Studies on Grafts of Embryonic Tissues of the Rat on the Chorio-Allantoic Membrane of the Chick.

II. Differentiation of meso- and entodermal derivatives.¹⁾

By

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With 3 textfigures.

In a former publication (Hiraiwa '27) I presented that the primordia of some tissues of quite young rat, 11-days of age and 3 mm. in length, showed the power of self-differentiation when isolated and transplanted on the chorio-allantoic membrane of the chick, and the differentiation of the ectodermal derivatives was further described. In the present paper the other derivatives are dealt with.

I referred to that Murphy ('12, '13) had grown rat embryonic tissue, and mouse and rat tumor tissue upon the chorio-allantoic membrane of the chick embryo but had given no detailed account of the results with rat embryonic tissue. After my return to Japan, I had the opportunities of looking over the extensive work of Kiyono and Sueyasu ('17) who had made transplantation of the embryonic tissues of chick, duck, and turkey as well as those of rat, mouse, guinea-pig, and rabbit on the chorio-allantoic membrane of the chick and duck. They demonstrated that those mammalian embryonic tissues had grown very well on the chorio-allantoic membrane of avian eggs, for about 12 days.

They stated at the conclusion "It is very interesting that the

1) This work was started at the University of Chicago under the direction of Prof. B. H. Willier and continued at Kyushu Imperial University, Japan. Contributions from the Zoölogical Laboratory, Kyushu Imperial University, no. 17.

embryonic tissues of higher animals grow and differentiate to the highest degree nourished by the embryo of lower animals when transplanted to the latter."

The donor embryos used by these investigators were of much later stages than mine. For instance, they used rat embryos measuring 15 mm., 20 mm., and 25 mm. in length.¹⁾ In my records the rat embryo of 15-days measured about 10 mm. and of 20-days about 30 mm. Comparing with these with Donaldson's data ('24), we may infer that their materials were about 16.5-day, 18.5-days, and 19.5-days old respectively. According to my observations the tissues and organs of rat embryo show rapid growth and differentiation after 15-days and quite a number of formed organs appear. So it is no wonder to see that they continue their development, if they are allowed to grow even when isolated.

Detailed account of meso- and entodermal derivatives.

1. Bone and cartilage.

I do not know the exact stages when the bone and cartilage make their first appearance. In the slides of the sections of 15-day embryo of the rat, any trace of formation of these is not found as yet. I think they may differentiate far later than 15-day stage. In the 11-day embryo, only mesenchymal cells were to be seen, densely aggregated at some places and not so dense elsewhere. But as indicated in the previous paper the cartilage and the bone showed very common appearance in the graft after nine days. Though they did not form definite structure, they differentiated from each level of the embryo but are relatively most abundant in grafts from anterior third and least abundant in posterior third. Bone was always associated with cartilage which appeared more frequently than the former. In Fig. 6 and Fig. 9 of the previous paper we see some parts of cartilage and bone tissue which surround the nasal sac, forming undoubtedly a part of nasal bone and cartilage. Bone and cartilage had no special relation to other donor tissue in the grafts, except that the nasal sac was surrounded by cartilage.

In the case of the chick graft by Hoadley ('25) bone and cartilage grew very well and in one case they occupied a large part of the graft.

1) Besides these they used the material from the rat embryos of the size of 55 mm. taken out just before birth. Judging from Donaldson's data which show that the rat embryo measures in average 39 mm. at birth, an embryo of 55 mm. must be extraordinarily abnormal.

Their differentiation was quite equal to that found in control animals of the same age.

2. Mesonephros.

In only one of the grafts developed from the middle third (series 7, graft 7) a nephric structure appeared. There is no absolutely reliable criterion by which mesonephros and metanephros can be distinguished from each other but judging from the level where this organ differentiated it is assumed to be mesonephros. The nephric structure in my figure is not so clear as the chick kidney in the experiments by Hoadley. In my case only tubules appeared and no Malpighian body was found (Fig. 1). This may be due, from use of foreign material, to the

Fig. 1.

Differentiated mesonephros from the graft 7, series 7, middle third, showing a necrotic figure in the center. $\times 20$.

antagonistic action of the host which attacked the grafted tissue so as to hinder its full development. As necrosis had begun I could not study the structure of the tubules in detail.

Hoadley ('26) states that the preprimordial segregates of the nephros become established as self-differentiating units at a definite time in ontogenetic development and moreover, the development of the constituent parts is characteristic and occurs

in grafts of certain definite regions of the early blastoderm. In the case of chick, grafts of 4-hour blastoderm formed only secretory tubules of kidney, while those of 6-hour blastoderm, when transplanted, formed both the secretory tubules and glomeruli. After 10 hours of incubation, grafts of appropriate level show the development of both the secreting elements and the collecting elements including the Wolffian ducts, and subsequent stages show the development of the entire organ. It cannot be decided whether those differentiated in my experiment are excretory tubules or collecting tubules because of the indistinctness of the structure. As the materials used as donor were very young, it is quite probable that those tubules in question are of secreting nature, judging from their early appearance.

In the center of the nephric structure there was a spot showing necrosis. It is considered that the mesonephros was allowed to develop for some time and thereafter, attacked by the host which had grown to

a certain degree. As to how another necrotic area outside of the mesonephros was formed I cannot explain.

3. Gut.

In the experiment of Hoadley ('25) the intestine of the chick differentiated very well in many cases and was proved to develop quite normally. But in general the entodermal derivatives seldom self-differentiate in the rat graft. The experiments of Kiyono and Sueyasu ('17) showed that the intestinal epithelium had grown to a certain degree but the growth was not so good as other tissues.

At eleven days, when grafting took place, the hind gut of the rat embryo was merely a blind tube surrounded by the entodermal epithelium. After nine days' growth on the chorio-allantoic membrane

the intestinal part of the gut which appeared from the posterior third of the embryo showed some degree of differentiation.

Fig. 2.

A cross section of the differentiated gut from graft 6, series 6, under low magnification. $\times 60$.

In the graft of series 6, graft 6, a typical case of differentiation of gut appeared. Several cross sections of convoluted gut were found, one of which is shown in Fig. 2. Though irregularly formed, traces of projections of the mucosa are found on the free inner surface of the tube. Under high magnification (Fig. 3)

they are proved to be the beginning of the formation of the villi. I tried to find any gland cells in mucosa of the gut but failed to do it. Outside the mucosa is a considerably thick layer of the submucosa surrounded by the inner circular and outer longitudinal layers of muscle. The arrangement of the elements of both layers of muscle is not regular in some place, yet they have their own characteristic features. There is no definite serous coat as the tube is embedded in the mesenchyme of the host, but the other structures of this gut in the graft are justly comparable to those which are found in the normal intestine of 20-day embryo of the rat.

Gut was found only in grafts of the posterior third and two cases

were found. No definite relation was noticed with other organs. And

Fig. 3.

Portion of the wall of the gut of Fig. 2. highly magnified M, mucosa; S, submucosa; C, circular layer of muscle; L, longitudinal layer of muscle. $\times 260$.

only this structure appeared but none of stomach, oesophagus, liver and pancreas.

Discussion.

In the previous paper I presented the data which show the power of self-differentiation of the primordia of the rat embryo, and described the differentiation of the ectodermal derivatives. I saw almost normal development of the hairs, which had no sign of existence when transplanted. The nasal pit which had been merely the depression of the ectoderm formed, after nine days in the graft, the nasal sac with fully differentiated olfactory epithelium.

Additional data concerning the meso- and entodermal derivatives convince me of the power of self-differentiation of the organ primordia of the rat embryo and show the fact of differential survival of the growing tissues which were transplanted into the very foreign tissues. Mesodermal derivatives are most abundant of all tissues, bone and cartilage especially well developing. The nephric element seemed to self-differentiate easily but was attacked soon by the host on account of being of a foreign origin. Next are the ectodermal derivatives. Among these skin differentiated very well, with mitoses in several places. Nasal sacs in cases developed very normally and central nervous system in all grafts appeared as a disorganized mass. Gut wall which rarely appeared was

the only self-differentiating tissue of the entodermal derivatives while no other organ came out from the entoderm of the embryo. The absence of digestive glands was notable.

If we use still earlier embryos as donors, dividing them in minute pieces of each level, we shall be able to measure the amount of organization of the primordia, by the amount and kind of tissues and organs which differentiate in the grafts of each level. This is the problem to be studied by further experiments.

Summary.

1. Additional data are presented, dealing with the differentiation of the meso- and entodermal derivatives of the eleven-day rat embryo, which developed in the grafts on the chorio-allantoic membrane of the chick after nine days.

2. Bone and cartilage showed very common appearance throughout the experiments, as in the case of the chick graft.

3. Mesonephros differentiated to some extent but soon underwent necrosis, being attacked by the host.

4. Gut is the only organ to appear of the entodermal origin, its degree of differentiation being well comparable to that of normal rat embryo of corresponding age.

5. That the tissues and organs which develop in the grafts of each level of the young embryo present a measure of the amount of organization attained by the element should be determined by further experiments using earlier embryo as donor.

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