An Experimental Study of the Development of the Notochordal Sheath

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WITH ONE PLATE

INTRODUCTION

The prime object of the studies to be described here was the investigation of the origin and epigenetic relations of the notochordal sheath. In addition, some data were collected concerning the effects on the development of the embryo of the removal of the notochord or its substitution by some other elongated strand of material, such as human hair. Many of the phenomena encountered in the latter studies have recently been described by Kitchin (1949) and Mookerjee (1952), so that it is with questions relating to the notochord sheath that we shall be chiefly concerned.

It has been generally accepted up to the present time that the sheath is a secretory product of the notochord. This view is based on the descriptive studies of a number of comparative anatomists who dealt with the formation of the sheath and the origin of the vertebral column. Of the older workers, one may mention Hasse (1892), v. Ebner (1896), Klaatsch (1895), and Schauinsland (1906). In more recent times Gadow (1933) came to the conclusion that the sheath is formed by the notochord cells, and so did Goodrich (1930) in his Studies on the Structure and Development of the Vertebrates. H. K. Mookerjee (1935) raised doubts as to Gadow’s interpretation of the development of the vertebral column, but did not question his views about the origin of the sheath. Tretjakoff (1927) was not satisfied by the prevailing ideas; he argued that the outer layer of the sheath (the elastica externa) was of mesoblastic origin, but he still held that the inner fibrous layer was secreted by the chorda.

From an entirely different point of view, the sheath has been interpreted by Studnička (1913) and Held (1921) as mesenchymal in origin. Williams (1942) also found reasons to support the view that the sheath originates from the adjacent sclerotomal tissues. In his studies on the self-differentiating powers of isolated anlagen Holtfreter (1939) found that chorda cells removed from early embryonic stages never developed a sheath. He concluded that the sheath-forming materials originate from the extra-chordal mesoblastic cells. The experiments to be described here fully corroborate this view, and further show that

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transplanted fragments of chorda can induce the laying down of a sheath by the mesoblastic cells in other regions of the body.

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MATERIALS AND METHODS

Embryos of *Triturus alpestris*, *T. palmatus*, and axolotl were used. Operations were carried out in Holtfreter solution, whose pH was kept at 7 by phosphate buffer. Healed embryos were transferred to \( \frac{1}{10} \) Holtfreter. All culture solutions were made up with 0.05 per cent. sodium sulphadiazine.

The excision of the notochord was carried out through the ventral wall of the embryo. This was placed with its dorsal side downwards in a shallow depression in a wax operating dish and a long incision made from anterior to posterior on the ventral surface. This was gradually deepened through the endoderm, until the archenteron cavity was reached. The two halves of the embryo were allowed to fall away from one another, and the notochord removed through the gap thus left open. In this way the whole chorda, or any part of it, can be excised with a minimum of damage to the neural plate or to the more lateral mesoderm. After the operation the two sides of the embryo were carefully brought back into contact, and the embryo left on its side for healing, which was usually quite successful; in cases in which healing was incomplete and yolky material protruded through the wound, death usually occurred within a few days. The excised chordal material could be carefully freed of adherent mesodermal cells after its removal, and this was always done to the fragments which were to be used as isolates or transplants.

In some embryos suitable lengths of human hair, sterilized by boiling in distilled water for 15 minutes, were substituted for the excised chorda. In most cases these moved from their original position during later development, or were even completely extruded from the embryo; but in some specimens they remained in place, occupying precisely the position from which the chorda had been excised.

The embryos were fixed in Bouin's fluid, sectioned at 10\( \mu \), and stained in Delafield’s haematoxylin.

FORMATION OF THE NOTOCHORDAL SHEATH

i. Isolation experiments

Pieces of presumptive notochord, carefully cleaned of any other adherent cells, were cultivated in isolation in Holtfreter solution for periods of up to 2 weeks. Isolates from stages as young as the late gastrula were able to carry out their histological differentiation nearly normally, the cells becoming vacuolated and polyhedral, but remaining somewhat smaller than they would in normal development (Mookerjee *et al.*, 1953). In isolates from the late gastrula or early neural plate stages the whole mass developed into such notochordal tissue, but no trace of a surrounding sheath was formed. The same was true with isolates from the stage with elevated neural folds (Plate, fig. A). In the normal embryo
the sheath is beginning to be laid down during the later stages of neural tube formation. Isolates from these or later stages therefore include some sheath. During their later development in saline solution no further deposition of sheath material occurs, although the notochord itself continues to differentiate normally. From early tailbud isolates one thus obtains well differentiated notochords surrounded by very thin sheaths.

If the isolated fragment is not wholly made up of presumptive chorda material, but includes the whole axis (neural tube, chorda, and somite mesoderm), a sheath develops round the chorda which is of a thickness comparable to that found in the normal embryo. Thus the failure of the sheath in pure chordal isolates is not due to the conditions of culture.

ii. Implantation experiments

When cleaned fragments of presumptive chorda, similar to those used in the isolation experiments just described, are implanted into other sites in the embryo, they develop sheaths whenever they are in a region containing mesoderm. This occurs not only in the trunk region of the body, but even in the head (Plate, fig. B), where there is no axial mesoderm but only head mesenchyme available. It seems that any mesodermal tissue is capable of depositing a sheath around an implanted chorda. On the other hand, in specimens in which the grafted chorda is, for part of its length, in close contact with the endodermal wall of the gut, it is found that the sheath is lacking in just that region.

iii. Notochordectomy experiments

Notochords were excised in mid-yolk plug, small yolk plug, and neural plate stages, as well as from later embryos.

The fusion of the somites and the thickening of the floor of the neural tube seem to be straightforward consequences of the absence of the chorda; they have been described not only by Kitchin (1949) and Mookerjee (1952), who removed the chorda surgically, but also by Lehmann (1934, 1937) and Cohen (1938), who suppressed chorda development by means of lithium. An attempt has been made to discover how far the effects are purely mechanical results of the absence of a central strand by substituting a relatively inert material (human hair) for the excised notochord. Only in comparatively few embryos did this remain in place during later development. In these specimens the hair effectively held apart the two rows of somites and prevented their fusion, and the neural tube then had its normal shape, with a thin floor (Plate, fig. C). The embryos, however, did not elongate appreciably better than those from which the chorda was simply removed without anything being substituted for it. The sheath of the notochord is absent round the implanted hair.

When the developing chorda is excised from younger stages (late gastrulae) the results are quite different (Mookerjee, 1952). The mesoderm mantle is at this time still plastic in its developmental capacities, and the missing organ is replaced. Fig. D of the Plate shows examples of embryos in which it is certain that
the presumptive notochord was removed from at least the greater part of the body, but in which a chorda of nearly normal size has been formed by regulation. The regulated chorda is always accompanied by its sheath.

Whenever a regulated notochord is found it is always accompanied by somitic mesoderm, though the converse is not true and somites may be found without notochord. This is of course what would be expected on Yamada's (1940) theory that the chorda represents a 'high' level on a gradient on which somites are the expression of a 'lower' level. The capacity of the mesoderm to regulate and produce a notochord after the original anlage has been excised gradually disappears during the early neural plate stages, and has vanished by the time the neural folds are well marked (Mookerjee, 1952). These results demonstrate that sheath formation never occurs without the presence of the notochord cells.

The embryos in which notochords have been reconstituted in this way can carry out a fair degree of elongation in the tail-bud and later stages, but are always considerably shorter than control embryos. This is probably not caused by any qualitative defect of the regulated notochords; in particular it is worthy of remark that the notochordal sheath, which as we shall see probably plays an important role in the mechanism of elongation, is quite normally formed. There is, however, a quantitative deficiency in the regulated notochords, which are considerably reduced in cross-sectional area. It is probable, in fact, that they are from the beginning smaller than normal in total volume, and that this is the basic reason for the comparatively slight elongation of the embryos.

**DISCUSSION**

The isolation experiments reported here fully confirm the view that the notochord does not form a sheath when other mesodermal tissues are absent. The transplantation experiments demonstrated that this layer is laid down around notochords which are placed among a typical mesoderm, but not around inert substances such as human hair even when the latter are in the position normally occupied by the chorda. The evidence therefore seems to be conclusive that the notochordal sheath is secreted by non-notochordal mesoblastic cells, and that this secretion is induced by contact with the chorda.

As was pointed out in the Introduction, this conclusion is contrary to the view of the origin of the sheath which has been usually accepted by comparative anatomists. It renders necessary a reconsideration of the relation between the structure of the fully developed sheath and its mode of secretion. The usual view at the present time may be presented in the words of Goodrich (1930): 'At an early stage the notochordal epithelium secretes a thin covering membrane in which intercrossing elastic fibres become differentiated. This is the elastica externa. Next is secreted, also by the notochordal epithelium, an inner and usually thicker fibrous sheath' (the elastica interna). By the notochordal epithelium Goodrich refers to the outer layer of cells in the differentiating notochord (cf. Mookerjee et al., 1953). Since, as we have seen, the sheath is not formed
by the notochord itself but by cells which lie outside and around it, one cannot consider that the outermost layer of the sheath was the first to be secreted and the inner ones were laid down at successively later periods. Moreover, such an idea does not take into account the fact that the diameter of the notochord increases considerably after the sheath first appears, so that the first-formed sheath would have to be very considerably stretched if it were to accommodate the material which eventually fits inside it.

Soon after the sheath is first formed the notochord can be pushed out from within it, leaving the sheath isolated like an empty sausage skin (Plate, fig. E). It is a thin tough membrane with a glistening surface, and is similar in structure to the more external layers (elastica externa) of later stages. By the time the sheath has thickened, the inner layer (elastica interna) is formed of thicker fibres and has a dull surface. Since the sheath is laid down from the outside, one must conclude that the glistening outer layer of the early stage does not simply persist in the same condition and constitute the similar-seeming layer of later stages, but that on the contrary it gradually becomes converted into more fibrous material, while a new glistening layer is laid down outside it. It seems probable that this transformation from the glistening to the dull condition is a result of active physiological processes, which also rearrange the material so as to increase the diameter of the sheath and thus accommodate the growing notochord.

The increase in diameter of the sheath does not, however, passively keep pace with the growing volume of the notochord. This is shown by the fact that isolated fragments of presumptive chorda, which are not clothed with a sheath, increase in diameter relatively more, and in length less, than they would do normally. The elongation of the notochord, in fact, seems to be a direct result of its confinement within a relatively inelastic sheath, which forces the increase in volume to take this form. This plays an extremely important role in the development of the embryo as a whole, since the extension of the dorsal axis of the body is dependent on the growth in length of the notochord.

SUMMARY

1. Pieces of presumptive notochord of amphibian embryos, carefully cleaned of other mesodermal cells, fail to form notochordal sheath when cultivated in isolation, but a sheath is formed around them when they are transplanted to any other site in the embryo in which they come in contact with mesoblastic cells. The sheath is absent from those regions of transplanted notochords which are in contact with non-mesodermal tissues such as endoderm.

2. No sheath is formed around pieces of human hair placed in the position from which the notochord has been excised.

3. If the presumptive notochord material is excised from late gastrulae of *Triturus*, a new notochord may be formed by regulation from the more lateral mesoderm. The regulated notochords are always provided with a sheath, and conversely the sheath is absent whenever the notochord is missing.
4. It is concluded that the sheath is laid down by extra-chordal mesoblastic cells, which are induced to carry out this secretion by contact with the notochord. It is pointed out that this contradicts the conventional view held by most comparative anatomists, who have attributed the secretion of the sheath to the external layer of notochord cells (the ‘notochordal epithelium’).

REFERENCES


EXPLANATION OF PLATE

A. Differentiation of notochord cells from a neural fold stage after 12 days' cultivation in Holtfreter saline. Note the absence of a notochordal sheath.

B. Embryo with notochord grafted in neural plate stage to the left of the main axis. Note the prominent sheath formed around the grafted notochord.

C. Cross-section of dorsal axis of an embryo in which a hair had been substituted for the notochord at an early neural plate stage. Note the normally shaped neural tube.

D. Section of an embryo to show the regulated notochord with a sheath.

E. Living notochord from a young larva. The cellular notochord has been removed from the sheath of the upper part of the notochord, and lies to the right of it.