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Behavior of Pigment Cells from Cultures of Neural Crest When Grafted Back into the Embryo.*

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Neural crest taken from chick embryos in early somite stages produces large numbers of pigment cells both *in vitro,*[1] and in grafts to the limb buds of older host embryos, the melanophores later appearing in the dermis and feathers of the hosts.[2] This work has been corroborated by Eastlick,[3] who transplanted limb buds with or without the neural crest, and found that the appearance of pigment was correlated with the presence of the neural component in the grafts.

The earlier cultures were short time cover slip preparations designed to test the capacity for differentiation of the tissue explanted. In view of the complex prospective potency of the neural crest in the embryo, it seemed advisable to attempt to develop pure cell strains in order to study the behavior of these cells under controlled conditions. For the past year, explants of early somite neural crest from colored breeds have been grown in small culture flasks in a standard medium of plasma and embryonic extract, according to the technic developed by Carrel and his associates. Such cultures grow rapidly during the first week *in vitro,* soon producing a ring of dark brown or black cells. Growth then continues at a slower rate, and strains of cells are produced which are uniform in appearance and behavior, being composed of typical branched melanophores which spread out upon the clot or become aggregated in clumps.

Cells from these cultures have been grafted to embryos of another breed than that of the donor, a single culture furnishing enough tissue to graft to as many as 30 host embryos, without entirely exhausting the original strain. When a graft is inserted into the limb bud of a white host (which is subsequently observed through a transparent window in the shell), the ectoderm heals over the tissue,

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1 Dorris, F., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 448; *Roux Arch.*, 1938, **138**, 323.

2 Dorris, F., *Anat. Rec.*, 1938, **70**, 91; *J. Exp. Zool.*, 1939, **80**, 315.

3 Eastlick, H. L., *Collecting Net*, 1938, **13**, 151; *Genetics*, 1939, **24**, 98; *Anat. Rec.*, 1939 a., **73**, Suppl. 2, 64; *J. Exp. Zool.*, 1939 b., **82**, 131.

which is still clearly visible as a dark mass below the surface. During the first 2 days, migration of melanophores from the graft occurs, and these are finally found in the dermis, epidermis, and developing feathers of the femoral tract in host embryos fixed at the 12th to 15th day of incubation. A total of 145 grafts have been made, the tissue being furnished by pure strain cultures of differentiated melanophores obtained from the neural crest of Australorp embryos (a Black Orpington breed), the hosts being White Leghorn or Rhode Island Red embryos ranging in age from 3 to 5 days of incubation. The work, which is being extended to include other breeds of fowl, will be reported fully elsewhere.

Two points brought out by these experiments are of special interest. One is the maintenance in grafts, of the slow growth rate established *in vitro*. In striking contrast to the behavior of neural crest grafted directly from the embryo, which produces pigmented areas covering the whole leg, shank, and foot, as well as parts of the dorsal and ventral trunk regions, neural crest melanophores from cultures formed patches of pigment identical in appearance, but very much smaller in extent, never involving even the greater part of the femoral feather tract. Even more interesting is the fact that these cells, although transplanted into an environment composed of embryonic tissues physiologically younger, and thus forming what has been described as an "embryonic field", are apparently unaffected, and continue to reproduce as pigmented cells, behaving in a manner quite similar to that seen *in vitro*. This stability of both differentiation and growth rate in the formed tissue, is in striking contrast to the extreme lability of the embryonic region from which the cells were originally derived.