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Studies on the Differentiating Potencies of the Dorsal Part of the Blastoporal Lip in *Triturus*-Gastrula III. Quantity of Material in the Notochordal Differentiation[1]

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Introduction

In the early publications of this series, the author has reported some information concerning the differentiating potencies revealed by means of explantation experiments of the dorsal part of the blastoporal lip (KATO, 1958, 1959). It has been disclosed that the differentiating potencies towards the notochord is gradually intensified with the shift of the presumptive notochordal material to the blastopore. However, no increase was found in the occurrence of the notochord when the material to be explanted was excised and cultured *in vitro* for a period required for shifting of the part to the blastopore, if it would be left in the intact embryo (KATO, 1959). Therefore, enhancement of the potency to differentiate into the notochord cannot be attributable to the mere time lapse, but some factor(s), whether it resides in the presumptive notochord or it comes from the surrounding tissues, would be concerned with the event. In addition, a quantity of the test material could be a variable for a rate of notochordal differentiation under the experimental conditions, as, for instance, LOPASHOV (1935) demonstrated that under the explantation conditions the dorsal lip can carry out the more complex differentiation when the more pieces are fused together. A similar quantity-dependent differentiation in explantion is well shown on other tissues such as the neural differentiation from the mouse embryonic shield, from the nodal region of the chick blastoderm (GROBSTEIN, 1952, 1955), from the amphibian neural plate (BRAHMA, 1959), somitic differentiation from the dorsal mesoderm of amphibian embryo (MUCHMORE, 1957), and the limb formation from the presumptive anlage of amphibian neurula (AMANO, 1956).

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Under these situations, it seems to be necessary to explore carefully a significance of quantity in the case of the notochordal differentiation from the dorsal blastoporal lip of *Triturus*. For this purpose the present experiments were performed.

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Material and Method

Two different parts distal and proximal to the dorsal blastoporal lip in the early gastrula of *Triturus pyrrhogaster* (stage 11 of OKADA and ICHIKAWA's table) were used for examination of the differentiating potencies. The proximal part occupies the presumptive prechordal plate just above the blastoporal lip, while the distal part, the presumptive notochordal area. They are abbreviated as part *aU* and *bU* respectively in the following description. Their precise location was already given elsewhere (KATO, 1958). Either single or several pieces previously fused together were sandwiched with two epidermal sheets stripped off from the ventral side of the early neurula (stage 15 or 16). The explants thus prepared were kept in the sterilized HOLTFRETER's solution for 10 to 15 days.

Results

The results obtained are arranged in Table 1 for the *aU* group, and in Tables 2 and 3 for the *bU* one. Fig. 1 provides a comparison between the results of both groups.

I. *Development of the part aU*

(a) *Explantation of a single piece* (*Series aU*-1) : Twenty cases were available in this series. Neither notochord nor muscle was recognized in all specimens, except for one which possessed a structure resembling to, but not a real notochord. Mesenchymal cells, though very few in number, were observed in 10 explants (50%), while other cells in these and other explants were a mass of undifferentiated cells laden with many yolk platelets (cf. Fig. A in Plate I).

(b) *Explantation of two pieces fused together* (*Series aU*-2) : Notochord occurred in 3 out of 17 available cases (18%, cf. Fig.C in Plate I), and muscle differentiation was not encountered in any case. Almost of all the explants had mesenchymal cells (16 cases, 94%), and they were more abundant

Tabl 1. Results of the explantation of the part *aU*.

No. of pieces fused together	1	2	3	5
No. of available cases	20	17	15	6
No. of explants containing identifiable tissues	10	16	15	6
No. of explants containing:				
Neural tissue	0	1	0	0
Notochord	1?*	3	1+1?*	0
Muscle	0	0	2	0
Mesenchyme	10	16	15	6
Undifferentiated cells	20	17	15	6
Pharynx	0	0	3	2

* The number with question mark indicates the number of explant containing the notochord-like tissue.

than in the explants of the previous series. Fragment of the neural tissue appeared in addition to the notochord in one explant; both structures appeared in close association (cf. Fig. C in Plate I).

(c) *Explantation of three pieces fused together* (*Series aU*-3) : Only one out of 15 available explants had the notochord, and a notochord-like fragment was seen in another case. A small number of muscular cells (usually less than ten) were noticed in two explants, one of which had the notochord above mentioned. Mesenchymal cells and the undifferentiated mass of cells were of the common occurrence. The quantity of mesenchymal cells contained was similar to that in the previous Series *aU*-2. Pharyngeal tissue surrounded by the mesenchymal cells was found in 3 specimens (20%).

(d) *Explantation of five pieces fused together* (*Series aU*-5) : All the explants consisted of the mesenchymal cells and an amorphous big assemblage of the undifferentiated cells, but neither notochord nor muscle was found (cf. Fig. B in Plate I). The pharyngeal structure developed amidst the mesenchyme in 2 explants (33%). Necessity of the mesenchyme for the pharyngeal and other endodermal differentiation was established by OKADA (1960a and b).

In this group of experiments, a positive relation between the differentiation and the quantity of the explanted material was detectable only for differentiation of mesenchyme ; i. e., fusion of more than two pieces was shown to be effective to promote the differentiation of the abundant mesenchymal cells in the explants (cf. Fig. 1). The same relationship might be acceptable in the case of the pharyngeal differentiation, since this type of differentiation was found only in the series in which more than three pieces were explanted together. However, there is a possibility that abundant mesenchymal cells provide favourable conditions for the pharyngeal differentiation of the endoderm.

II. *Development of the Part bU*

(a) *Explantation of a single piece* (*Series bU*-1) : As is shown in Table 2, only 4 explants out of 18 available cases had the notochord (22%) and in 2 of them a small fragment of the neural tube appeared (cf. Fig. D in Plate I). Mesenchymal cells, though poor in quantity, were found in 14 cases (78%), and an amorphous assemblage of undifferentiated cells was recognized in all cases.

(b) *Explantation of two pieces fused together* (*Series bU*-2) : In this series the explants usually accomplished very complex differentiation with the formation of various structures. That is, the notochord was the most common occurrence and it appeared in as many as 12 out of 19 available cases (63%). The muscle was found in 8 cases (42%), the mesenchyme in 16 explants (84%), and the neural tissue in 10 specimens (53%). Besides, every explant possessed an amorphous fragment (often) or a mass (rarely) of undifferentiated cells. Inspecting Table 3, it seems to be a rule that the muscle and the neural tissue can differentiate in the presence of the notochord.

(c) *Explantation of three pieces* (*Series bU*-3) : Most of the explants had the notochord usually accompanied with the neural and/or muscular tissue (7 out of 8 available cases, 88%, cf. Table 2 and Fig. E in Plate I). In this series the muscular tissue showed also a tendency to appear with the notochord (5 cases, 63%), as was seen in the previous series (cf. Table 3). Mesenchymal cells were always found. A small aggregate of undifferentiated cells was often observed.

(d) *Explantation of five pieces* (*Series bU*-5) : Frequencies in the occurrence of the notochord and the neural tissue were nearly the same as in Series

Table 2. Results of the explantation of the part *bU*.

No. of pieces fused together	1	2	3	5
No. of available cases	18	19	8	6
No. of explants containing identifiable tissues	14	19	8	6
No. of explants containing:				
Neural tissue	2	10	4	3
Notochord	4	12	7	4
Muscle	0	8	5	1
Pronephros	0	0	0	1
Mesenchyme	14	16	8	6
Undifferentiated cells	18	19	8	6

Table 3. Tissues differentiated in the explants of the part *bU*.

Type of explants*	No. of pieces fused together: 1	2	3	5	Total
No. of explants showing the notochord, muscle and neural tissue, separately or together	4	12	7	4	27
C+N+M	0	6	4	1	11(40%)**
C+N	2	4	0	2	8(30%)
C+M	0	2	2	0	4(15%)
C	2	0	1	1	4(15%)

* C+N+M indicates the explant in which the notochord, neural tissue and muscle developed; C+N, the explant containing the notochord and neural tissue; C+M, the explant with the notochord and muscle; and C, the explant with the notochord alone.

** Percentage indicates the frequency in the occurrence of each type against the number of available cases.

bU-2 and *bU*-3; namely, there occurred the notochord in 4 cases (67%), and the neural tissue in 3 out of 6 cases (50%). But the muscular differentiation was found only in one specimen (17%). All explants contained the mesenchymal cells and a fragment or a mass of undifferentiated cells. In this series, the pronephric tubules which never occurred in other experimental series appeared in one case.

Throughout the results obtained in this group, the following points may be remarked: (1) the frequencies in the occurrence of the notochord and muscle increase in the explants made of more than two pieces, and (2) the differentiation of the muscle depends upon the presence of the notochord.

Discussion

Enhancement of the developmental potencies by increasing the quantity of materials

As cited in introduction, the significance of material quantity in the histogenesis and organogenesis has been pointed out by several authors. Their experimental results commonly showed the necessity of a certain amount of cells to undergo the subsequent differentiation. In the present experiment the same situation was proved to be hold true for the differentiation of the dorsal blastoporal piece isolated from the early gastrula. When the explant was composed of more than two pieces, it achieved generally an advanced differentiation of some definable tissues, regardless of the difference of origin of the explants (cf. Table 2 and Fig. 1). That is, in the case of the explants from the part *aU*, they showed a differentiation of the mesenchymal cells, while

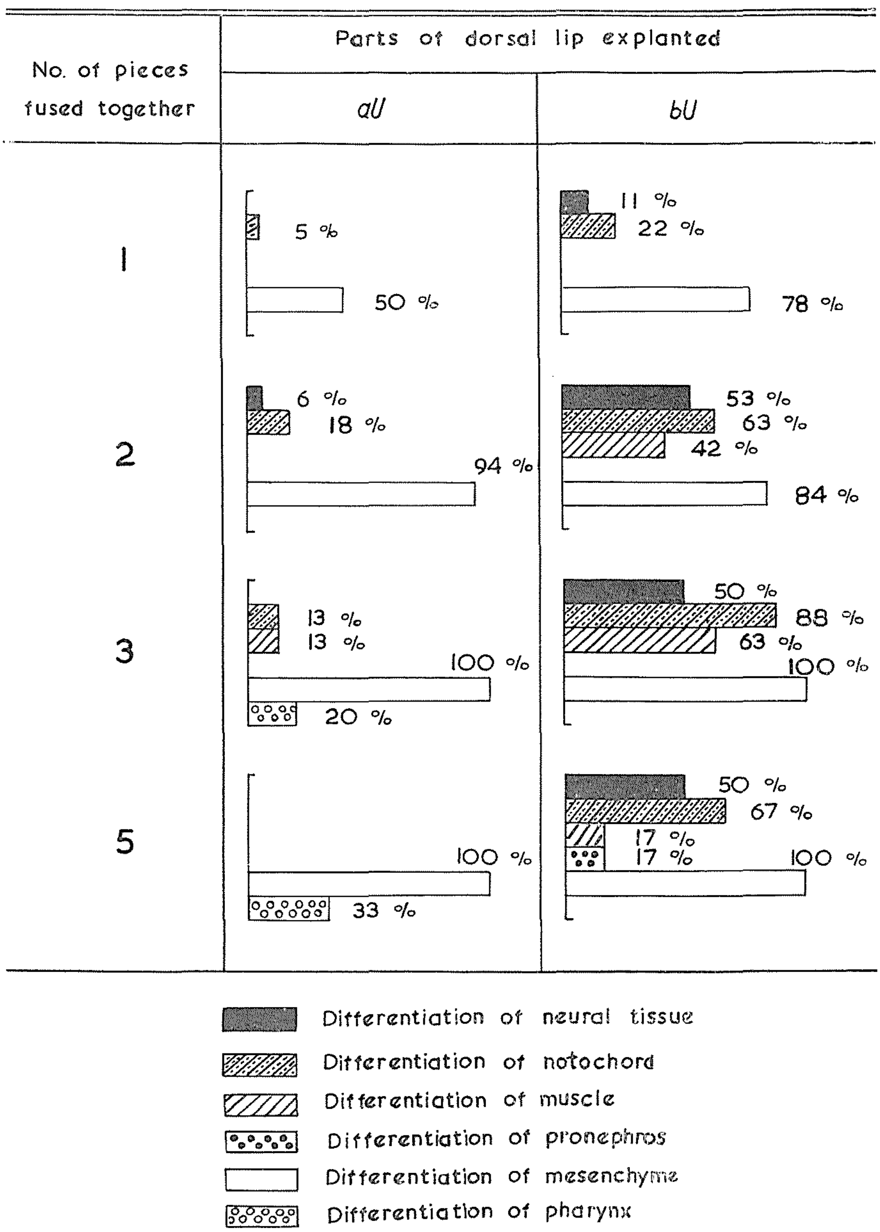

Fig. 1. Comparison of the frequencies of different tissues obtained in each experimental series.

in the case of part *bU*, they indicated often the production of such tissues as the notchord, muscle and neural tissue (cf. Fig. 1). This difference in the results implys that the part *bU* is more potent than the part *aU* in terms of producing the various tissues. The mere increase in number of pieces to be fused together did not necessarily yield more various differentiatons, because kinds of the tissues found in the explants composed of increased number of pieces were not different from those in the explants of a single piece (KATO, 1959). In contrast, LOPASHOV (1935) reported that the greater the number of pieces of the dorsal lip explanted together was, the more the variety of tissue differentiation was obtained. According to his paper, only the notochord and muscle appeared when the explant was made of less than four pieces, but in addition to them the neural tissue occurred when more than five pieces were fused together. The discrepancy between LOPASHOV's and present results may be due to the difference in size of the test-piece. Judging from his figure, Lopashov's piece is larger and liable to contain the materials of both parts of *aU* and *bU* in the present experiment, including a bit of presumptive somitic region. Consequently, his piece was endowed from the first with more presumptive fates than ours.

To sum up, it will be safe to state that increase of the piece number affords favourable conditions for the explant to realize its developmental potencies. However, it is still doubtful whether the quantitative increase of the explant may cause any qualitative difference in its differentiation.

Plasticity in the differentiating potencies of the presumptive notochordal area and the notochordal differentiation

The mere increase of pieces derived from the part *bU* in one explant enhances the realization of the differentiation of various tissues; especially the increase in the rate of notochordal formatioon is the most marked one, whereas the part *aU*, when the number of pieces to be fused together is increased, does not show any appreciable increase in the rate not only of the notochordal differentiation but also of the other one, except for the mesenchymal differentiation. Thus, it seems probable that the realization of various potencies in the part *bU* may have some stimulative effect on the notochordal forming potency. In this connection, it may be interesting to note that the notochord is rather scarcely encountered in the absence of the neural tissue (cf. Table 3). This result is in good accordance with that of the previous experiment in which the possible effect of the neural tissue upon the notochordal differentiation was shown (KATO, 1957). In that case, the notochordal formation was induced even from the part *aU* of the young gastrula when it was explanted together with the neural tissue from the neurula. Thus, it may be inferred that under some adverse experimental conditions the notochordal formation may require some kind of stimulation from the neural tissue. In fact, the positive role of the neural tissue upon differentiation of the the other mesodermal derivatives has been pointed out by some authors (HOLTZER *et al*, 1954a, b and 1956 ; MUCHMORE, 1958).

Summary

1. The piece taken from the presumptive area of the head mesenchyme and the foregut of the early gastrula indicated the increased rate of occurrence of the mesenchymal differentiation, but not of the notochordal differentiation when more than two pieces were fused together in explantation.

2. When more than two pieces taken from the presumptive notochordal area of the early gastrula were explanted together, the marked increase was found in the occurrence of the notochord, muscle and neural tissue.

3. The notochordal differentiation occurred generally coupled with the differentiation of the neural tissue, and it was discussed that the neural tissue seems to have some stimulative effect on the notochordal differentiation.

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Explantation of Plate I

Abbreviations : Ch, notochord ; Mus, muscular bundles ; Mes, mesenchymal cells ; N, neural tissue ; Nt, neural tube; U, mass of undifferentiated cells.

Fig. A. Mass of undifferentiated cells and mesenchyme in the explant of the single piece from the part *aU*.

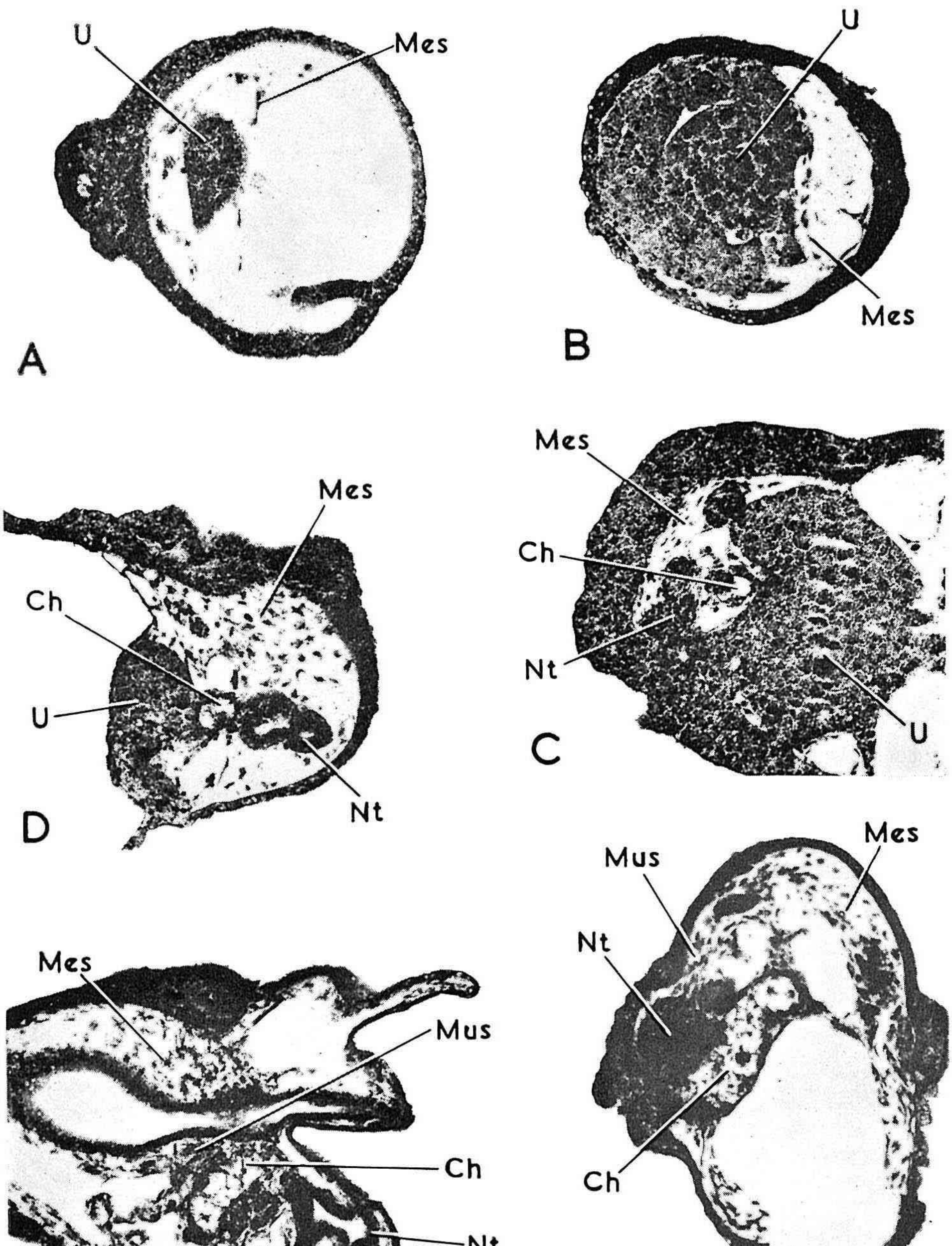
Fig. B. Mass of undifferentiated cells and mesenterial tissue in the explant made of 5 pieces from the part *aU*.

Fig. C. Differentiation of the notochord and neural fragment in the explant made of 2 pieces from the part *aU*.

Fig. D. Differentiation of the fragmental notochord and neural tube in the explant of a single piece from the part *bU*.

Fig. E. Differentiation of the notochord, muscular bundles and undefinable neural structure in the explant made of 3 pieces from the part *bU*.

Fig. F. Differentiation of the notochord, muscle and neural tube in the explant made of 5 pieces from the part *bU*.



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