SOME OBSERVATIONS ON THE LYMPHATICS OF THE NASAL MUCOUS MEMBRANE IN THE CAT AND MONKEY[1]

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In the rabbit, cat, and monkey, experiments have shown that certain dyes and proteins (T-1824, trypan blue, egg albumin, and serum albumin) can be found in cervical lymph following intranasal instillation (Yoffey & Drinker, 1938; Yoffey *et al.* 1938). It was further shown in the rabbit and monkey that vaccinia virus, although it did not immediately enter the lymph stream following intranasal instillation, did so after a preliminary period during which the virus became established in the nasal mucosa; subsequently a steady stream of virus was found entering the blood through the cervical lymph ducts at all times from 12 hr. to 7 days after virus had been dropped into the nose (Yoffey & Sullivan, 1939). It was thought that a histological study of the lymphatics of the nasal mucosa might throw some further light on these observations, and accordingly the present work was undertaken.

For dyes or proteins to reach the lymph stream, they must pass through the intact mucous membrane, a layer of submucous connective tissue, and the endothelium of the lymphatic capillaries. That dyes and proteins can pass through lymphatic endothelium is well established (Drinker & Field, 1933). That protein in small amounts can pass through the intact intestinal mucosa is also usually accepted. Since the first observations of Uhlenhuth (1900) and Ascoli & Viganò (1903) the problem has been investigated by many workers, and extended to cover several kinds of protein. Reviews of the literature are given by Ratner & Gruehl (1934), and Alexander *et al.* (1936). As far as the nasal mucous membrane is concerned, Baum & Trautman (1925–6) described the presence of numerous "stomata" of considerable size, through which the submucous lymphatics were alleged to be in free communication with the exterior. We have been unable to see stomata in freshly fixed preparations, and it seems certain that, when present, they are due to post mortem autolysis at various points in the nasal mucous membrane.

[1] The expense of this research was aided in part by a grant from the Proctor Fund of the Harvard Medical School, for the Study of Chronic Diseases.

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MATERIAL AND METHODS

In several dogs and cats a rich lymphatic plexus was demonstrated, by the method of interstitial injection, in the lateral wall and septum of the nose. These preparations, however, tended to be somewhat patchy. The preparations about to be described were made on one cat and three monkeys (*Macaca mulatta*). In one monkey the posterior pharyngeal wall was exposed 20 min. after death, and at several points interstitial injections were made of small amounts of a 1 : 2 dilution of Higgins' India ink. The pharyngeal wall was then excised and fixed in Zenker, being kept flat by pinning to a cork sheet.

The best injections of the nasal lymphatics were made from the subarachnoid space. In one monkey and one cat under general anaesthesia (intraperitoneal nembutal) a small hole was drilled in the cranial vault and a fine needle inserted into the subarachnoid space. Through this 3·0 c.c. of Higgins' India ink (diluted 1 : 2 with normal saline) were slowly injected. Unfortunately no record of the injection pressure was made, but it was noted that toward the end of the injection increased pressure was necessary, which must have been well above the normal pressure of the cerebrospinal fluid. In a third monkey a similar injection was made of a 0·5 % solution of potassium ferrocyanide and iron ammonium citrate.

After the animals had been killed the head was skinned, cut off, and the mandible and palate removed to allow better access of fixative to the roof of the nose. Fixation was in 10 % formol-saline, to which, in the case of the monkey injected with potassium ferrocyanide and iron ammonium citrate, 1 % of hydrochloric acid was added. After 2 weeks the vault of the skull was sawn off, and also the base of the skull behind the pituitary fossa. The frontal lobes of the brain were dissected away, leaving undisturbed the olfactory bulbs lying on the cribriform plate. The specimens were then returned to fixative for another 2 weeks, after which they were transferred to 5 % nitric acid, in which they were left, with frequent changes, until decalcification was complete. Each specimen was then trimmed so as to leave behind only the olfactory bulbs, the cribriform plate, and the roof of the nasal cavity. In the cat, some of the many laminae in the roof of the nose (ethmoturbinals) were cut off and cleared by the Spalteholtz method. The remainder, and also the blocks of tissue from the two monkeys, were then embedded in celloidin, and serial sections cut at 12μ. Sections were counterstained most effectively with carmine.

LYMPHATICS IN THE POSTERIOR WALL OF THE NASOPHARYNX

Pl. I, fig. 1 is a low-power camera lucida drawing of the mucous membrane from the posterior wall of the nasopharynx of a monkey. The huge size and number of the lymphatics are sufficiently obvious. This heavy distribution of lymphatics is characteristic of the nasopharynx in general, a circumstance doubtless determined by the fact that the nasopharyngeal mucosa suffers from hyperaemia and mild irritation probably more often than any other part of the

body. It is thus constantly flooded with proteinized fluid from the blood capillaries, and this fluid must be carried off by the lymphatics since it will not return directly to the blood capillaries. An analogous situation is presented by the vagina, particularly in monkeys. These animals experience an oedema of the vulvar region which extends up into the vagina at each menstrual period. The oedema fluid is rich in protein, and a complicated and very extensive collection of lymphatics is necessary to remove it (Wislocki, 1939).

LYMPHATICS OF THE SEPTUM AND LATERAL WALL OF THE NOSE

Pl. I, fig. 2 shows the lymphatics in one of the numerous ethmoturbinal laminae in the roof of the cat's nose. The lamina consists of a very thin bony plate with mucous membrane and submucous lymphatics on either side. Looking through the Spalteholtz-cleared lamina the two superimposed layers of lymphatics are therefore seen, a fact which becomes clearer on noting the obliquely cut edge of the lamina, where one layer projects beyond the other. In Pl. I, fig. 3 these laminae are cut at right angles to their surface, and it is evident that the lymphatic plexus is disposed not only in a plane parallel to the mucous membrane, but also at right angles to it, i.e. it possesses depth as well as surface extent. This is brought out more clearly at a higher magnification (Pl. II, fig. 4).

It is significant that the lymphatic vessels are closely applied to the columnar epithelium, with usually only a very thin layer of subepithelial connective tissue intervening. Dyes, proteins, viruses, or bacteria must therefore come into contact with the submucous lymphatics as soon as they pass through the epithelium. It can thus be readily understood why it is that vaccinia virus, from a primary focus in the mucous membrane, can so constantly be found in cervical lymph (Yoffey & Sullivan, 1939). Similar considerations apply to the finding in cervical lymph (rabbit) of type III pneumococci after nasal instillation (Schulz *et al.* 1938). It is possible that the virus of the common cold disseminates from the nasal mucosa in the same way.

Pl. I, fig. 3 and Pl. II, fig. 5 are from the cat and monkey, respectively, in which India ink was injected into the subarachnoid space. The sections pass through olfactory bulb, cribriform plate, and roof of nose, the olfactory bulb being cut at right angles to its long axis. In each specimen olfactory nerve bundles may be seen passing through foramina in the cribriform plate; particles of India ink are also passing through the same foramina, surrounding the nerves. In the cat (Pl. I, fig. 3) there is present, immediately below the cribriform plate and on either side of the lamina perpendicularis of the ethmoid, a dense accumulation of India ink in the tissues, suggesting a sudden extravasation; from this focal accumulation the ink passes freely into the submucous lymphatics. As far as we can make out in these sections (cat) there is perfectly free communication between the perineural space and the connective tissue spaces below the cribriform plate. This apparently free communication may

have been artificially produced by the injection pressure rupturing a thin perineural sheath. Unfortunately there is such a dense mass of ink that intimate structural details are difficult to make out.

In the monkey (Pl. II, fig. 5) ink does not seem to have passed through the cribriform plate quite so freely, but again the perineural location of the ink is perfectly evident (Pl. II, fig. 6). In this particular preparation (Pl. II, fig. 5) the mucous membrane of the roof of the nose has come away. In the monkey injected with potassium ferrocyanide and iron ammonium citrate the mucous membrane remained *in situ*, and a well-marked submucous lymphatic plexus was present, exactly like that figured by Weed (1914, Pl. IV, fig. 8).

DISCUSSION

The method employed in the preparation of the specimens depicted in Pl. I, figs. 2, 3, Pl. II, figs. 4–6, while devised primarily for the purpose of injecting the nasal lymphatics, raises once again the question of the precise nature of the communication between these lymphatics and the subarachnoid space—a question of great theoretical and practical interest. It seems to be unquestionable that, after injection into the subarachnoid space, simple or colloidal solutions, as well as suspensions of particulate matter, may readily be forced down the cranial nerves between the nerve sheaths (which are continuous with the membranes reflected from the brain) and the nerve fibres. In the present instance it is the olfactory nerve with which we are primarily concerned. Increased injection pressure, as in the present experiments, may force down much more fluid, but apparently some comes down even when pressures are normal. Weed (1914) showed that if he made subarachnoid injections leading to the deposition of granules of Prussian blue, marked deposits of the dye accumulated in the perineural spaces about the olfactory nerves, and great masses of it in the subarachnoid cul-de-sac just above the cribriform plate. If the original ferrocyanide mixture used by Weed was injected into the subarachnoid space under high pressure, fluid could be made to pass out through this olfactory pathway so rapidly as to drip from the nose. When a dye, such as trypan blue, is injected into the subarachnoid space with just enough pressure to get it in, after a short time—15–30 min.—one may observe that not only has the mucous membrane of the olfactory area become very blue, but that the colour also extends down the deep lymphatics and through the cervical lymph nodes. It is a dramatic demonstration of the ease with which the cervical lymph pathway can be injected from the inside of the cranium.

The intimate details of what happens are apparently as follows. Dye or India ink injected in the subarachnoid space passes downward along the outside of the nerve, separating off the nerve sheath, until eventually, if undue pressure is employed, it may track along the nerve as far as the mucous membrane, to be forced out at the surface of the mucosa at points of emergence of olfactory nerve terminals; or it may break through the sheath and fill the meshes of the loose connective tissue of the submucosa. This apparently is what has happened

in the cat preparation (Pl. I, fig. 3). Once in the submucous connective tissue, dye or India ink promptly enters the very numerous and large lymphatics which characterize the entire nasopharyngeal mucous membrane and, *via* these channels, soon reaches the large cervical lymphatic trunks.

As the nerve passes through the dura mater and arachnoid, there runs beside it an extension of the subarachnoid space which gradually narrows as the dura and arachnoid come closer to the pial covering of the nerve, with which they blend outside the skull to form the perineural sheath. The prolongation of the subarachnoid space which extends down between the dura and arachnoid on the one hand, and the nerve with its pial investment on the other, acts as a fluid wedge when the pressure in the subarachnoid space is increased. Presumably this wedge first strips the progressively thinning dura-arachnoid away from the nerve, and ultimately ruptures it. It is clear, however, that in the absence of such a fluid wedge extending between the dura-arachnoid and the nerve, a communication between the nasal lymphatics and the subarachnoid space would be difficult to demonstrate, and this fact is brought out most strikingly when one considers the passage of substances in the reverse direction, i.e. from the nose to the interior of the cranium.

Experimental evidence indicates that the upward path into the brain case (leaving out of consideration living particles) is traversed only with the greatest difficulty. In fact, apart from simple crystalloids (Le Gros Clark, 1929; Faber, 1937–8), it has not been shown to occur. Thus Le Gros Clark (1929) left a 0·5 % solution of trypan blue in the nose of a rabbit for 24 hr., and at the end of that time found no sign of dye within the cranium. Our own experience with trypan blue and T-1824 has been similar (Yoffey & Drinker, 1938). In no case was dye ever found inside the cranium of cats, dogs, or rabbits after it had been left in the nose for several hours. These experiments were all the more convincing since the cervical lymph ducts were cannulated at the same time, and it was therefore possible to show that dye was present in the lymph in high concentration over many hours. Evidently, then, dye can readily pass from subarachnoid space to nasal lymphatics, but not in the opposite direction. How is this one-way passage ensured?

Any tendency for fluid which has penetrated the nasal mucosa to enter the perineural channels is apparently obviated by the fact that the neural sheath fits tightly against the nerve filaments, and even if pressure could be applied, it would be almost impossible to force anything in; the only effect would be to push the sheath more tightly against the nerve. But it seems unlikely that this sleeve valve would ever be subjected to great pressures; before a high pressure could be attained, fluid in the submucous connective tissue spaces would drain off into the submucous lymphatics.

There is a further point to be considered. The cerebrospinal fluid is at a considerably higher pressure than cervical lymph. Thus Weed & Hughson (1921–2) found in seventy-seven cats under ether an average cerebrospinal fluid pressure of 119 mm.; this pressure underwent only slight fluctuations, e.g.

11 mm. in an animal observed for 2 hr. As to cervical lymph, McCarrell (1939*b*) found in six dogs pressures ranging from −28 to 32 mm. of water, the negative pressure which sometimes develops being due to suction of lymph into the great veins. Cerebrospinal fluid, therefore, is at about three or more times the pressure of cervical lymph; it is presumably this pressure gradient which accounts for the fact that some cerebrospinal fluid normally drains away into the lymph. According to Weed (1922) "the absorption of the cerebrospinal fluid is a twofold process, being chiefly a rapid drainage into the great dural sinuses, and in small part a slow indirect escape into the true lymphatic vessels". If there is a slight but continuous seepage of fluid through the cribriform plate into the nasal lymphatics, dye which had penetrated the mucosa would be swept on into the lymph vessels rather than pass upwards into the interior of the cranium. However, it seems unlikely that this current can be very large, and that much cerebrospinal fluid leaves the subarachnoid space in this way. For one would have to explain the passage of simple solutions (Le Gros Clark, 1929; Faber, 1937–8) from the nose to the interior of the cranium on the basis of diffusion, which it would be difficult to visualize if there were a copious flow of cerebrospinal fluid away from the brain.

The argument thus far has been based on the generally accepted view that there is a complete and uninterrupted perineural sheath. If it should turn out that the perineural sheath is incomplete, then the passage through it of cerebrospinal fluid would offer no difficulty; the failure of dyes to pass from the nose into the cranium would have to be explained on the grounds that their diffusion rate is less than that of the outward current of cerebrospinal fluid. If, however, the perineural sheath is complete, one must assume that it thins down sufficiently to allow cerebrospinal fluid to pass through; its thickness and physical properties must be very like those of capillary endothelium. In other words, it must function as a semipermeable membrane through which colloidal dyes can pass from the subarachnoid space downwards aided by the pressure of the cerebrospinal fluid, but cannot pass through from below upwards against this pressure. Crystalloids such as potassium ferrocyanide and iron ammonium citrate could pass through in either direction.

A thin perineural sheath could readily be ruptured, a possibility which has already been mentioned in discussing the apparently free communication between the perineural spaces and the submucous connective tissue spaces in the cat (Pl. I, fig. 3). The evidence of the present experiments is not sufficient to enable one to decide definitely whether the perineural sheath is normally complete or not. If the perineural spaces open freely into the submucous connective tissue of the nose, one would expect a measurable amount of cerebrospinal fluid to drain away normally by this route, the amount being appreciably increased if the pressure of cerebrospinal fluid is raised. Since lymph from the nose drains finally into the cervical lymph duct (Yoffey & Drinker, 1938) this cerebrospinal fluid should give rise to a free and spontaneous flow of cervical lymph.

Previous experiments have shown that a spontaneous flow of cervical lymph does occur with fair regularity in the monkey, though not so frequently in the cat. On the other hand, a comparison of Pl. I, fig. 3 and Pl. II, fig. 5 would lead one to anticipate just the opposite result; for judging by the escape of India ink from the perineural spaces, the quantity of cerebrospinal fluid draining into the nasal lymphatics should be greater in the cat. It has already been emphasized that in view of the increased injection pressures used, these findings should be interpreted with caution. More accurate information might well be obtained by the technique of McCarrell (1939*a*), obtaining first a steady flow of cervical lymph, and then noting the effects upon this lymph flow of experimentally induced changes in the pressure of the cerebrospinal fluid.

SUMMARY

1. In the nose and pharynx of the cat and monkey there is a rich lymphatic plexus which drains into the deep cervical lymph duct.

2. The vessels of this plexus are in very close contact with the nasopharyngeal epithelium.

3. The plexus may readily be injected from the cranial subarachnoid space with India ink, or a solution of potassium ferrocyanide and iron ammonium citrate.

4. There is a brief discussion of the permeability of the perineural sheath, and the precise mode of communication between the subarachnoid space and the nasal lymphatics.

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EXPLANATION OF PLATES I AND II

PLATE I

Fig. 1. Camera lucida drawing of a section through the posterior wall of the nasopharynx of a monkey. Note the numerous large lymphatic channels, filled with India ink, close to the epithelium.

Fig. 2. Portion of one of the ethmoturbinal laminae of a cat, in which India ink was injected into the cranial subarachnoid space. The lamina has been cleared by the Spalteholtz method, so that viewed by transmitted light the submucous lymphatic plexus is seen on both sides. At the left and lower borders the lamina has been cut obliquely, and the plexus on one side is seen projecting beyond that on the other. ×22.

Fig. 3. Section through olfactory bulb, cribriform plate, and roof of nose, in a cat in which India ink was injected into the cranial subarachnoid space. Note ink passing through foramina in cribriform plate, on either side of the lamina perpendicularis of the ethmoid. Note also the dense masses of ink immediately below the cribriform plate, and the widespread filling of the submucous lymphatic plexus. *A*, olfactory bulb; *B*, cribriform plate; *C*, fibres of olfactory nerve and India ink passing through cribriform plate; *D*, nasal cavity; *E*, ethmoturbinal lamina; *F*, nasal septum. ×7.

PLATE II

Fig. 4. Portion of Pl. I, fig. 3 at a higher magnification. Note the lymphatic plexus immediately adjoining the columnar epithelium. ×25.

Fig. 5. Section through the olfactory bulb, cribriform plate, and roof of nose, in a monkey which had received an injection of India ink into the cranial subarachnoid space. Compared with the cat (Pl. I, fig. 3) the ink appears to have come down in smaller quantities. The mucous membrane of the roof of the nose has come away during the preparation. *A*, olfactory bulb; *C*, olfactory nerve bundle surrounded by India ink passing through a foramen in the cribriform plate; *D*, roof of nasal cavity; *M*, marrow cavity in upper end of nasal septum. ×7.

Fig. 6. Portion of Pl. II, fig. 5 at higher magnification. Same lettering. Note the ink tracking down in the perineural space. ×25.

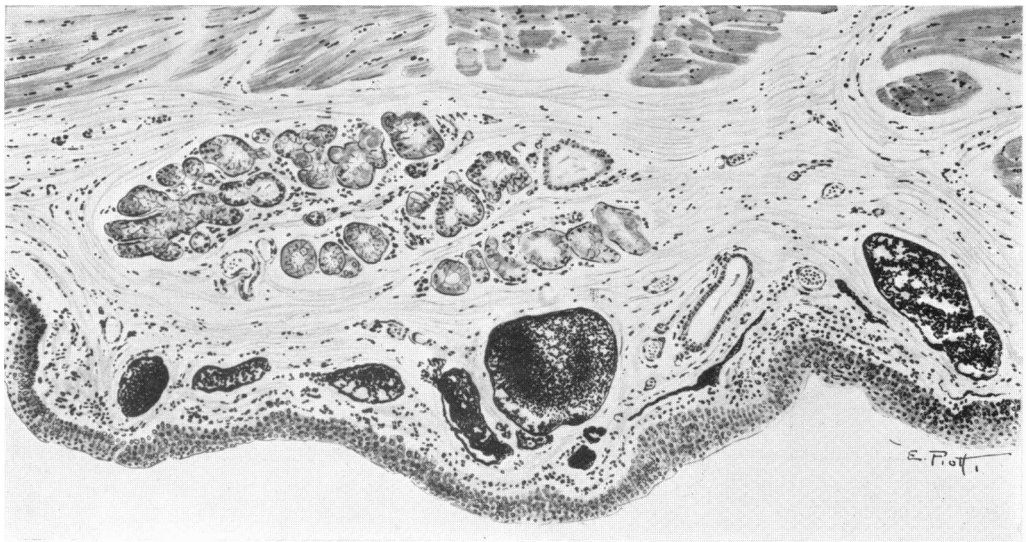


Fig. 1.

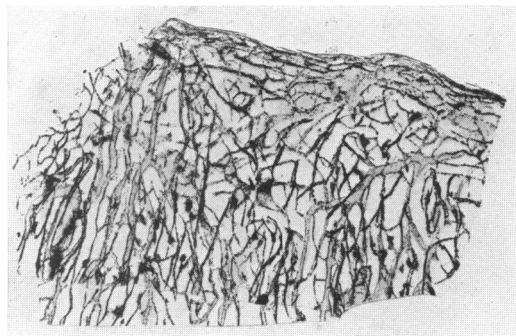


Fig. 2.

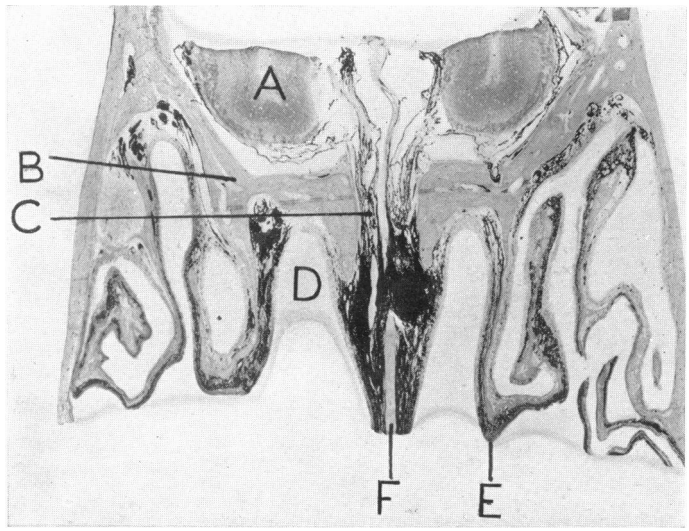
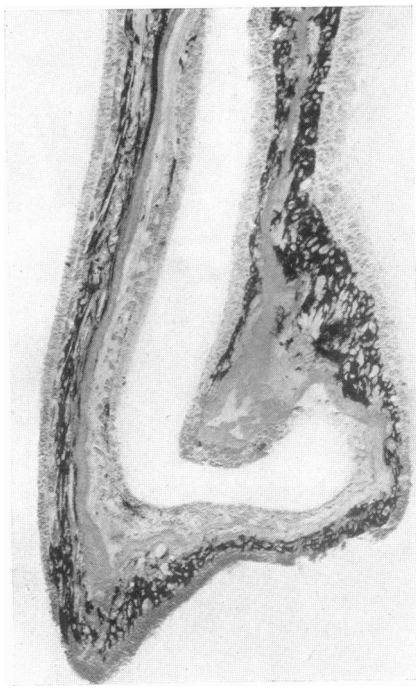


Fig. 3.



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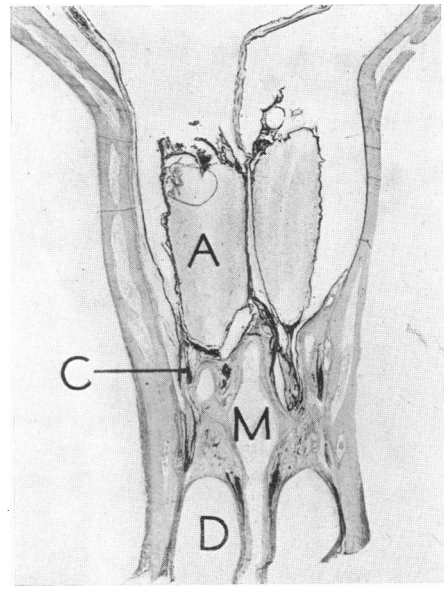

Fig. 5.

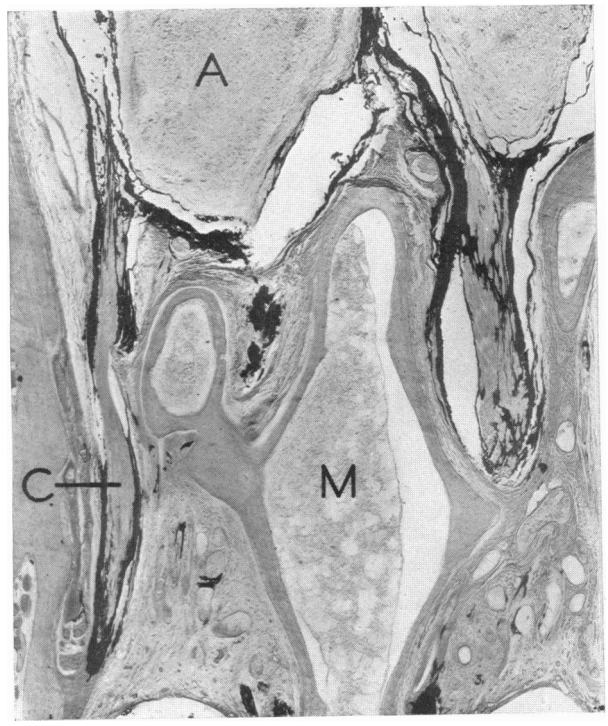

Fig. 6.