DIFFERENTIATION OF THE EMBRYONIC TISSUES OF THE FISH, *ORYZIAS LATIPES*, TRANSPLANTED ON THE CHORIO-ALLANTOIS OF CHICK[1]

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TWELVE FIGURES

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I. INTRODUCTION

In three previous experiments (Oka, T.B., 1931, 1938 a, b), it was ascertained that melanophores and lipophores are formed in tissue cultures, in which the tissues of non-somite embryos and of the blastula blastoderms of *Oryzias latipes* (teleost) are cultivated, and that they differentiate to three kinds of melanophores and two kinds of lipophores, of characteristic size and amount and velocity of deposition of melanin and lipochrome granules, affected by the allelomorphic genes B, B′, b and R, r respectively. In addition, it was ascertained that these melanophores and lipophores developed only in cultures made with such media as the embryonal tissue extract of chick or the blood-plasma of chick, toad, frog, and red carp, but not in such fluid media as Ringer's and Lock's solution.

It was originally planned to ascertain whether the characteristic formation of melanophores and lipophores which are affected by the genes B, B′, b and R, r respectively, may be attained in cases in which

[1] The subject of this paper was read before the monthly meeting of the Zoological Society of Japan on May 26, 1934.

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the tissues of non-somite embryos of the fish of various strains are transplanted on the chorio-allantoic membrane of White Leghorn chicks, and this was satisfactorily established.

The present paper deals chiefly with the growth and differentiation of the transplanted fish tissues, since there is no experiment on fish-chick grafting in the literature. Murphy (1913–'26), Kiyono and Sueyasu (1917), Hiraiwa (1927, 1930), and Nicholas (1933) have reported experiments on the transplantability of the tissues of different species, but all of them are the transplantations of mammalian embryonic tissues on the chorio-allantois of birds, that is to say, transplantability between warm-blooded animals.

It is a pleasure to express my hearty thanks to Prof. T. Goda who kindly offered me every facility of his laboratory, to Prof. M. Kume, of the Tokyo Women's College for Teachers, who kindly instructed me in the technique of the chorio-allantoic transplantation employed in the Institute of Prof. B. H. Willier, and also to Drs. K. and J. C. Dan, of the Misaki Marine Biological Station, who were kind enough to read through the manuscript.

II. MATERIAL AND METHODS

The present experiments were carried out in the summer of 1932. The methods employed here for transplanting the embryonic tissues of the fish, *Oryzias latipes*, into the chorio-allantoic membrane of the chick was essentially similar to that improved by Prof. Willier (1924).

As host, the embryos of White Leghorn were always used, and as donor, the blastula blastoderm and the non-somite embryo (Fig. 1) of the fish of a black strain were transplanted in toto. The embryos of variegated red (B'B'RR) and red (bbRR) strains were also transplanted for comparison.

After preparing a chick embryo of nine days' incubation at 37°C., a fish embryo, freed from the yolk and the chorion of its eggs, is transplanted at the junction of two or three large allantoic blood-vessels of the chorio-allantoic membrane of the chick, avoiding transplantations directly over the host embryos. The hen's eggs thus operated are placed with the operation window down on a cotton-bed, and are left overnight in the incubator which is intentionally kept at 37°C., and in the next morning

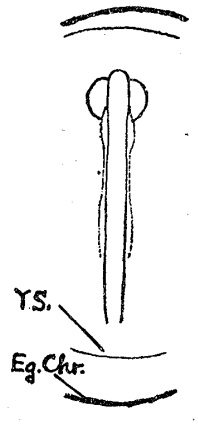

Fig. 1. A pre-somite embryo of *Oryzias latipes*. Eg. Chr., egg chorion; Y.S., yolk sphere.

the eggs are turned upside down on the bed keeping the window upside. The transplants thus treated were found to have been incorporated in the chorio-allantois of the chick after two days.

It should be noted that the temperature 37°C. is nearly optimum for the development of the chick, but it is found to be a little above the optimum for the growth of the embryos of this cold-blooded teleost. Several tests were made at 34°C., but no positive graft was obtained; the host chick hardly survived. If only the temperature is raised without any operation, blastula blastoderms of this fish were able to develop at 37°C. or even at 40°C. If it rises above 43°C., the fish embryos are killed.

III. FORMATION OF MELANOPHORES AND LIPOPHORES IN THE GRAFTS

Successful grafts, although they are so minute in size that they are liable to be overlooked, can usually be identified by groups of melanophores formed in them.

a) Melanophores. Melanophores are formed in all the grafts of the black strain (BBRR) transplanted at the non-somite stage. It should be pointed out that in normal embryos of the black strain of the fish, the melanophores had not as yet been formed in the non-somite embryos, and only at about the 8-somite stage at the earliest, they appear on the heads of the embryos. The melanophores formed in the grafts are more or less branched and large in size and filled with melanin granules (Fig. 2).

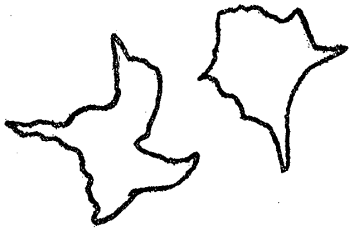

Fig. 2. Melanophores formed in the fish-chick grafts. × ca. 1030.

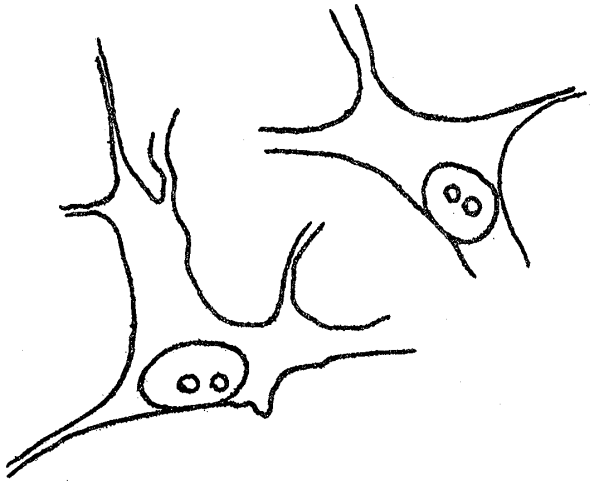

Fig. 3. Melanophores formed in the head of 8-somite embryos of the black strain of the fish. × ca. 1030.

It is of interest to note here that the melanophores are a little larger and more branched compared with those formed in the culture made with the embryonal tissue extract and the blood-plasma of White Leghorn chicks, but they are smaller than those formed in the cultures made with the blood-plasma of red carp (Oka, T. B., 1938 a, b).

b) Lipophores. The formation of lipophores, on the contrary, is rather surprisingly infrequent in these experiments, and only in the graft E several lipophores were found. The graft had grown for four days on the host chick. It should be noted that in normal embryos the lipophores first appear in the head of the embryos at about the 13-somite stage. In other favorable grafts such as the grafts D and C no lipophores could be found.

The lipophores formed in the graft E were found superimposed with melanophores (Fig. 4). They were large in size and filled with lipochrome granules, although they were less branched than those in normal embryos. But these lipophores in the graft E are larger in size compared with those formed in the cultures made with the embryonal tissue extract and blood-plasma of the chick, while they are smaller than those formed in the cultures made with the blood-plasma of red carp (Oka, 1938 a, b). The rarity of lipophores in the fish-chick grafts is of questionable significance.

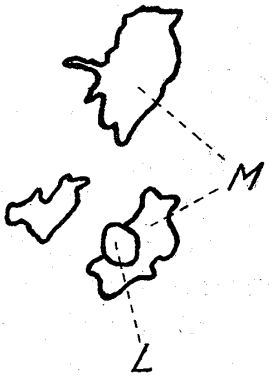

Fig. 4. A lipophore (L) superimposed with a melanophore (M) formed in graft E. × ca. 1030.

Since melanophores and lipophores appeared in the grafts, it seems apparent that some mesodermal cells in the non-somite stage must have undergone self-differentiation. Though this process could not be traced in the present experiment, it is possible to do so in tissue cultures.

IV. DIFFERENTIATION OF SPINAL CORD, MUSCLE FIBERS AND NOTOCHORD IN GRAFT D

The graft D (Fig. 5), which had been reared for eight days, was the most favorable one in this connection. The tissues such as spinal cord, muscle fibers and notochord were found well differentiated. The graft was readily identified by a group of melanophores and by the richly vascularized host capillaries at the junction of large chorio-allantoic blood vessels (Fig. 5). It appeared slightly opaque, with local bulges, and could be clearly recognized. It measured about 157 μ in the

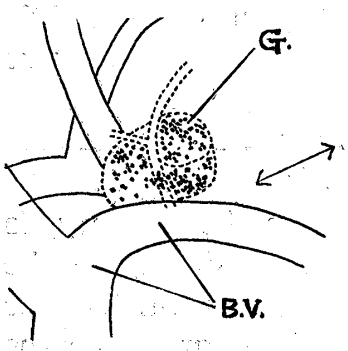

Fig. 5. A sketch of graft D. Black spots indicate the melanophores formed. An arrow indicates the direction of sectioning.

long axis and 107 μ in the short axis. It should be repeated that there were scarcely any lipophores even in such a well grown graft.

The graft D was fixed in Bouin's solution and cut into serial transverse sections of 10 μ in thickness. The direction of sectioning being shown by an arrow in Fig. 5.

Sections were stained with Delafield's hematoxylin and counterstained with eosin.

Microscopically examined, the transplanted embryos were found to have been incorporated into the mesenchyme of the chorio-allantoic membrane and were covered with its chorionic epithelium as in the chick-chick grafts (Fig. 6). The tissues of the grafts were easily identified, as the cells of the donor were smaller than those of the host.

In the following lines will be given a description of the tissues found to have undergone progressive growth and differentiation in graft D and in others.

a) Spinal cord. As is shown in Fig. 6, the spinal cord in graft D has grown large in size with a large canalis centralis, although they are irregular in shape and are scattered coarsely (Fig. 7).

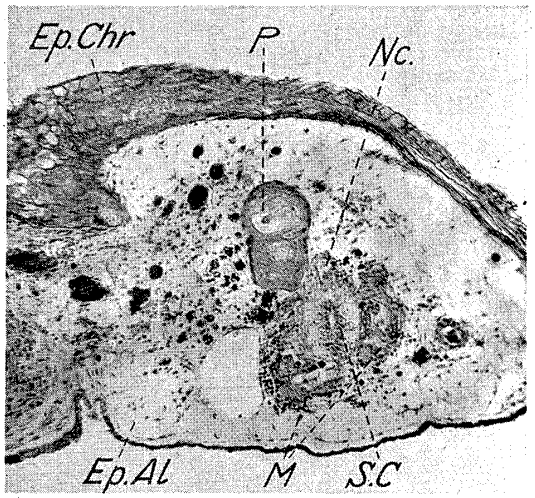

Fig. 6. A photomicrograph of a section through graft D, in which a fish embryo incorporated completely in the mesenchyme of the chorio-allantoic membrane of the chick is shown. Ep. Al., allantoic epithelium; Ep. Chr., chorionic epithelium; M. muscle; Mel., melanophores; Nc., notochord; P., pearl; S.C., spinal cord.

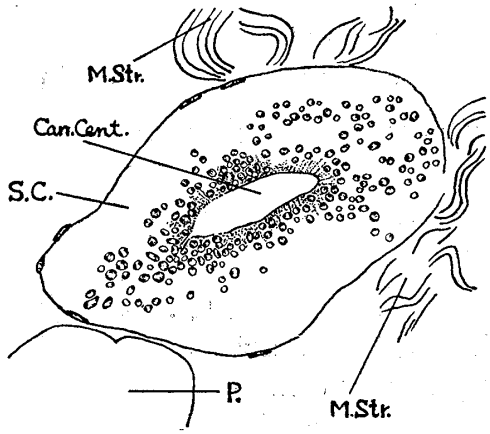

Fig. 7. A Section through the spinal cord differentiated in graft D. × ca. 414. Can. Cent., canalis centralis; M. Str., striated muscle; P., pearl; S. C, spinal cord.

In a normal spinal cord at about the same stage of development, the nuclei are round and expanded in shape and are closely arranged surrounding the canalis centralis. The spinal cord on non-somite embryos at the time of transplantation is small, with a narrow canalis centralis, and its constituent cells are somewhat polygonal in shape, and the nuclei are distributed rather uniformly in it. From this evidence, it may be concluded that the spinal cord of the fish embryo is capable of growing and differentiating in the grafts.

b) Muscle fibers. On both sides of the spinal cord in Fig. 6, there are found bundles of muscle fibers composed of both striated (Fig. 8, M. Str.) and non-striated muscle fibers.

It is beyond doubt that these two types of muscle fibers have been differentiated from polygonal somitic cells of non-somite embryos.

Fig. 8. Striated muscle fibers differentiated in graft D. × ca. 3000. M. Str., striated muscle fibers.

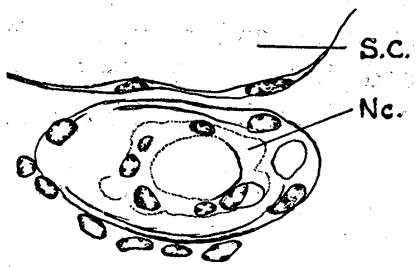

Fig. 9. A section through the notochord differentiated in graft D. × ca. 1058. Nc., notochord; S. C., spinal cord.

c) Notochord. Below the spinal cord in Fig. 6, there is found a notochord (Fig. 9). The wall of this notochord has become thicker and its content is beginning to disappear as it does in normal development. It is quite obvious that the embryonic notochord of the fish has grown and differentiated during the eight days in the graft D.

d) Aside from the above mentioned tissues, there are found several tubule-like tissues at the right side of the notochord (Fig. 6). It is, however, hardly possible to identify them.

V. PULSATION OF THE EMBRYONIC HEART IN GRAFT H

In graft H, it was surprising to discover under a binocular dissecting microscope that the heart of the transplant was beating rythmically (9,30 a.m., August 15, 1932)[1]. The pulsation continued for about 15 minutes, in Ringer's solution of 1/2 concentration, after the graft was removed from the host chick. The frequency of pulsation, unfortunately, was not measured.

[1] Prof. Goda had a kindness to observe the pulsation of the graft H.

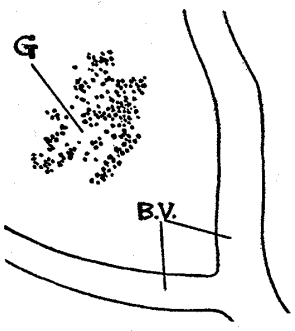

Fig. 10. A sketch of graft H. Black spots indicate the melanophores formed. The graft exhibited pulsation after removed from the host chick.

The graft H was removed from the host on the morning of the third day after it was transplanted, as the temperature of the incubator had accidentally fallen to 28°C. The graft was readily identified by a group of melanophores, and it looked slightly opaque, showing an obscure contour. The graft had grown at the junction of large chorionic blood vessels and was vascularized with capillaries of the host membrane. In normal embryos of the fish the embryonic heart commences to pulsate at about the 13-somite stage.

Histologically examined, the graft H had been partly incorporated in the mesenchyme of the membrane, and a part of it facing the chorionic side remained uncovered by the chorionic epithelium (Fig. 11). Tissues of the heart, spinal cord, notochord and tubule-like structures could also be found.

Heart (Fig. 12). The rythmic pulsation of the graft H can safely be taken as an evidence for the fact that the primordium in the non-somite embryo of the fish differentiated and became functional after being transplanted, since, beside the embryonic heart, there is no tissue that can exhibit any kind of pulsation in such a young embryo.

In the section there is found a sac-like tissue that seems to be the embryonic heart (Fig. 11). This is a simple sac composed of one layer of large cells having swollen nuclei. The heart of normal embryos, in comparison, is a simple sac enclosed by one-cell layered epithelium.

This is the only case, however, in which the author has any evidence of differentiation and functioning of the heart among the present fish-chick grafts.

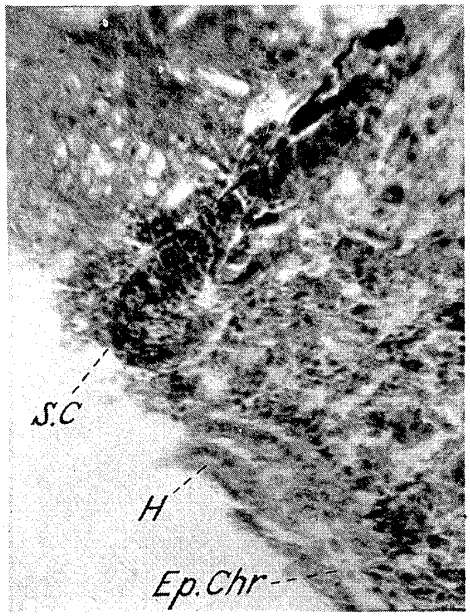

Fig. 11. A photomicrograph of the section through graft H, showing the heart pulsated in the graft. Ep. Chr., chorionic epithelium; H., heart; Mel., melanophore; S. C., spinal cord.

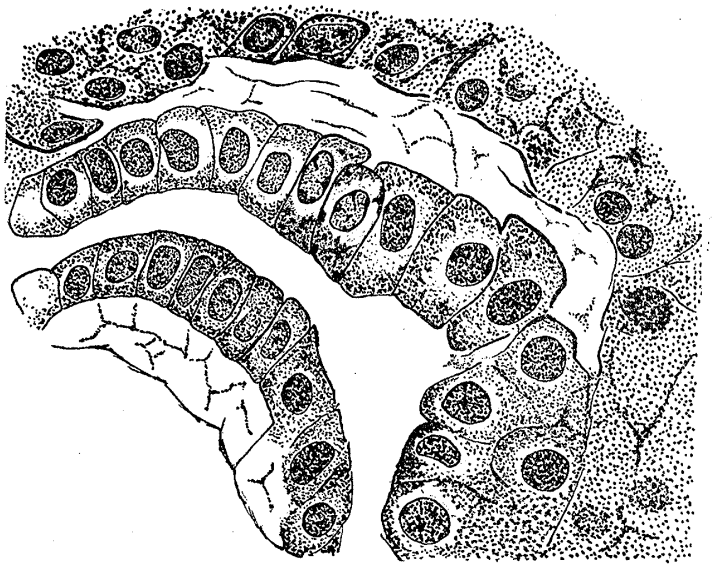

Fig. 12. A section through the heart differentiated in graft H. Pulsation of the graft was expressed by this heart. × ca. 966. A photomicrograph of the section is shown in Fig. 11.

In passing, a section of the spinal cord in the graft is shown in Fig. 11. It is surrounded by melanophores well differentiated as usual. The graft F, grown in the same host as the graft H about 1 cm. away, did not exhibit any sign of pulsation.

VI. TRANSPLANTATION OF BLASTULA BLASTODERM

At the end of the summer of 1932, the transplantation of a whole blastula blastoderm of the fish of a black strain was attempted. Six cases of such transplantation were successfully made. The eggs thus operated were incubated for five days. Among these eggs three embryos were found to have survived, but no melanophores indicative of successful graft were found. The incorporation of the blastula blastoderm apparently hardly took place in these cases.

SUMMARY

1. Non-somite embryos of the fish, *Oryzias latipes*, are readily incorporated in the chorio-allantoic membrane of White Leghorn chick, while its blastula blastoderms were not successfully incorporated in the present experiments.

2. Embryonic tissues of the fish, having been incorporated in the chorio-allantois of the chick and incubated intentionally at 37°C. for

two to eight days, develop in spite of the odd combination between a cold-blooded animal and a warm-blooded animal.

3. Melanophores are always formed in the fish-chick grafts.

4. Lipophores are found only in one graft.

5. Spinal cord and notochord are able to continue differentiation.

6. Muscle fibers, both striated and non-striated, are found differentiating from the polygonal somitic cells.

7. Pulsation of the differentiated embryonic heart of the fish was observed after two days in one case.

8. The positive grafts in the present transplantations are, apparently after all, only simple survivals of the incorporated tissues, and the tissues which thus remain surviving undergo growth and differentiation.

9. If ever the transplanted tissues successfully remain surviving, they cannot, sooner or later, be prevented from degeneration.

LITERATURE CITED

Detwiler, S. R. (1932). Growth acceleration and regulation in heteroplastic spinal-cord grafts. Journ. Exp. Zool. **61**; 245.

Hiraiwa. Y. K. (1927). Studies on grafts of embryonic tissues of the rat on the chorio-allantoic membrane of the chick. I. Differentiation of ectodermal derivatives. Journ. Exp. Zool. **49**; 441.

—— (1930). Studies on grafts of embryonic tissues of the rat on the chorio-allantoic membrane of the chick. II. Differentiation of meso- and entodermal derivatives. Folia Anatomica Japonica **8**; 157.

Hunt, E. A. (1932). The differentiation of chick limb buds in chorio-allantoic grafts, with special reference to the muscles. Journ. Exp. Zool. **63**; 57.

Kiyono, Kenji and Sueyasu, Y. (1917). Ueber die Implantation der Embryonalgewebe verschiedener Tierspezies in die Hühner- und Entenembryonen. (Japanese with German Resumé). Kyoto Igaku Zassi **14**; 594.

Minoura, T. (1921). A study of testis and ovary grafts and their effects on the embryo. Journ. Exp. Zool. **33**; 1.

Kume, Matazo (1935). The differentiating capacity of various regions of the heart rudiment of the chick as studied in chorio-allantoic grafts. Phys. Zool. **8**; 73.

—— (1936). 鶏胚の前腸門附近に於ける肝組織形成能力に關する研究. 動雑, **48**; 679.

—— (1937). Preliminary note on the heart-forming area in the chick embryo of somite stages. Zool. Mag. (Tokyo) **49**; 206.

Murphy, J. B. (1913). Transplantability of tissue to the embryo of foreign species. Its bearing on questions of tissue specificity and tumor immunity. Journ. Exp. Med., **17**; 482.

—— (1914). Studies in tissue specificity, II. The ultimate fate of mammalian tissue implanted in the chick embryo. Ibid, **19**; 181.

—— (1914). Factors of resistance to heteroplastic tissue grafting. III. Studies in tissue specificity. Ibid, **19**; 513.

—— (1916). The effect of adult chicken organ grafts on the chick embryo. Ibid, **24**; 1.

—— (1926). The lymphocyte in resistance to tissue grafting malignant disease, and tuberculous infection. Monographs Rockf. Inst. Med. Res. **21**.

Nicholas, J. S. (1933). The development of embryonic rat tissues upon the chick chorio-allantois. Journ. Exp. Zool. **66**; 193.

Oka, T. B. (1931). Effects of the triple allelomorphic genes in *Oryzias latipes*. Journ. Facul. Sci. Imp. Univ. Tokyo, Sect. IV, **2**; 171.

—— (1938). Melanophores and lipophores differentially formed, in tissue cultures of embryonic tissues of *Oryzias latipes*, affected by the genes. Jap. Journ. Zool. (in press)

—— (1938). Embryos formed in tissue cultures of blastoderms of *Oryzias latipes*, containing melanophores and lipophores formed differentially affected by the genes. Jap. Journ. Zool. (in press).

Sandstrom, C. J. (1932). The growth and differentiation of duck kidney tissue on the chorio-allantoic membrane of the chick and duck. Phys. Zool. **5**; 989.

—— (1936). Reciprocal chorio-allantoic transplants of embryonic duck and chick kidney transplants. Biol. Bull. **70**; 36.

Waterman, A. J. (1932). Culture in vitro and transplantation of young rabbit embryos. Anat. Rec. **54**; 72.