On a former occasion the author ('55) has pointed out that the differentiation processes of neural tissue consist in at least two independent phenomena, neuralization of the presumptive ectoderm and characterization of the neuralizing tissue. It has further been found that the mesodermal tissues play an important rôle in the latter process (Takaya '55). In order to investigate this rôle of the mesodermal tissues, a large number of explantation experiments were carried out with the brachial part of the neural plate, the mesodermal substrate of which had been partly or wholly removed. The results disclosed that among mesodermal tissues the mesenchyme bears special significance for the determination of the types of differentiating neural tissue, since it was found that the types of neural tissue produced varied greatly, depending mainly on the presence or absence of mesenchymal tissue. In the following, a brief account of the results will be given. In this experiment, embryos of Triturus pyrrhogaster, Rana japonica, R. nigromaculata, and Bufo vulgaris formosus were used as material, and the operation was performed on neurulae with high medullary folds.

Experimental. In explants of the brachial part of the neural plate, the mesodermal substrate of which had been thoroughly removed, neural tissue was frequently produced alone, without being accompanied by any mesodermal tissue. In such cases the neural tissue always formed a solid or globular mass, containing a large number of neural cells (Fig. 1). In these neural masses neither white matter nor any other structures were differentiated to indicate the polarity. However, these masses did not consist of a random accumulation of neural cells. On the contrary, they were made up of multiple layers of neural cells, the nuclei of which were more dense in the innermost layer than in the outer ones. Presumably the first corresponds to the germinative ependimal layer, and the second to the mantle layers. Between the innermost layers there was generally a lumen, but it was very narrow, like that shown in Fig. 1. There were a few exceptional specimens which formed a lumen of considerable width (Fig. 2), but there was none which formed an expanded lumen.
Even in those explants which failed to differentiate mesodermal tissues, epidermis was produced, but this was always very thin, forming an expanded vesicle. There were several specimens in which epidermis was lacking, probably on account of the lack of a mesodermal component to contribute the epidermal differentiation. In these specimens, even in the absence of an epidermal covering, neural masses were always found to be produced. From these facts, we may be safe in stating that the massive proliferation is a peculiar tendency of the neural tissue which is manifested when the tissue is present alone, unaccompanied by any mesodermal tissue.

In a few specimens, neural tissue was found to be produced together with a piece of notochord or with a small mass of muscle tissue. No mesenchymal tissue was found in these explants. The notochord and muscles found were well differentiated and were closely attached to the neural tissue. Especially it was noted that the notochord was very frequently imbedded in the neural tissue (Fig. 3). Even in the presence of these mesodermal tissues, as long as mesenchyme was absent, the neural tissue invariably formed a mass (Figs. 2 and 3). However, between these cases and those of the simple formation of the neural tissue, there was a difference in one respect, i.e., in that white matter was found to be produced in the contact area between the neural and mesodermal tissues (Figs. 2 and 3). Generally more white matter was produced in the area of neural tissue which was in contact with the muscles than in the area attached to the notochord. At any rate, the occurrence of such cases may be interpreted as indicating that the presence of notochord or muscle tissue does not necessarily interfere with the massive proliferation of differentiating neural tissue. On the contrary, no such proliferation of the

Fig. 1. A mass of neural cells formed in the total absence of mesodermal tissue

Fig. 2. A mass of neural cells formed together with muscle tissue. White matter (dotted area) is produced in the contact area.

Fig. 3. A mass of neural cells formed together with notochord. White matter (dotted area) is produced in the contact area.
neural tissue was found in any of the explants in which the neural tissue was formed together with mesenchymal tissue.

In explants of the neural plate prepared by removing the notochordal area of the underlying mesoderm, generally no notochord was produced. In these explants muscle differentiation also failed very frequently, and the explants were full of a large amount of mesenchyme (Takaya '56). Even in these cases neural tissue was always developed, but it usually formed an expanded vesicle with thin walls (Fig. 4). Also narrow tubes of neural tissue were sometimes met with (Fig. 5). In all these cases neural cells were very scarce and the walls were frequently loaded with many yolk platelets even after 20 days of cultivation. It seems very likely that the proliferation of the neural cells is suppressed or becomes very difficult in the mesenchymal tissue. There were a few specimens in which neural tubes with relatively thick walls were produced. Even in such cases the tubes consisted mainly of matrix and a very few neural cells. As shown in Fig. 6, most of the neural cells found were situated at the inner border of the walls which represented the germinative layer. In consideration of these facts, we are inclined to assume that the proliferation of the neural tissue is greatly reduced or almost entirely suppressed if the tissue is encircled by abundant mesenchyme.

From the above description, it becomes apparent that the differentiation of the neural tissue may be greatly modified by the presence or absence of the mesenchymal tissue; whereas neural vesicles with thin walls are produced in the presence of mesenchyme, in its absence masses of neural cells are developed. These two types of neural differentiation may be in contrast to each other, since proliferation of the neural cells is marked in the formation of the neural mass, while no proliferation occurs in the production of the
neural vesicles. Presumably, between differentiating neural tissue and differentiating mesenchymal tissue, there exists an intimate connection which results in the suppression of the inherent faculty of the neural tissue to proliferate neural cells.

**Consideration.** The two types of neural tissue described above, i.e., the mass type and the vesicle type, are not rare. They have been frequently reported by previous workers to be produced in the course of embryonic transplantation and explantation experiments. However, it must be emphasized that in our experiment these two types of neural tissue were produced from one and the same source, i.e., from the brachial part of the neural plate. This part of the neural plate naturally forms the spinal cord, if it is left in its normal site, but in explantation, the same tissue actually develops into tissue masses or vesicles. From this fact, it may be concluded that the neural plate ectoderm is not yet determined, at least in the beginning of its differentiation, to produce any specific type of neural tissue. As has been pointed out by the author ('55), the determination of a specific character may be accomplished during the later course of differentiation.

One of the most potent factors in neural differentiation may be the proliferation of neural cells. Concerning this neural proliferation, our results have shown that it always occurs in neural tissues which are isolated from other tissues, especially from mesenchymal tissues. Explants of the neural plate ectoderm, if cultivated in the absence of any other tissues, always showed active proliferation with the resulting production of a mass of neural cells. Therefore, it can be stated that the proliferating faculty is immanent in the neuralizing tissue itself. However, this inherent potency of the neuralizing tissue is shown to be suppressed when the tissue is surrounded by abundant mesenchyme. In consequence of this suppression, neural differentiation always results in the production of vesicles with thin walls. Whether such suppression of the neural proliferation is the result of an influence issuing directly from the mesenchymal tissue or comes into existence by the co-operation of neural and mesenchymal tissues, is a question which can not be decided by the present results alone. It is certain, however, that the presence of abundant mesenchyme around the neural tissue is sufficient to suppress the proliferation of the latter tissue. Mesodermal tissues other than mesenchyme, such as notochord and muscle, were shown to exert no suppressing influence even when they were in close contact with the neural tissue. But these mesodermal tissues were found to have another kind of effect upon the neural tissue. The formation of white matter was always found in the areas of neural tissue in direct contact with
these mesodermal tissues. From these facts of our experiment, it may be said that the differentiating tendency of the neural tissue becomes altered in accordance with the nature of the mesodermal tissues which are brought into contact with it. Presumably, close association of two kinds of tissue will give rise to a new tendency, at least on the side of the neural tissue, which it would otherwise not possess.

Although the two types of neural tissue described above appear quite different from each other, they are of the same constitution, in that they both commonly possess the neurocoel and ependimal layers. In the mass type, the neurocoel is compressed through massive proliferation of the ependimal layers, while in the vesicle type, it expands, apparently as the result of failure of proliferation of the ependimal layers. By this comparison, it appears reasonable to assume that the form into which the neural tissue is determined depends mainly upon the different degrees of proliferation of the neural cells in the ependimal layers, and the degree of neural proliferation is, in turn, influenced greatly by the mesenchymal tissue immediately surrounding the neural tissue.

References

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