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Neuro-notochordal Relationship in the Development of the Exlpanted Pieces taken from the Dorsal Lip of *Triturus*-Gastrulae[1)]

By

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Introduction

Although data have not yet shown any particular substance for chorda-mesoderml formation in Amphibian egg, it has been taken for granted that at the beginning of the early gastrular stage the notochordal primodium is already determined to achieve "bedeutungsgemäss" differentiation (recent review, HOLTFRETER and HAMBURGER, 1955). Nevertheless, this primodium, under some experimental conditions, has been shown to be still highly modifiable in terms of producing various kinds of tissues besides the notochord and muscle. The present author has already reported elsewhere that the primodium loses such plasticity and becomes definite as it shifts towards the blastopore (KATO, 1958, 1959). IKUSHIMA (1959, 1961) also indicated a correlation between the congregation movement of the dorsal blastoporal tissue and the notochordal differentiation. It appears, therefore, that the enhancement of the notochord forming potency in the notochordal primodium might link closely with the morphogenetic movement on gastrulation.

On the other hand, the results of our previous experiments would seem to suggest that the presence of the neural tissue is significant for the enhancement of the notochord forming potency in the dorsal blastoporal material (KATO, 1959). The present paper is concerned with a closer analysis of realization of the notochord forming potency in the dorsal blastoporal area.

The author's grateful thanks are due to Prof. M. ICHIKAWA of the University of Kyoto, under whose supervision and encouragement this work was carried out. His hearty gratitudes should be noted here to Dr. T. S. OKADA for his helpful comments on the work, and also to Drs. T. SHIN-IKÉ and N. IKUSHIMA for their kind advice.

Material and Method

Embryos of *Triturus pyrrhogaster* were used as material. Operation scheme

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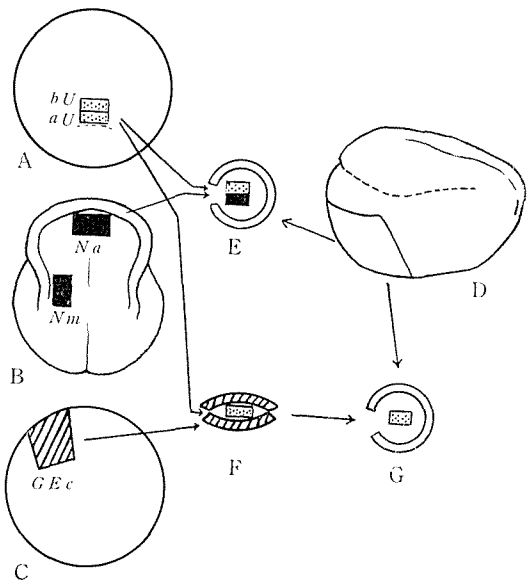

Fig. 1. Scheme of operation showing the location and size of the dorsal blastoporal piece (A), of the neural tissue to be combined (B) and of the presumptive ectoderm to be used as a temporal envelope (C). In the experiments of Group I the dorsal blastoporal piece alone (*aU* or *bU*) is cultured in the ectodermal vesicle from the neurula (D). E indicates the experiments of Group II where the dorsal blastoporal piece is cultured together with the piece from the neural plate in a ectodermal vesicle of neurula. G shows those of Group III where the same piece is cultured in the belly ectoderm from the neurula after pre-cultured in the sandwich of the presumptive ectodermal pieces for 24 hours (F).

is given in Fig. 1, in which the location of the piece to be tested is also shown. The pieces were taken from the dorsal area proxial or distal to the blastopore of the earliest gastrula (stage 11 of OKADA and ICHIKAWA's standard table). They will be referred to as *aU* and *bU* respectively in the following description. The presumptive fate of the part *aU* is the prechordal plate and foregut, whlie that of *bU* is the notochord.

The experiments consisted of three groups : (1) explantation of the test piece wrapped with the mesoderm-free belly ectoderm taken from the early neurula (control group), (2) explantation of the piece wrapped with the same ectoderm together with a developing neural tissue isolated either from the most anterior part (indicated as *Na* in Fig. 1) or from the middle part (*Nm*) of the mid-neurula at stage 18, (3) explantation of the piece wrapped with the ectoderm after 24 hours' pre-culturing in the sandwich between the presumptive ectodermal pieces taken out of the early gastrula (*GEc*). All explants

thus made were kept in the sterilized HOLTFRETER's solution for 10 to 15 days, and fixed in BOUIN's mixture for the histological examination.

Results

The results of 3 groups are diagrammatically shown in Fig. 2.

I. *Control experiments*

(a) *Explantation of aU (Series aU)* : As is given in Table 1, all of 17 specimens showed no differentiation of the notochord and muscle. But the mesenchymal tissue was found sometimes (7 cases, 41%). All explants contained a mass of undifferentiated cells laden with many yolk platelets (Fig. A in Plate I).

(b) *Explantation of bU (Series bU)* : Unlike the results of the previous series, differentiation of the notochord and/or muscle occurred in some explants (notochordal differentiation in 4 and muscular one in 2 out of 18 available specimens. cf. Table 1). Mesenchyme developed more frequently (11 cases, 61%). Besides these, a small fragment of the neural tissue was encountered in 4 specimens (22%) which were provided with the notochord (cf. Fig. B in Plate I). Undifferentiated mass of cells was observed in all cases.

II. *Explantation of the piece together with the neural material*

(a) *Explantation of aU together with Na (Series aU plus Na)* : Although no notochordal differentiation was found in the explants of *aU* alone (Series *aU*), the same piece in this series displayed the development of the notochord

Table 1. Differentiation of the dorsal blastoporal piece explanted alone.

Experimental series	No. of cases	Notochord	Muscle	Mesenchyme	Undifferentiated cells	Undefinable neural tissue
Series aU	17	—	—	7(41%)	17(100%)	—
Series bU	18	4(22%)	2(11%)	11(61%)	18(100%)	4(22%)

in 10 out of 26 available specimens (38%, cf. Table 2). But, the added *Na* had nothing to do with the differentiation of the muscle and mesenchyme; the results concerning these tissues being similar to those of explantation of *aU* alone. A mass of undifferentiated cells was encountered in all cases.

It must be mentioned that the notochord, if appeared, was always in close contact with the archencephalic (2 cases), the deuterencephalic and/or undefinable neural structure (8 cases) developed from the added neural tissue. In the specimens lacking the notochord the added neural tissue developed always into either the archencephalic or undefinable structure. Since the anterior part of the plate is destined to give rise to the archencephalic structure in its

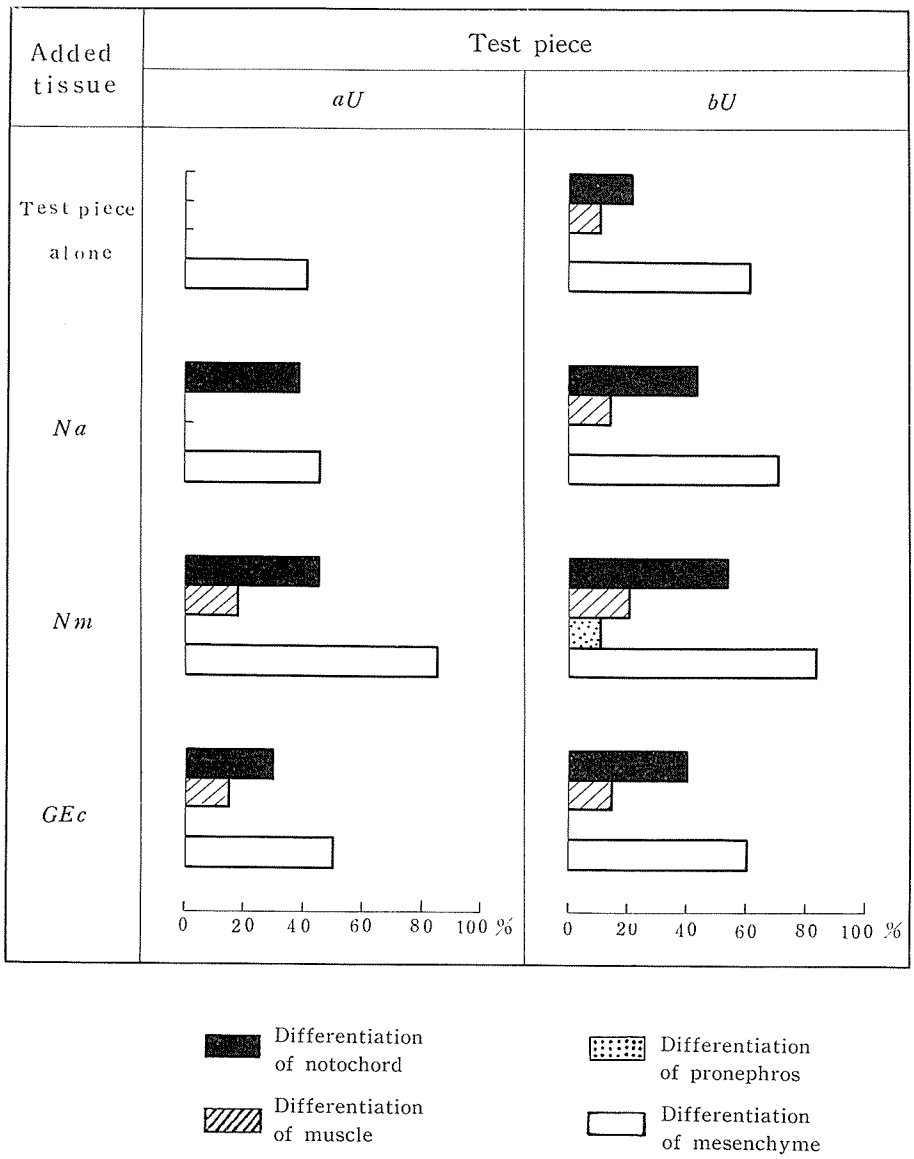

Fig. 2. Comparison of the developmental frequencies of various mesodermal tissues obtained in each experimental series.

normal development (MANGOLD and WOELLWARTH, 1950 ; WOELLWARTH, 1952), the "bedeutungsfremde" differentiation into the deuterencephalic structure in the case where the notochord differentiation had occurred may be ascribed, as TAKAYA (1956a, 1956b, 1959) had already pointed out, to the effect of the mesenchyme having developed adjacently to it.

(b) *Explantation of aU together with Nm (Series aU plus Nm)*: In the frequency of the notochordal formation no marked difference was recognized between the present and the previous series of experiments (10 out of 22 available explants, 45% ; cf. Table 2 and Fig. 2). But, the muscle fibres which

Table 2. Differentiation of the dorsal blastoporal piece explanted together with the neural tissue.

Experimental series	No. of cases	Noto-chord	Muscle	Pro-nephros	Mesen-chyme	Undiffer-entiated cells	Archen-cephalic struc-ture	Deuteren-cephalic structure and/or spinal cord	Undefin-able neural tissue
Series aU plus Na	26	10 (38%)	—	—	12 (46%)	26 (100%)	10 (38%)	4 (15%)	12 (46%)
Series aU plus Nm	22	10 (45%)	4 (18%)	—	19 (86%)	22 (100%)	—	18 (82%)	4 (18%)
Series bU plus Na	14	6 (43%)	10 (14%)	—	10 (71%)	14 (100%)	2 (14%)	3 (21%)	10 (71%)
Series bU plus Nm	19	10 (53%)	4 (21%)	2 (11%)	16 (84%)	18 (95%)	—	13 (68%)	6 (32%)

were never found in the previous series were noticed in 4 of the present cases (18%) in close association with the neural tissue. Fig. E in Plate I is a representative specimen in which the notochord and muscle fibres are seen between the spinal cord and the deuterencephalic structure.

(c) *Explantation of bU together with Na (Series bU plus Na)*: As compared with the test of the part *aU*, the mesodermal differentiation occurred slightly more frequently in this series; i. e., the notochord in 6 cases (43%), muscle in 2 cases (14%), and mesenchyme in 10 cases (71%) out of 14 available specimens. The neural tissue developed was the deuterencephalic structure in general (Fig. F in Plate I). Although in some cases no definable mesodermal structure appeared, the archencephalic differentiation from the added neural piece was always observed (cf. Fig. G).

(d) *Explantation of bU together with Nm (Series bU plus Nm)* : The frequency in the occurrence of the mesodermal structure was slightly higher than in the previous series ; i. e., in 10 out of 19 available cases the notochord was produced (53%), the muscle, in 4 cases (21%) and the mesenchyme, in 16 specimens (84%). Pronephric tubules, which were not found in any series of

the previous experiments, were encountered in 2 specimens. The neural tissue differentiated into the deuterencephalic structure and/or spinal cord according to its presumptive fate.

III. *Explantation of the piece pre-cultured in the vesicle of presumptive ectoderm*

The results of the two groups of previous experiments demonstrate that addition of the neural material provides the good conditions under which the notochordal formation from the part *aU* and *bU* occurs more frequently. On the other hand, there is a possibility that the aging of the ectoderm utilized as an envelope exerts some different effect on the notochordal differentiation from its primodium; i. e., the test piece displayed the notochordal formation more frequently, when pre-cultured in the envelope of the neural competent ectoderm taken from the early gastrula, than when it was pre-cultured in the incompetent ectoderm isolated from the neurula (KATO and OKADA, 1956 ; KATO, 1958, 1959). Surely in the former case the neural induction always occurred. Therefore, in the present series of experiments the young ectodermal piece (*GEc*) was exclusively used as the wrap of pre-culturing the test piece. After 24 hours' culturing, the test piece was dissected out, wrapped again with the incompetent neurular ectoderm and cultured further. Thus, the test piece would be expected to receive some influence coming from the induced neural tissue during the period of pre-culturing.

(a) *Explantation of aU* (*Series aU in GEc*): A possible effect of pre-culturing in *GEc* envelope was inferred from a comparison of the results between this and previous series (control group) ; i. e., in the present experiment the notochord was found in 6 out of 20 available cases (30%) against zero percent in the previous series (cf. Table 3 and Fig. H in Plate I). In addition, a small number of muscle fibres developed in 3 cases (15%). As expected, any sign of the neural development was not recognized, but the gastrular ectoderm used as the first envelope showed without exception a part of palisadal structure at the time of isolating the test piece.

(b) *Explantation of bU* (*Series bU in GEc*): In this experiment a considerable increase was also noticed in the frequency of the notochordal formation.

Table 3. Differentiation of the dorsal blastoporal piece explanted after contact with the competent gastrular ectoderm for 24 hours.

Experimental series	No. of cases	Notochord	Muscle	Mesenchyme	Undifferentiated cells	Undefinable neural tissue
Series aU in GEc	20	6(30%)	3(15%)	10(50%)	20(100%)	—
Series bU in GEc	20	8(40%)	3(15%)	12(60%)	19(95%)	1(5%)

Fourty percent of the available cases (8 cases) contained the notochord (cf. Table 3). Muscle fibres were recognized in some specimens (3 cases, 15%), and mesenchyme appeared in as many as 12 cases (60%). There was an explant in which both the notochord and a neural fragment were found. This neural fragment should be derived from the induced neural material accidentally included in the test piece, when it was isolated from the enveloping ectoderm after pre-culturing. In fact, the enveloping ectoderm showed the palisadal structure towards the neural tissue when it was separated from the test piece.

To sum up, the results of this group apparently indicate the positive effect through the neural induction of the young gastrular ectoderm on the notochordal differentiation from the blastoporal piece. But the effect is not so strong as in the case of adding the developing neural tissue. This may be due to the short period of time during which the test piece will receive the influence from the developing neural tissue, because if the test piece is continued to culture in the envelope of young gastrular ectoderm, it can develop into the notochord with higher frequency than that found in the present group of experiments (cf. KATO, 1958).

Discussion

Enhancement of the notochord forming potency by the developing neural tissue

The present experiments have disclosed that the frequency of the notochordal development from the dorsal blastoporal piece, when cultured *in vitro*, is increased either by addition of the developing neural tissue or by a temporal contact with the neural competent ectoderm of the early gastrula. But, addition of the differentiated neural tissue from the tail-bud embryo is ineffective in enhancing the notochordal formation from the same piece (KATO, 1957). It will be stated, therefore, that the simultaneous presence of the developing neural tissue is favourable for the differentiation of notochord from the blastoporal piece.

ROUND and FLIKINGER (1958) suggested the migration of RNA from the chordamesoderm to the overlying ectoderm during the course of gastrulation from the result that the decrease of RNA in the chordamesoderm is accompanied with the increase of RNA in the ectoderm. The points whether RNA decreasing in the chordamesoderm causes the intensification of the notochord forming potency of the area, and if so, whether the addition of the developing neural tissue helps this decrease are still uncertain.

Significance of the simultaneous presence of the neural potency for the enhancement of the notochord forming potencey

The meaning of the developing neural tissue and the neural competent ectoderm for the notochordal differentiation from the presumptive area does

not necessarily imply that it is indispensable to be contact with each other throughout the whole course of gastrulation and notogenesis. Before reaching to the blastopore, the presumptive notochordal material is not rigidly determined, but when it once occupies the region just above the blastopore, it is definitely determined to undergo the notochordal formation even by itself. In other words, during this process of migration the plasticity in terms of producing the neural tissue has been missed from the area (KATO, 1959).

Naturally it arises a question of whether the different factor or factors operate in enhancing the notochord forming potency of the presumptive notochordal material in the two cases, namely when this material shifts to occupy the proximal area to the blastopore of the intact embryo and when, as in the present experiments, it is cultured together with the developing neural tissue for the same period of time. In this connection, it will be worth mentioning that the notochordal formation from the undetermined material (part *bU*) can be enhanced by means of increasing a number of the test pieces fused together (KATO, 1963). But even in this case the notochordal development occurred exclusively in the case when the neual tissue is simultaneously induced. The fact seems to indicate that the presence of the neural potency in this material (part *bU*) is concerned in elevating the notochord forming potency which is otherwise unable to realize the notochordal differentiation. Under these considerations, it may be not so unreasonable to assume that addition of the neural tissue causes the test piece to perform the notochordal differentiation by means of elevating the notochord forming potency in it.

This supposition may be intensified from the following fact. That is, the non-notochordal material in the dorsal blastoporal region (part *aU*) can manifest the notochord forming potency under some experimental conditions (TAKAYA, 1953; KATO and OKADA, 1956; KATO, 1957, 1958; MASUI, 1960), but it can hardly be possible to realize the notochordal differentiation by means of increasing a number of test pieces, as in the case when the notochordal area (part *bU*) is multiplied (KATO, 1963). No notochordal formation in this case may be due to the lack of the neural differentiation from this part *aU*. So far as we know, the notochordal formation from this material is nearly impossible unless the neural tissue is present whatever the experimental conditions are applied to (KATO, 1957, 1958, 1959, 1963; Series *aU plus Na*, *aU plus Nm*, and *aU in GEc* in the present experiments). Therefore, the view will be right that the presence of the neural potency in the part *bU* may play some important role for the elevation of the notochord forming potency in it. But, the physico-chemical nature involved in the phenomenon remains to be proved.

Progressive intensification of the notochord forming potency during gastrulation

There are some experimental data which suggest the relationship beween

morphogenetic movement and intensification of the notochord forming potency. The previous experiments of the author (KATO, 1958) demonstrated that the nearer the part moves towards the blastopore, the higher the notochord forming potency in it. IKUSHIMA (1959, 1961) has maintained also the importance of the aggregation and congregation movement of cells in the blastoporal area for the differentiation of the notochord. These apparently indicate that the presumptive notonchordal material is more rigidly determined as it shifts towards the blastopore during the course of gastrulation.

However the movement of cells is not prerequisite to the notochordal differentiation, because the non-notochordal cells (part *aU*) just above the dorsal blastoporal lip of the early gastrula can differentiate more often into the notochord than the presumptive notochordal cells of the part *bU*, provided that the test pieces are cultured in the envelope of the young gastrular ectoderm (TAKAYA, 1953; KATO, 1958). What does this paradox mean? Here, it may be interesting to cite that the cells situated at the site corresponding to our part *aU* show specifically higher and faster incorporation of labelled amino acids than in other parts of embryo (SIRLIN, 1960). This high amino acids metabolism in the proximal part to the blastopore would mimic the physico-chemical activity of cells which are moving towards the blastoporal lip.

In short, the determination of the notochord in the presumptive chorda-mesodermal area will be assumed to occur as follows: First, the notochord forming potency spreads over the dorsal part of the blastopore as a labile state, secondly, the potency becomes restricted gradually in the presumptive notochordal area with its gradual intensification as the gastrulation movement proceeds. Eventually it is determied in fixed state when the area arrives just above the blastopore. The intensification and determination of the potency may link in nature with the special metabolism of this blastoporal area.

Role of the neural tissue in the mesodermal differentiation

The present experiments indicate that addition of the developing neural tissue causes the explanted blastoporal lip to develop more often into the notochord, muscle and other mesodermal tissues. The similar phenomenon has been reported by several authors. For instance, YAMADA (1939) demonstrated that the muscle differentiation from the explanted somitic material depends on the simultaneous development of the neural tissue from the competent ectoderm. This phenomenon was further analyzed by MUCHMORE (1958), and positive effect of developing neural tissue was confirmed. Both of the maturation process of the myotome cells and the development of the vertebral cartilage depend also on the inducing substance coming from the spinal cord (HOLTZER and DETWILER, 1953, 1954; HOLTZER, LASH and HOLTZER, 1956). The role of the developing neural tissue in the process of mesodermal differentiation may be different in respective developmental system which occurs at the different stage of development. Further investigation is required on the problem of

whether the same substance(s) coming from the developing neural tissue can call forth the cartilage differentiation on one hand and the notochrdal differentiation on the other, depending on the difference of origin and stage of the mesodermal material. Anyhow, it is likely that the role of developing neural tissue revealed by the previous and present experiments is to raise the special metabolism of cells to the level at which the notochord forming potency can work preferentially among other potencies.

Summary

(1) The explanted pieces taken from the dorsal blastoporal lip of the early gastrulae differentiated more often into the notochord in the presence of the developing neural tissue than in the case of its absence.

(2) The notochordal formation from the test piece was found to increase when it was previously put in contact with the neural competent gastrular ectoderm for 24 hours.

(3) The factor(s) concerning the progressive intensification of the notochord forming potency in the dorsal blastoporal part was discussed on the basis of the results obtained from the present and previous experiments of the author.

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Explanation of Plate I

Key of abbreviations: A: Archencephalic structure, D: Deuterencephaic structure, Mes: Mesenchyme, Ms: Muscle cells, N: Notochord, S: Spinocaudal structure, U: Undifferentiated cells, Un: Undefinable structure of neural tissue.

Fig. A. Differentiation of mesenchyme and undifferentiated cells from an explant of *aU* alone.

Fig. B. Differentiation of notochord, mesenchyme and fragmental neural tissue from an explant of *bU* alone.

Fig. C. Differentiation of notochord, mesenchyme and archencephalic structure from an explant of *aU plus Na*.

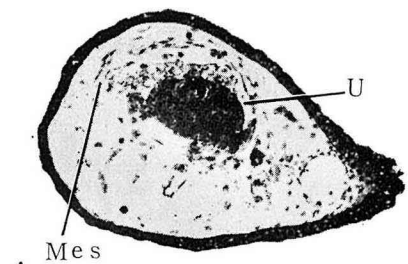
Fig. D. Explant from *aU plus Na*, showing archencephalic formation and undifferentiated mass with mesenchymal cells.

Fig. E. Explant from *aU plus Nm*: *Nm* differentiated into the spinocaudal and deuterencephalic structures, and *aU*, into notochord, muscle fibres and mesenchyme with undifferentiated cells.

Fig. F. Explant of *bU plus Na*, showing notochord in contact with the typical deuterencephalic structure.

Fig. G. Explant of *bU plus Na*: *bU* remains undifferentiated mass of cells. Each test piece occurs separately and *Na* shows the develoment of archencephalic structure.

Fig. H. Differentiation of notochord and mesenchyme from an explant of *aU* contacted temporarily with *GEc*.



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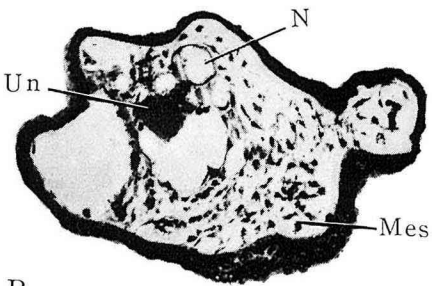

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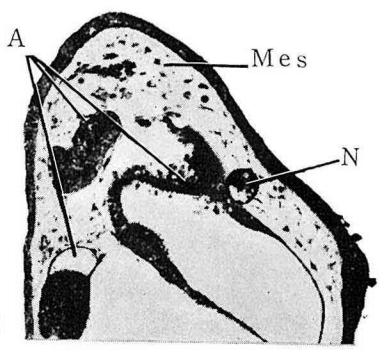

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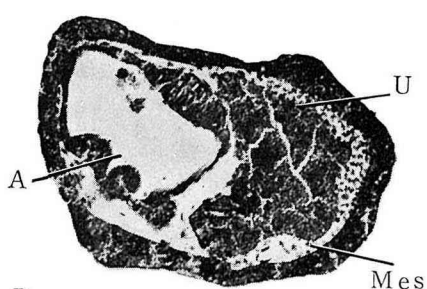

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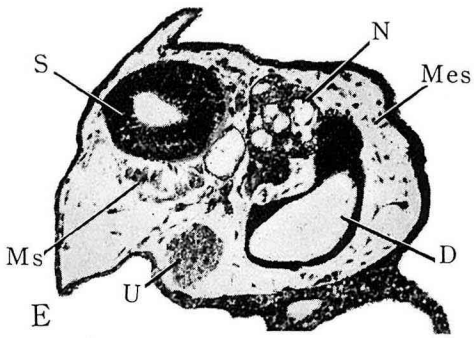

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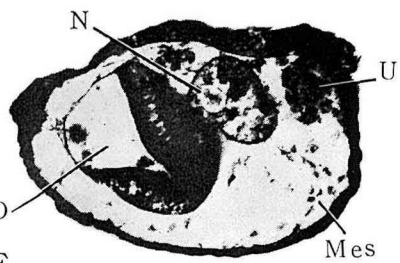

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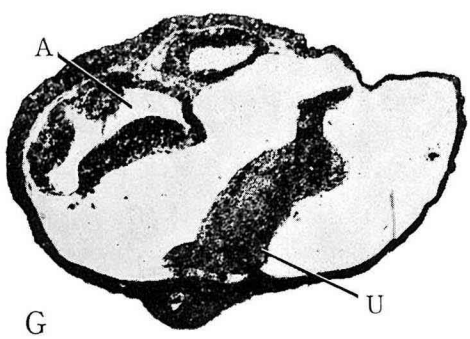

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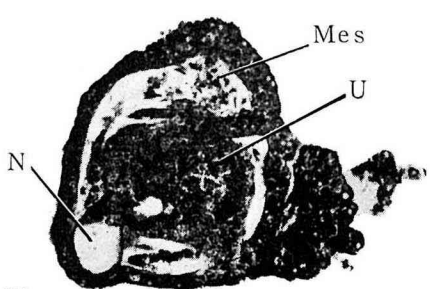

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