THE FATE OF A RADIO-OPAQUE MEDIUM INJECTED INTO THE SCIATIC NERVE*

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Considerable controversy exists concerning the relationship between the tissue spaces of the central nervous system and meninges, and those of the remainder of the body. It has been suggested that some materials may reach the central nervous system by routes other than through the hematoencephalic barrier. One suggested pathway is the tissue spaces of peripheral nerves. Other work in this laboratory (4) indicates that tetanus toxin may ascend via this route.

It was hoped that the relationship between the tissue spaces of peripheral nerves and the tissue spaces found centrally would be further clarified by determining the course of inert materials injected into peripheral nerves in a number of species of mammals. Consideration was given to radio-opaque media and visual dyes.

METHODS AND MATERIALS

Twenty-four cats, 21 rats and 10 opossums were used in this experiment. All animals were anesthetized with intraperitoneal pentobarbital (nembutal), and the sciatic nerve was exposed by a lateral incision of the thigh. Pantopaque†, a radio-opaque oil which is nontoxic to the nervous system, was injected into the nerve at the middle of the thigh with a 30 or 33 gauge needle mounted on a tuberculin syringe. In some of the experiments Oil Red O was dissolved in the pantopaque. This made it possible to follow the course of the material visually as well as radio graphically. The volume injected was approximately 0.1 cc. in cats and opossums and 0.05 cc. in rats. All injections were directed toward the periphery.

In 7 cats, 5 rats, and 2 opossums, injections were made into the extrafascicular connective tissue surrounding the nerve. In the remainder of the animals the injection was into the nerve fascicle. Following injection, a series of radiograms were taken after varying time intervals ranging from 30 seconds to 48 hours. At the termination of some experiments, 8 to 48 hours following injection, the animal was sacrificed. A laminectomy was performed and the location of the dye in the sciatic nerve and vertebral canal was recorded.

The above procedures were repeated on 7 dead animals. Intrafascicular injections were made into the sciatic nerves of 2 cats, 2 rats, and 2 opossums within 5 minutes after death. The animals had been killed by an overdose of nembutal, and in all of the cases the aorta was sectioned after cessation of heart beat. In one cat the injection was performed 10 hours after death. The material was followed visually and with radiograms.

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† Pantopaque is a trade name for Ethyl Iodophenylundecylate which proved to be a superior injection medium for neurography. Thorotrast (thorium dioxide solution) and a solution of Evans Blue were both found to be unsatisfactory.
Plate 1

Fig. 1. Extrafascicular injection of pantopaque in the cat. Arrow indicates injection medium.

Fig. 2. Intrafascicular injection of pantopaque in the cat. Arrows indicate injection medium.

Fig. 3. Intrafascicular injection of pantopaque in the opossum. Arrows indicate injection medium.

Fig. 4. Intrafascicular injection of pantopaque in the rat. Arrows indicate injection medium.
An attempt was made to demonstrate the relationship of the various connective tissue planes at the intervertebral foramen by preparing serial microscopic sections through this area from a normal animal. Two blocks of tissue were removed from a cat which had been perfused with 10 per cent formalin immediately following death. The blocks, each containing a portion of 2 adjacent vertebrae and the intervening foramen, were immersed in formalin for 24 hours, decalcified in an electro-decalcifier, and embedded in nitrocellulose. Fifty micra cross and longitudinal sections were cut and then stained with Mallory's triple connective tissue stain.

RESULTS

Radiograms taken one minute (and in a few cases 30 seconds) after intrafascicular injection consistently showed the pantopaque in the vertebral canal of cats and opossums. In all cases, the pantopaque in the vertebral canal appeared as a "pool" dorsal to the center of the lower lumbar vertebrae (Plate 1, figs. 2 and 3). Radiograms taken later in the sequence showed only relatively small changes in the position and volume of the pantopaque. In most cases a cephalic extension of the pantopaque for a few centimeters in the vertebral canal was apparent within 1 hour after injection. After 48 hours, a general fading of the injection medium could be detected. This was most apparent in the area of the injection site. Sullivan and Mortensen (15) reported that the peripheral portion of the nerve lost its opacity 5 or 6 hours after an injection of brominol.

In dissections, pantopaque colored with Oil Red O, could be observed visually in a single fascicle from the injection site to the intervertebral foramen and in the subdural space. The globules of the colored medium were easily moved by exerting slight pressure on the dura mater. These globules escaped if the dura was incised. By cutting deeper, a leakage of cerebrospinal fluid could be observed.

In rats there was some difficulty in observing the injection medium both visually and radiographically due to the small amount used and the smaller size of the structures involved. Observations, nevertheless, indicated that the material behaved in the same manner as in the cat and opossum (Plate 1, fig. 4).

The results following extrafascicular injections were the same in the cat, rat

<table>
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<th>Number of Animals</th>
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<th>Kind of Injection</th>
<th>Fate of Medium</th>
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<tr>
<td>14</td>
<td>Living cat</td>
<td>Intrafascicular</td>
<td>Moved centrally to subdural space</td>
</tr>
<tr>
<td>7</td>
<td>Living cat</td>
<td>Extrafascicular</td>
<td>Remained in area of injection site</td>
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<tr>
<td>2</td>
<td>Dead (5-10 min.) cat</td>
<td>Intrafascicular</td>
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<td>Intrafascicular</td>
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Fig. 1. Cross section through the intervertebral foramen of a cat (X 30). Mallory’s Triple Connective Tissue Stain was employed. S.N.—spinal nerve, S.C.—spinal cord, D.M.—dura mater. Arrow indicates delamination of dura mater, one lamina fusing with the connective tissue of the nerve and the other with the vertebral periosteum of the centrum. Fig. 2. Cross section through the intervertebral foramen of a cat (X 100). Mallory’s Triple Connective Tissue Stain was employed. S.N.—Spinal nerve, D.M.—dura mater. Arrow indicates delamination of dura mater, one lamina fusing with the connective tissue of the nerve and the other with the vertebral periosteum of the centrum.
and opossum; however, they were strikingly different from the intrafascicular injections (Plate 1, fig. 1). Little, or no, central movement of the injected medium could be demonstrated immediately or even after 48 hours. A slight diffusion outward from the injection site could be noted. When this type of injection was performed, the nerve "ballooned" at the site of injection as the connective tissues around the nerve were separated by the injection pressure. Alford and Schwab (1) reported this ballooning effect, but apparently did not understand its significance. Their study suggested that where ballooning was encountered the injection was extrafascicular.

Intrafascicular and extrafascicular injections in animals dead for short periods of time behaved in the same manner as in living animals. In the cat which was dead for 10 hours, however, intrafascicular injections of pantopaque revealed no central movement. The results of all experiments are summarized in Table 1.

Study of sections taken through intervertebral foramina indicate that, as the spinal nerve roots pass out of the cord, the dura forms a sheath around them as far as the ganglion in the intervertebral foramen. At this point the sheath divides. One lamina continues with the nerve and blends with its outer connective tissue; the other turns back and fuses with the vertebral periosteum of the vertebrae (Plate 2, figs. 1 and 2).

DISCUSSION

Key and Retzius (10) stated that there is a contripetal flow of the fluid in the tissue spaces of the peripheral nerves to the subarachnoid space. Numerous other workers (Funaoka (7), Defrise (3), Mentell (11), Brierly et al (2), Tarlov et al (16), Moore et al (13)) have supported this theory, but few have mentioned a communication with the subdural space.

Sullivan and Mortenson (15) and French, Strain, and Jones (6) stated that the position of the needle tip relative to the peripheral nerve fasciculus is the determining factor as to whether an injected medium will ascend to the intermeningeal spaces. This investigation supports the above hypothesis, for only following intrafascicular injection did the injected material ascend. French, Strain, and Jones (6) also reported that the carbon particles, which were present in their injection medium, were located in the center of the nerve fasciculus in a cavity produced by displacing the axis cylinders laterally. This also was noted only after intrafascicular injections.

The results obtained in this laboratory following extrafascicular injection indicate that the material remains for as long as 48 hours at the site of injection. These results can be correlated with the recent work of Rohlich and Weiss (14) on the peripheral nerve barrier. These workers described a membrane two cells thick surrounding the individual nerve fascicles. They referred to it as the "perilemma" and stated that it is selectively permeable. The sites of the injections in the current study were outside the perilemma (extrafascicular) and inside the perilemma (intrafascicular). Since the membrane is selectively permeable, the injection media cannot penetrate this barrier following extrafascicular injection and, therefore, cannot ascend in the intrafascicular tissue spaces.
The results obtained in this study indicate that the barrier around peripheral nerves is impermeable or only slightly permeable to pantopaque.

The identical results that were obtained when using animals dead for short periods of time could be explained on the basis of residual muscle tone and tissue fluid pressure. The material failed to ascend the nerve when injected into the animal dead for a long period of time. This was probably due to the loss of muscle tone and tissue fluid pressure, and subsequent collapse of the nerve connective tissue spaces. Alford and Schwab (1), utilizing as an injection medium a solution of ½ per cent each of iron ammonium citrate and potassium ferrocyanide, reported central movement of the injection medium in living animals for varying distances. In animals dead for long periods of time, however, the material reportedly moved centrally to the subdural and subarachnoid spaces. It should be pointed out that these investigators injected large quantities of the injection medium. This procedure may have introduced a high injection pressure which reopened the collapsed channels and forced the material centrally.

Considerable disagreement exists concerning the central communication of the connective tissue spaces of peripheral nerves. There are 4 possibilities as to the fate of injected material: the material may fail to ascend; the material may enter the subdural space; the material may pass into the subarachnoid space; or the material may pass via the dorsal and ventral roots to enter the spinal cord. Injected media may employ one or any combination of these possibilities.

French, Strain, and Jones (6) stated that, when a radio-opaque medium is injected into the peripheral nerve, it appears first in the subdural space and then ruptures into the subarachnoid space at, or near, the arachnoid cuff. Alford and Schwab (1) stated that injected media may enter the subarachnoid and subdural spaces, and Homen and Latinen (8) recovered virulent strains of streptococcus from the subdural and subarachnoid spaces following their injection into the sciatic nerve of rabbits. The results of the experiments reported here support only in part the findings of these latter workers in that all of the injected material was followed into the subdural space after intrafascicular injection and was never detected in the subarachnoid space. It is possible, however, that under higher injection pressures the material may rupture through the arachnoid cuff and enter the subarachnoid space. It is probable that the direct connection between the tissue spaces of peripheral nerves is with the subdural space. The subarachnoid communication described by previous workers was the result of membranal rupture.

The injected material was never found to continue into the fascicles of the dorsal and ventral roots to the spinal cord. This is significant in view of the results obtained by numerous investigators (Howe and Bodian (9), Matzke and Fedinec (12)) who support the theory that neurotropic toxins and viruses ascend directly to the central nervous system via some element of the peripheral nerves. The failure of pantopaque to follow this pathway may be due to the fact that the path of least resistance is to the subdural space. Small amounts of injected material, however, may follow the dorsal and ventral roots to the spinal cord, but minute volumes of the injected medium could not be detected either radiographically or visually. The evidence is not conclusive that the route to the
subdural space from the peripheral nerve connective tissue spaces is a patent pathway. The fact that the injection medium, under minimal pressure, ascended rapidly would indicate that it is certainly a pathway of very little resistance, however, and possibly patent.

Numerous investigators have attempted to demonstrate a continuity between the tissue spaces of peripheral nerves and the intermeningeal spaces by utilizing radio-opaque material. Nevertheless, many of the compounds used were unsatisfactory media for neurography and failed to clearly demonstrate the communication on the radiograms. French and Strain (5), and French, Strain and Jones (6) did, however, obtain high quality radiograms which clearly demonstrated a communication. Their results indicated a continuity of the nerve tissue spaces with one of the intermeningeal spaces, and their interpretation was that the radio-opaque material was in the subarachnoid space.

Histologic sections through the intervertebral foramen indicate that the dura mater splits into 2 lamellae. One fuses with the vertebral periosteum and the other with the connective tissue surrounding the nerve. The connective tissue around the nerve is continuous, in turn, with the peripheral nerve perineurium and possibly the perilemma. This arrangement of connective tissue planes at the intervertebral foramen would allow material injected into the fascicle to ascend deep to the dura; namely the subdural space. On the other hand, material injected outside of the perilemma would be blocked at the intervertebral foramen by the fusions of the dura mater.

Speculation concerning the anatomical relationships of the tissue spaces which permit the ascent of injected media to the subdural space raises a question. Work in this laboratory had indicated that the perilemma of Rohlich and Weiss (14) also surrounds the fascicles in the dorsal and ventral roots. If the perilemma of the nerve roots is continuous with the perilemma of the peripheral nerve fascicles, then the perilemma would have to rupture for material to enter the subdural space. Due to the low injection pressure employed, it is believed that no membranes were ruptured. This would seem to indicate that the perilemma ensheathing the fascicles of the nerve roots is not continuous with the perilemma found peripherally.

It is probable that the perilemma of the nerve roots fuses with the arachnoid cuff whereas the perilemma of the peripheral nerve fascicles fuses with the dura mater. This would eliminate the perilemma as an obstacle to the passage of material from the connective tissue spaces of peripheral nerves to the subdural space. Further histological study of the perilemma in the area of the intervertebral foramen will be necessary to increase our understanding of its role regarding the passage of substances from the connective tissue spaces of peripheral nerves to the intermeningeal spaces.

SUMMARY

Intraneural injections of a radio-opaque medium and subsequent study by radiographic, visual, and histological techniques support the following statements: 1. A direct pathway, which offers very little resistance to injected media, interconnects the intrafascicular connective tissue spaces of peripheral nerves.
with the subdural space. 2. No direct pathway exists between the peripheral nerve tissue spaces and the subarachnoid space. 3. Injected media will ascend peripheral nerves only if it is administered directly into the intrafascicular tissue spaces of the nerve. 4. Media injected into the extrafascicular tissue of peripheral nerves remains localized at the site of injection. 5. Injected material behaves in an identical manner when animals dead for short periods of time are utilized. 6. Central movement of material injected into the peripheral nerves cannot be obtained when utilizing animals dead for long periods of time. 7. The selectively permeable membrane which surrounds the individual fascicles of peripheral nerves is impermeable or only slightly permeable to pantopaque. 8. The dura mater divides into two laminae at the intervertebral foramen: one lamina fuses with the outer connective tissue of the peripheral nerve, while the other fuses with the vertebral periosteum.

REFERENCES