THE FATE OF THE NOTOCHORD AND DEVELOPMENT OF THE INTERVERTEBRAL DISC IN THE SHEEP, WITH OBSERVATIONS ON THE STRUCTURE OF THE ADULT DISC IN THESE ANIMALS.

By E. W. CARLIER, M.B., *Senior Assistant to the Professor of Physiology in the University of Edinburgh.* (PLATE XX.)

IN the course of my general histological work I happened to notice that the nucleus pulposus of the intervertebral disc of the adult sheep was not always situated in the centre, but had more frequently an eccentric position in the disc; I therefore resolved to investigate the subject from an embryological point of view.

E. H. Weber[1] was amongst the first to notice the gelatinous part of the intervertebral discs, and Luschka[2] first pointed out that this gelatinous matter is referable to the notochord. Dursy,[3] on the other hand, denies that the chord participates in the formation of the nucleus pulposus, while Heiberg[4] and Kölliker support Luschka's statement. Balfour[5] states that in mammals the notochord becomes first constricted in the centres of the vertebræ, and disappears in these after ossification; that in the intervertebral discs it remains unconstricted, and after undergoing histological changes remains throughout life as part of the nucleus pulposus in the axis of the intervertebral ligaments. He also observed a slight swelling of the notochord near the upper and lower margins of each vertebra.

Kölliker[6] believes that the notochord in man and higher mammals is not a cartilaginous rod, though it may be somewhat analogous. He states that the notochord remains for a considerable time in the intervertebral discs, and he describes, in new-born animals, a cavity in each disc equalling half the disc in length, occupied by a kind of soft mucous jelly coming mostly from the chord, but to some extent also from the softened elements of the disc itself, the remains of the chord being in patches, with nucleated cells full of vacuoles filled with fluid

[1] Meckel's *Archiv*, 1827.

[2] Luschka, "Die Altersveränderungen des Zwischenwirbelknorpel" (Virchow's *Arch.*, t. ix. p. 311, 1856).

[3] Dursy, *Z. Entw. d. Kopfes d. Mensch.*, &c., Tübingen, 1869.

[4] Heiberg, *Schenk's Mittheil., Wien. Inst.*, liv. ii., 1879, p. 119.

[5] Balfour, *Embryology*, vol. ii. p. 460.

[6] Kölliker, *Embryology* (French edition), 1882, pp. 415–439.

surrounded by a striated tissue containing round or stellate cells, sometimes like mucous tissue, sometimes like soft cartilage in appearance. With regard to the rabbit, he says the notochord is eccentric in position, being placed nearest the back; nodes are seen on the chord corresponding to the intervertebral discs. At a somewhat later period the backward projection is more marked. The disposition of the cells of the chord is remarkable. Within the vertebræ the cells are elongated, fibre-like; in the expansions of the notochord the cells are elongated in the long axis of the discs, as if the cells of the chord had been pushed into the discs by the bodies of the vertebræ; and no doubt the rapid growth of the cartilage produces a drawing out of the cells of the chord situated in it, which would therefore accumulate in the discs. The swellings of the chord in the thoracic region are smaller than in other regions. Subsequently the cells of the chord produce cartilage. The sheep seems to differ from the rabbit mainly in exhibiting an earlier tendency to form cartilage.

The Notochord.

I procured many sheeps' embryos, which I prepared according to methods to be afterwards detailed. I would merely state that most of the sections were made in a vertical-antero-posterior direction, and that, by using eosine and iodine-green as a double stain, I was enabled to stain fibrous tissue of a pink, and cartilage of a green colour.

I first examined the *lumbar region* only, with the following results:—In very young embryos the notochord runs in a straight line down the middle of the vertebral column, but ere long a change is observable. In an embryo 1¼ inch in length the notochord runs nearer the anterior than the posterior surface of the column, and shows a slight bending forwards in each intervertebral segment (Pl. XX. fig. 1). In an embryo 3¼ inches long this anterior position of the notochord is much more marked, while the slight bending in the disc is transformed into a well-marked V-shaped curve, with its apex pointing anteriorly (Pl. XX. fig. 5). In an embryo 5 inches long there is little change. In one 7 inches long the appearance is similar, with this exception, that the vertebræ, having now become almost completely ossified, the notochord has disappeared in them, remaining only in the intervertebral segments, where the apex of the V appears to be spreading out laterally to form a discoid flattened mass.

I next examined a fœtus just after birth, in which the notochord can be seen situated anteriorly, little of it remaining,

however, except the apex of the V, the limbs having gradually been absorbed. In the adult condition the apex of the V alone remains as the nucleus pulposus.

If the *dorsal region* be now examined, a similar series of changes may be observed in the notochord, with this exception, that the chord is placed nearer the *posterior* than the anterior surface of the vertebral column, with the apices of its V-shaped bends directly *backwards* (Pl. XX. fig. 3), the nucleus pulposus in the adult being also nearer the posterior than the anterior aspect.

There is a regular transition between these two extremes of position in the mid-dorsal and mid-lumbar regions, so that if the chord be traced downwards, it is seen gradually to leave the posterior aspect, to cross the middle line, and finally in the lumbar region to reach to the anterior aspect of the vertebral column. At the junction of the dorsal with the lumbar region, one or two intervertebral segments may be observed in which no V-like bending can be seen, the notochord spreading out equally in either direction (Pl. XX. fig. 4).

In the *cervical region* the arrangement is similar to that in the lumbar region, only less marked, and the chord is more central in position. The transition between its position in the cervical to that in the dorsal region takes place in a similar manner to the transition between the dorsal and lumbar regions.

In the *sacral region* the position of the notochord is again found nearer the dorsal than the ventral aspect of the vertebræ, but the bends instead of being V-shaped are now C-shaped, with the convexity directed dorsally (Pl. XX. fig. 6). The transition from the lumbar to the sacral region is not gradual, but abrupt, the notochord crossing obliquely within one of the vertebræ.

If we consider the spinal column in its whole length, we see that in the cervical region the notochord is nearly central in position with the apices of its V-shaped bendings projecting anteriorly; that it crosses the middle line at the junction of the cervical with the dorsal region, the disc between the 7th cervical and 1st dorsal vertebra exhibiting a spreading of the notochord in all directions. That in the dorsal region the notochord lies near the posterior surface of the spinal column, with the apices of

the V-like bendings directed backwards, the bendings projecting more and more until a maximum of backward projection is reached in the mid-dorsal region, then less and less till the notochord again crosses the axial line at the junction of the dorsal with the lumbar region; one or two discs in this region showing the notochord swelling out all round. That in the lumbar region the chord lies near the anterior surface of the vertebral column, with its V-shaped bends pointing anteriorly, a maximum of anterior projection being reached in the mid-lumbar region; that the chord again crosses the axial line at the junction of the lumbar with the sacral vertebræ, the chord crossing in the first sacral vertebra obliquely from before backwards; and that in the sacral region the chord lies quite near the posterior surface of the spinal column, the bends passing from a V to a C-shape. The slight swelling of the chord near the two extremities of each vertebra, as described by Balfour, is also visible in most cases. From the foregoing it is seen that the notochord follows very closely the curves of the vertebral column, and it would appear that in the dorsal and sacral regions the portions of the vertebræ situated on the ventral aspect of the notochord grow more rapidly than those on its dorsal aspect, whilst in the lumbar and cervical regions it is the opposite.

The tissue surrounding the notochord is at first composed of a mass of undifferentiated embryonic cells, which, however, soon become irregular in shape, and produce a jelly-like matrix. Next, the cells dispose themselves in rows, arranged concentrically around a spindle-shaped mass of similar but somewhat denser tissue. This mass has one of its poles projecting into the hollow of the V-shaped bend of the notochord, and by its growth would appear to press upon and force the notochord to assume its peculiar shape (Pl. XX. figs. 3, 5, 6); while in fig. 4, where no spindle-shaped body can be seen, the chord has spread itself out into a plate in the centre of the loose connective tissue which is arranged concentrically around it. Whether this unequal growth of the vertebral column produces the bending of the notochord, or whether the bending of the notochord necessitates the unequal growth of the column I must leave undecided.

As development proceeds, the notochord disappears in all

those parts of the head and spinal column that are converted into bone; therefore in the head no trace of the notochord ever remains in the adult. It is in the intervertebral segments, and in them alone, that any remains of the notochord can be found during adult life.

The cells of the notochord, which are at first somewhat rounded, become spindle-shaped or irregularly elongated, as described by Kölliker, and as shown in the figures. They again assume a rounded form, produce cartilage capsules, and ultimately undergo degenerative changes before the end of the first year; in which degenerated condition they seem to remain throughout life. They will be further alluded to in treating of the adult disc.

Development of White Fibro-Cartilage as seen in the Intervertebral Disc of the Sheep.

The loose embryonic cells which surround the notochord at first gradually produce a jelly-like matrix, resembling that of mucous tissue. Fibrils subsequently make their appearance in this matrix, and the tissue then closely resembles that form of fibrous tissue known as Wharton's jelly, in which the cells are irregular in shape, and scattered amongst the fibrils. The fibrils become more numerous and arrange themselves in lamellæ, around a spindle-shaped mass, produced in the centre of the disc. Possibly this, by its growth, bends the notochord, and with it the lamellæ on the opposite side, which therefore also became V-shaped (Pl. XX. figs. 3, 5). Gradually, as age advances, this spindle-shaped mass opens out, becoming looser in texture, and ultimately indistinguishable from the rest of the disc. This fibrous tissue undergoes a change with advancing age, whereby its chemical nature is altered. The cells begin to secrete hyaline capsules around them of a cartilaginous nature, and the fibres and matrix of the connective tissue gradually become infiltrated with chondrogen: consequently they are no longer stained pink, but purplish and then green, in the eosin-iodine green stain. This change can be seen occurring in embryos of the length of 7 inches; it is more marked at birth, and is complete before the end of one year.

From the foregoing it will be seen that the centre of the disc is not entirely composed of altered notochord, as has been described, but of an altered fibrous tissue. The peripheral parts of the disc are from the first composed of fibrous tissue, staining red in eosine (Pl. XX. fig. 1). It is arranged in lamellæ which are continuous with the periosteum of the vertebræ, and which are in all cases more numerous on the anterior than on the posterior aspect of the discs (Pl. XX. figs. 2, 3, 4, 5, 6). The peripheral lamellæ retain this character throughout life, being composed of a true fibrous tissue, as described by Klein, and not of cartilage as many have stated. The greater abundance of true fibrous tissue on the ventral aspect of the whole vertebral column, binding the vertebræ firmly together on that aspect, is doubtless of service in resisting the strain due to the weight and movements of the body.

The Adult Disc.

In the adult disc two structural elements may be considered—the fibrous and cellular.

The fibrous element, of which the greater part of the disc is composed, is arranged in lamellæ, many of which encircle the whole disc; some, however, exist only on the anterior, become thinner at the sides, and never reach the posterior aspect (fig. 8, 2, 4, 5). The lamellæ show a concentric arrangement around a mass of tissue, the remnant of the notochord. The lamellæ vary in thickness, those near the surface are the thinnest, but many thin lamellæ may be found scattered among those that are thicker and more centrally placed. The lamellæ vary in direction; the outer lamellæ are arranged concentrically (fig. 8, 1); immediately internal to these are lamellæ which are also concentric but which bulge out posteriorly, and so resemble the shape of an elbow-piece of ancient armour (fig. 8, 3). Within these are other lamellæ, the central parts of which bulge outwards all round the disc, and have therefore the shape of two truncated hollow cones united by their bases (fig. 8, 6); while within these again are lamellæ which in vertical section are seen to enclose an ovoid mass containing the remains of the old notochord. These, unlike the other lamellæ, never reach the adjacent surfaces of the vertebræ between which the disc is placed (fig. 8, 7). All the other

lamellæ are firmly attached to the bone. Within the elliptically-arranged lamellæ there is a felted fibrous mass, the fibres of which run in all directions.

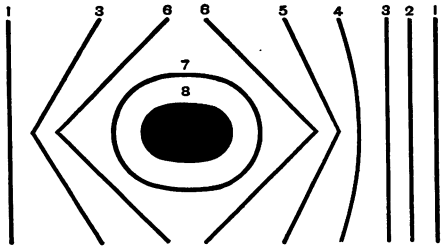


FIG. 8.—Schematic representation of the arrangement of the fibrous lamellæ in an intervertebral disc, from the dorsal region of an adult sheep. 1, concentric lamellæ; 2, 4, 5, lamellæ incomplete on the posterior aspect of the disc; 3, lamella of the shape of an elbow-piece of ancient armour; 6, lamella of the shape of a double truncated cone; 7, ovoid lamella; 8, centrum of felted fibres containing the remains of the notochord.

Arrangement of Fibres in the Lamellæ.—In the outer lamellæ the bundles of fibres are arranged obliquely, in such a manner that the fibres of two neighbouring lamellæ cross each other at a wide angle. In the lamellæ, which are disposed like an elbow-piece, the same arrangement occurs anteriorly, but the fibres gradually become less and less inclined, until in the middle line posteriorly, they are parallel and run vertically. In the lamellæ arranged like truncated cones, the fibrous bundles cross one another anteriorly at a more acute angle, whilst posteriorly they resemble those of the last-mentioned lamellæ. In the ovoid lamellæ the fibres are arranged in a manner closely resembling the lines of longitude on a globe. In the central mass the fibres run in all directions, thus giving a tangled or felted appearance to it.

From a mechanical point of view the arrangement above described forms a firm yet elastic whole—most rigid at its periphery where the lamellæ are most vertically placed, more elastic towards the centre where the lamellæ are placed like carriage springs, breaking any shocks which the vertebral column may sustain in the direction of its length. Yet the external lamellæ are not devoid of elasticity, as the oblique decussating arrangement of the fibres will allow of a certain amount of compression of these lamellæ in a vertical direction.

Such an arrangement must be highly beneficial to animals such as the sheep, that fight by butting with the head.

The fibres of the different lamellæ exhibit a remarkable difference in their staining properties, which points to a difference of chemical composition. Those of the lamellæ nearest the periphery of the disc stain of a deep pink with eosine and picrocarmine, swell up and become transparent when treated with acetic acid, and become converted into gelatine on prolonged boiling. They are, therefore, of the nature of white fibrous tissue. The fibres of lamellæ, situated internal to these, acquire a purplish tint on double staining with eosine and iodine-green, swell up imperfectly in acetic acid, and are only partially converted into gelatine on boiling, the jelly forming in little patches, whilst the remaining tissue swells and becomes white and opaque like cartilage. They therefore partake of the nature both of white fibrous tissue and of cartilages. The fibres of lamellæ still more centrally placed (Nos. 5, 6, 7, &c., in fig. 8) acquire a green instead of a purple colour on double staining with eosine and iodine-green; they are unaffected by acetic acid, and become swollen, white, and opaque on boiling. They have, therefore, the composition of cartilage, and yield no gelatine. It is peculiar, to say the least, that in the green parts it is not the hyaline interstitial substance of the matrix that stains so vividly, as one would be led to expect from the behaviour of hyaline cartilage stained in a similar manner, but the fibres imbedded in it. Since these fibres, as already stated, stain red in the young embryo, they must have become infiltrated with chondrogen.

The Cellular Element.—In the outer lamellæ the cells are reduced to thin plates; their nuclei alone being visible, they resemble in appearance the cells of adult tendon as seen in transverse section; if, however, lamellæ be torn from a fresh disc, teased in acetic acid, and stained in magenta, the cells may be seen in rows between the bundles of fibres, and clasping them; they closely resemble the cells which are found in the tendons of the tails of young rats when treated in a similar manner. They are, however, more irregular in shape, and here and there one may find a thin cartilage capsule enclosing a cell.

In all the inner lamellæ the cells closely resemble those of cartilage, each cell being surrounded by a hyaline capsule. Many are rounded in shape, but most cells are branched. The cells are arranged in rows, as one would expect from their arrangement in the fœtal condition. In profile view, the cells often appear spindle-shaped, some showing very long processes which occasionally anastomose with processes of neighbouring cells. In surface view, the cells present a more or less oval outline, with several branches. The cartilage capsule enclosing each cell is prolonged around the processes. Sometimes two cells are enclosed in a single capsule, each cell being surrounded by a thin daughter capsule of its own, as in hyaline cartilage.

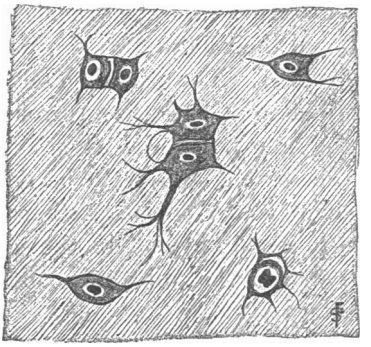


FIG. 9.—Branched cells of white fibro-cartilage from the region corresponding to 6, fig. 8.

The cells in the loose tissue, between the lamellæ, often have a striking appearance. They are scattered irregularly, and on surface view show numerous very long and much branched processes. They are also surrounded by cartilage capsules extending to the very tips of the processes (fig. 9). Some cells also exhibit signs of recent division, and are best seen near the upper and lower surfaces of the disc, where the lamellæ are wider apart than in its centre.

Remains of the Notochord.—I have already alluded to an ovoid mass, somewhat centrally placed in the disc, and have spoken of it as a tangled mass of fibres, but when examined more closely, it is seen to consist of cells embedded in a matrix

of hyaline cartilage surrounded by a tangle of fibres. These cells are all that remain of the notochord, and occupy the position of the apex of the V-like bend of the notochord seen in the embryo. The cells in these groups often have a degenerated appearance, many small cells being situated in one enlarged cell-space, similar to those that occur so frequently in the calcified costal cartilages of old persons. Some of these small cells may exhibit a thin capsule, but many of them are quite devoid of such a capsule, and resemble broken-down nucleated particles of protoplasm. This appearance of the *nucleus pulposus* has long been known; but besides these masses there are other cells of the notochord which seem to have escaped notice hitherto.

Towards the centre of the disc some cells may be seen that appear to have penetrated into the lamellæ, broken them up somewhat, and altered their nature, causing a fogged or misty appearance of their fibres. The cells themselves resemble those of cartilage, and are often of large size, surrounded by cartilage capsules. How the misty material is produced I am quite unable to say. These cells appear to be derived from a few cells of the notochord, which do not participate in its general bending, but seem to remain behind in the centre of the disc; these cells will be seen in the embryonic condition on referring to Pl. XX. fig. 1, *m*.

Attachments of the Intervertebral Disc to the Vertebræ.—At the margins of the disc the bundles of fibres of the lamellæ are continuous with the fibres of the periosteum of the vertebræ. Further in a line of demarcation is visible between the epiphysis and the disc; this line is evidently a sectional view of a plate of tissue, which separates the disc proper from the bone. The line is irregular, and stains of a violet or dark green colour; the irregularities which it presents are due to little elevations or depressions in the plate, caused by the presence in these localities of blood-vessels or little groups of cells, which indent its surface (Pl. XX. fig. 7). There are two of these plates, in one at the upper and one at the lower surface.

The fibres of the lamellæ which abut against these plates may be traced through them into the bone matrix, where they fade away, and by this means a very firm attachment of the discs to the vertebræ is secured.

My best thanks are due to Mr S. Fowler who has kindly made the drawings for me from microscopical preparations.

Methods.

All the embryos which I obtained were fixed with Kleinenberg's picro-nitric acid solution, the process being subsequently completed with alcohol. The sections were all cut in gum with a freezing microtome. Adult tissues were placed in a bone-softening fluid compound of 52 grammes of chromic acid dissolved in 1000 c.c. water, to which 10 c.c. commercial nitric acid is subsequently added. This fluid was changed every second day till the bone was sufficiently soft, after which the tissues were well washed in water and preserved in spirit.

The following method of staining appears to me to yield the best results, especially when dealing with adult tissues. Sections prepared as above are placed in methylated spirit for two or more days, after which they are placed in water to wash out the spirit, and transferred to water solution of eosine (1:1200) for one hour, then washed in distilled water to remove the superfluous stain, and placed in aqueous solution of iodine green (1:1200) for an hour, then washed in distilled water for a few minutes, and mounted in balsam by the usual method. The stain is permanent in balsam. For embryonic tissues half an hour in each dye is sufficient, but the preparations soon fade. In this way an excellent double stain is produced, the fibrous tissue becoming a brilliant pink, whilst cartilage is stained green; where fibrous tissue and cartilage intermingle the general tint is purplish.

When stained thus the individual fibres of the central portions of the disc stand out remarkably, each being quite distinct from the matrix in which it is imbedded. For cells this method is not very satisfactory, since they tend to shrivel, but with care this may be avoided. The cells stain red, their nuclei green. For the cell capsules this stain is invaluable, the finest processes being stained green, and standing out sharply from the surrounding matrix. For the cells an aqueous solution of hæmatoxylin is perhaps best, and should be used with glycerine as a mounting fluid. Unfortunately the colour fades after a time.

[Explanation of Plate.

EXPLANATION OF PLATE XX.

d. Dorsal aspect of vertebral column.
v. Ventral aspect of vertebral column.
b. Cartilaginous vertebra.
c. That part of the intervertebral disc that becomes converted into white fibro-cartilage.
f. That part of the intervertebral disc that remains throughout life as white fibrous tissue.
n. Notochord.
s. Spindle-shaped body.
p. Perichondrium.
m. Cells of notochord which do not participate in the general bending.

Figs. 1 to 6 are vertical antero-posterior sections of the vertebral column of embryo sheep.

Fig. 1. Embryo sheep, 1½ inch long. Mid-lumbar region, showing a slight bending of the notochord towards the ventral aspect. × 300.

Fig. 2. Embryo sheep, 3 inches long, cervical region, showing the V-shaped bend of the notochord directed towards the ventral aspect. × 100.

Fig. 3. Embryo sheep, 5 inches long. Mid-dorsal region, showing the V-shaped bend of the notochord directed towards the dorsal aspect. × 50.

Fig. 4. Embryo sheep, 5 inches long. Junction of dorsal and lumbar regions, showing the notochord spread out equally in all directions. × 50.

Fig. 5. Embryo sheep, 3¼ inches long. Mid-lumbar region, showing the V-shaped bend of the notochord directed towards the ventral aspect. × 100.

Fig. 6. Embryo sheep, 9 inches long. Sacral region, showing the notochord directed dorsally, the V-like bend being replaced by an arc-like curve.

Fig. 7. Junction line between disc and vertebra. *b*, bone of vertebra; *c*, hyaline cartilage; *j*, junction line; *f* white fibro-cartilage of disc.

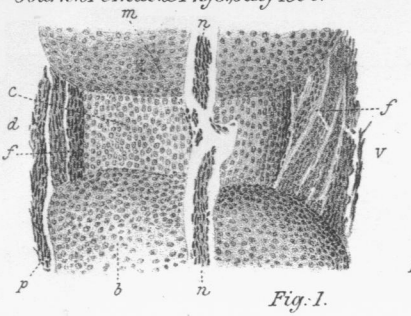


Fig. 1.

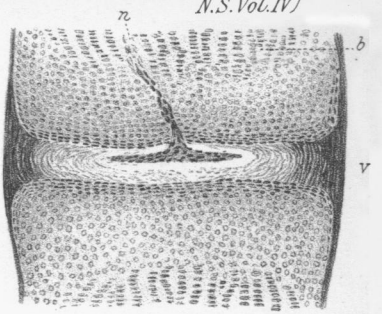


Fig. 4.

Fig. 2.

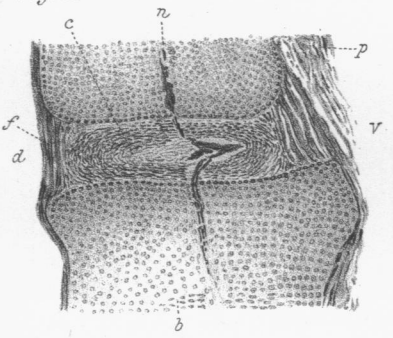


Fig. 5.

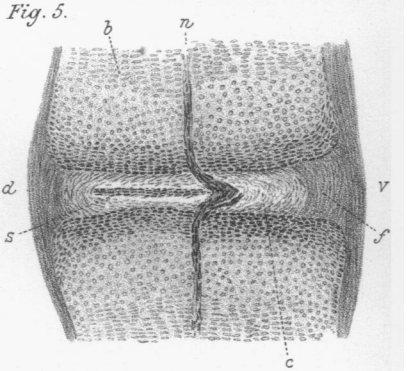


Fig. 3

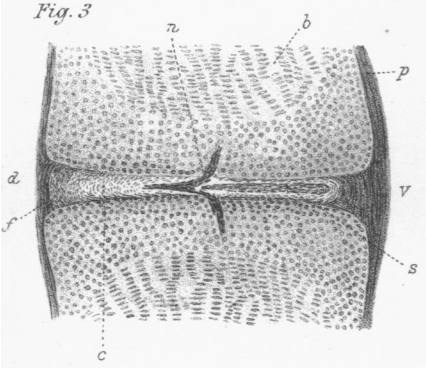


Fig. 6.

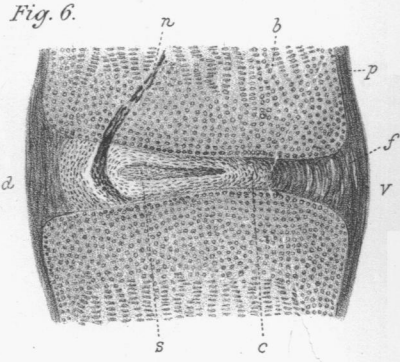
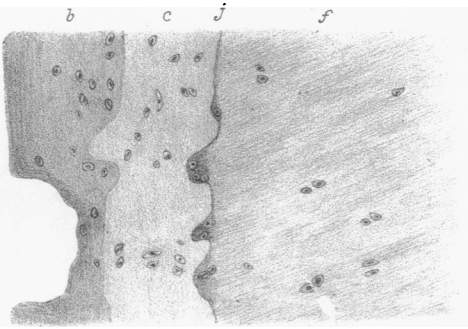


Fig. 7.



S. Fowler del.

F. Huth, Lith^r Edin^r

NOTOCHORD.