 (1931).