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THE PRELYMPHATIC PATHWAYS OF THE BRAIN AS REVEALED BY CERVICAL LYMPHATIC OBSTRUCTION AND THE PASSAGE OF PARTICLES

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Summary.—Light and electron microscopy was used to examine portions of the brain, the circle of Willis, and the internal carotid arteries of normal cats and rabbits, of sham-operated ones, and of those whose cervical lymphatics had been ligated. Carbon was injected into the cerebral cortex of some lymphoedematous animals. It was found that lymphatic ligation produced oedema of the brain, and a dilatation of the prelymphatic spaces around the vessels. Carbon was traced in these from the injection site, around the minor and major vessels, in the adventitia of the internal carotid artery, entering lymphatics adjacent to it, and finally in the draining lymph nodes. The oedema and dilated spaces were not present in the control animals. This was taken to indicate that there is a continuous system of non-endothelialized spaces and potential spaces—the prelymphatics—draining the brain into the cervical lymphatics. The protein in these spaces appeared to be increased if the lymph-oedema had lasted three weeks as compared to 24 hours, indicating that one of the major roles of this system is the removal of protein.

IT IS well known that there are no true lymphatics in the brain and retina. However, spaces which have been termed " prelymphatics " have repeatedly been demonstrated in these organs when the lymph flow in the collecting lymphatics in the neck has been obstructed (Csanda *et al.*, 1974; Csanda and Obal, 1974; Földi, 1968; Földi *et al.*, 1968*a*; Földi *et al.*, 1968*b*; Földi *et al.*, 1968*c*; Kozma *et al.*, 1972; Obal *et al.*, 1973; Varkonyi *et al.*, 1969; Varkonyi *et al.*, 1970) as well as in normal animals (Frederickson and Low, 1969; Gärtner, 1968) and in man (Cervos-Navarro and Ferszt, 1973). These spaces have been seen around all sizes of vessels, from the capillaries to the internal carotid arteries. They occur in the adventitia of the larger vessels and in the basement membrane region of the smaller ones. The lymphatic obstruction also causes an oedema of the brain. These experiments have therefore been interpreted as indicating that there is normally a passage of fluid (and presumably protein) from the brain to the cervical lymphatics via a connected series of spaces, and potential spaces, situated peripherally to the blood vessels, which eventually drain into the true lymphatics around the great vessels in the neck. The physiological importance of this system has been demonstrated by the experimental oedema produced when they are obstructed; such obstruction also occurs in man, causing the syndrome of " lymphostatic encephalopathy " (Földi, 1969; Földi, 1972).

This concept of the prelymphatics has aroused some controversy. While similar systems have been described in other tissues *e.g.*, the intestine (Kalima and Collan, 1976) cortical bone (Deysine, 1976), the tongue (Casley-Smith, 1976) and many other regions (Rodbard and Taller, 1969). It has been objected that these are simply spaces in the interstitial tissue, which are present everywhere, and hence do not justify any special title. Certainly in some primitive animals, *e.g.* amphioxus (Casley-Smith, 1971) it can be

seen that the whole vascular system and all the spaces in the tissues are in direct continuity. In higher animals this is no doubt also true, however it would seem that this series of interstitial spaces and potential spaces is of considerable importance in the normal functioning of the drainage of the brain and retina. Hence, if only for brevity, it appears that they warrant a specific name.

A more serious objection has been that of Fiedler (1975). He considers that many of these spaces are artefacts caused by immersion fixation, since he finds similar, although lessened, evidence of brain oedema and dilated spaces in both normal animals and those with ligated cervical lymphatics when he uses perfusion fixation. It is certainly true (Sjöstrand, 1967) that perfusion fixation avoids some of the artefacts produced by immersion; however, it is equally true that it introduces its own artefacts, in particular oedema, unless the colloid osmotic pressure of the perfusant is increased by the addition of macromolecules (Bohman, 1970). Even then there is a difficulty: oedema can be artefactually produced by insufficient colloid osmotic pressure, or artefactually removed by too much. It would appear safest to use both immersion and perfusion. The former has frequently been employed; the present report concerns the latter—with increased colloid osmotic pressure. While the fact that Fiedler found similar spaces in both normal and lymphoedematous animals might appear to indicate that they are not important for the drainage of the brain, the fact that he did not increase the colloid osmotic pressure of his perfusant probably meant that artefactual oedema was produced in both cases, thus masking the effect of cervical lymphatic ligation.

There is yet a further objection to the suggestion that the prelymphatics of the brain are important for its drainage. Courtice and Simmonds (1951) found that protein passed from the subarachnoid space of cats and rabbits mainly via the arachnoid villi, but also via the lymphatics near the cribriform plate and, slightly, via the dorsal root ganglia. They made no mention of the prelymphatic system, although this is probably in continuity with the subarachnoid space (Brightman, 1966; Gardner, 1972), and did not report any passage of the protein along the internal carotid artery. These results are, however, not incompatible with the concept of prelymphatics. There is a basement membrane lining the glial border fronting the subarachnoid space, and the interstices between the cells are long, winding and narrow. In the absence of brain oedema it is evident that the rate of penetration of material from the space to the prelymphatics must be very slow. Material injected into the subarachnoid space will obviously pass preferentially by the other routes and will not model macromolecules in the interstices of the brain itself. In addition, Courtice and Simmonds (1951) do not positively record examining the internal carotids and the lymph nodes draining the lymphatics around them, while Stober (1972) states that subarachnoidly injected carbon is concentrated perivascularly and passes to the cervical lymph nodes, not to the nasal mucosa.

We therefore decided to re-examine the question of the prelymphatic system in the brain. We used perfusion fixation (with raised colloidal osmotic pressure) of the brains of cats and rabbits, both normal animals, sham-operated ones, and those whose cervical lymphatics had been ligated 24 h or 3 weeks before death. In order to positively show whether drainage occurred from the brain to the cervical lymphatics and to discover if there were more than one path, Indian ink was injected into the cerebral cortices of the animals whose lymphatics were ligated 24 h before death.

MATERIALS AND METHODS

Two normal rabbits and 2 normal cats were used, and 2 of each class of animal were sham-operated. Two rabbits had their cervical

lymphatics ligated 24 h before death, and 2 cats had theirs ligated 3 weeks before death. The animals weighed ~ 2 kg and were fed normal diets. The ligations involved dissecting out all the cervical lymph nodes and passing a ligature around them; a wedge-shaped portion of subcutaneous tissue was also extirpated from both sides of the neck. The lymphoedematous rabbits had 0·1 ml of Pelikan ink (c11/1431a—Gunther Wagner, Hannover) injected ~ 2 mm below the surface of one cerebral hemisphere via a needle which was sealed into the skull, and cut off and sealed after the injection with dental cement. This procedure prevents gross CSF pressure alterations due to leakage through the skull.

The animals were anaesthetised with pentobarbitone and perfused through an internal carotid artery for 6 h at 100 mmHg pressure and at 20° with Karnovsky (1965) formaldehyde-glutaraldehyde fixative. (The colloid osmotic pressure was raised by the addition of 4% polyvinylpyrrolidone—PVP.) Portions were taken of the cerebral cortex (both near the injection site and remote from it), the hypothalamus, the anterior and posterior pituitary, the circle of Willis, and the internal carotid artery outside the skull. Small pieces (~1 mm^3) were post-fixed in 2% osmium tetroxide, dehydrated in ascending concentrations of acetone (starting at 70%) and embedded in araldite. Semi-thin sections were examined under phase-contrast. Thin sections were stained with uranyl acetate and lead citrate (pH 11).

RESULTS

Macroscopically the brains of all the animals whose lymphatics had been ligated, and none of the others, were quite oedematous, with flattened sulci. Those animals which had been injected with carbon had this in the injection site, some in the subarachnoid space, and much around the major arteries inside the skull, the internal carotids outside the skull, and in the lymph nodes adjacent to these. No carbon was visible in the region of the cribriform plate, or in the submandibular lymph node. The light microscope showed that the animals with ligated lymphatics had oedema of the brain (Fig. 2), while the normal ones and the sham operated ones did not (Fig. 1). (The anterior and posterior pituitaries were normal in all cases.) The animals injected with carbon had this diffusely throughout the tissue adjacent to the injection site, but not in remote areas. They also showed carbon localized around the minor vessels (Fig. 2), in the adventitia of the major ones (Fig. 3, 4), and in the adjacent lymph nodes. The carbon appeared to be in two or three dilated spaces in the adventitia around the perimeter of the major vessels. These occupied about a quarter to a half of the circumference. In the lymphoedematous animals without carbon (Fig. 5) these spaces were also visible. The material in the spaces seemed somewhat more dense in those animals which had had their lymphatics ligated 3 weeks before. In the normal and sham-operated animals the spaces were minimal or not visible.

The electron microscope confirmed the findings with the light microscope. In particular, it confirmed that the material in the perivascular spaces of the injected animals was indeed carbon (Fig. 6–9). It also showed that this was present inside the brain in non-endothelialized spaces; *i.e.* in the interstitial spaces, which were partly occupied by the basement membrane, around the minor vessels (Fig. 6), and in the interstitial spaces in the adventitia of the major vessels (Fig. 7, 8). In the internal carotid artery outside the skull the carbon was also often in spaces of this kind, but occasionally we were fortunate enough to see it actually entering the lymphatics around these vessels (Fig. 9). As usual (Casley-Smith, 1976) this occurred via open endothelial intercellular junctions, although some was also contained in vesicles in the endothelium.

There was a moderate amount of proteinaceous material in the spaces of the animals whose lymphatics had been ligated for 3 weeks (Fig. 11, 12). This was perhaps rather less than is normally seen in lymphoedema (Casley-Smith *et al.*, 1974) but was more than appeared in the animals which had only had lymphoedema for 24 h (Fig. 7, 8). The oedema present in the cerebral cortex and hypothalamus of these 2 groups of animals also seemed to contain similarly different amounts of

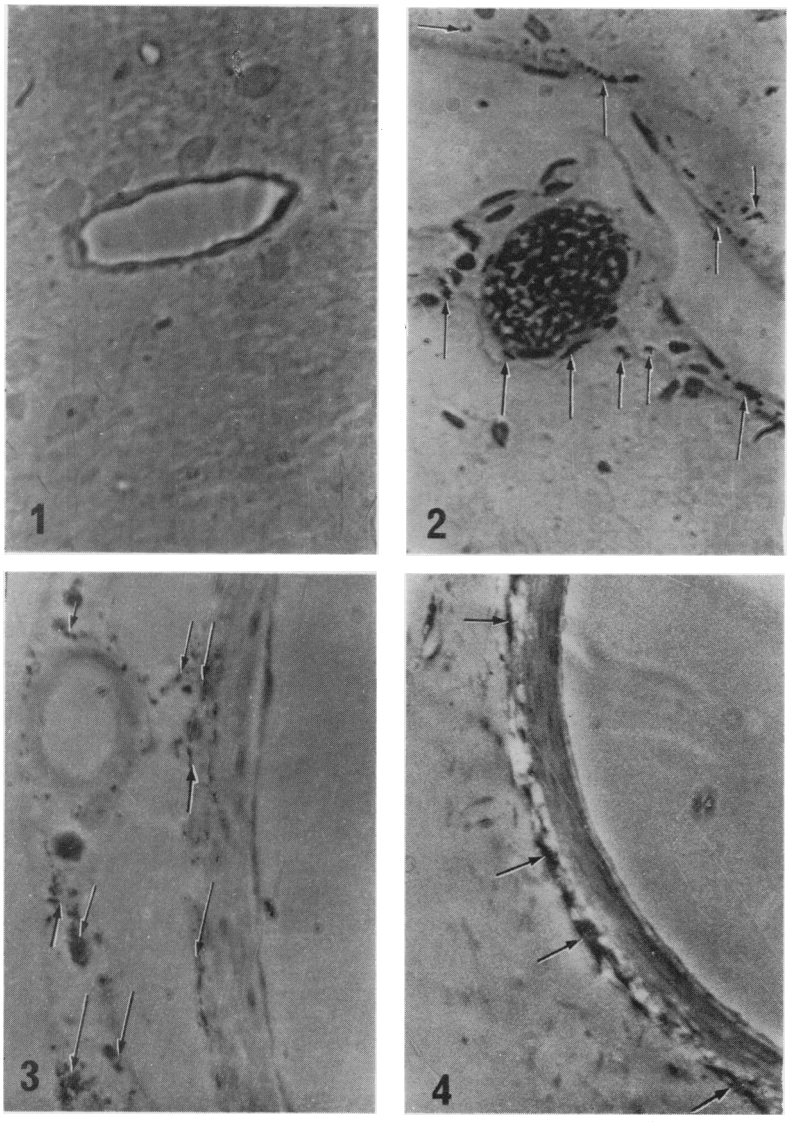

FIG. 1.—A light micrograph of normal rabbit cerebral cortex. × 835.

FIG. 2.—A light micrograph of rabbit cerebral cortex 24 h after the lymphatics were ligated and after the injection of carbon. The cortex is quite oedematous and there are 2 vessels which have carbon (arrows) around their periphery. Some is also visible deeper in the tissue. × 835.

FIG. 3.—A light micrograph of an artery in the circle of Willis of a rabbit as in Fig. 2. There is a considerable amount of carbon (some of which is indicated with arrows) in the periphery of the artery, where there is also a large space, which contains a vasa vasorum. × 835.

FIG. 4.—A light micrograph of an internal carotid artery, outside the skull, of a rabbit as in Fig. 2. There is a considerable amount of carbon in a space in the adventitia. × 83.

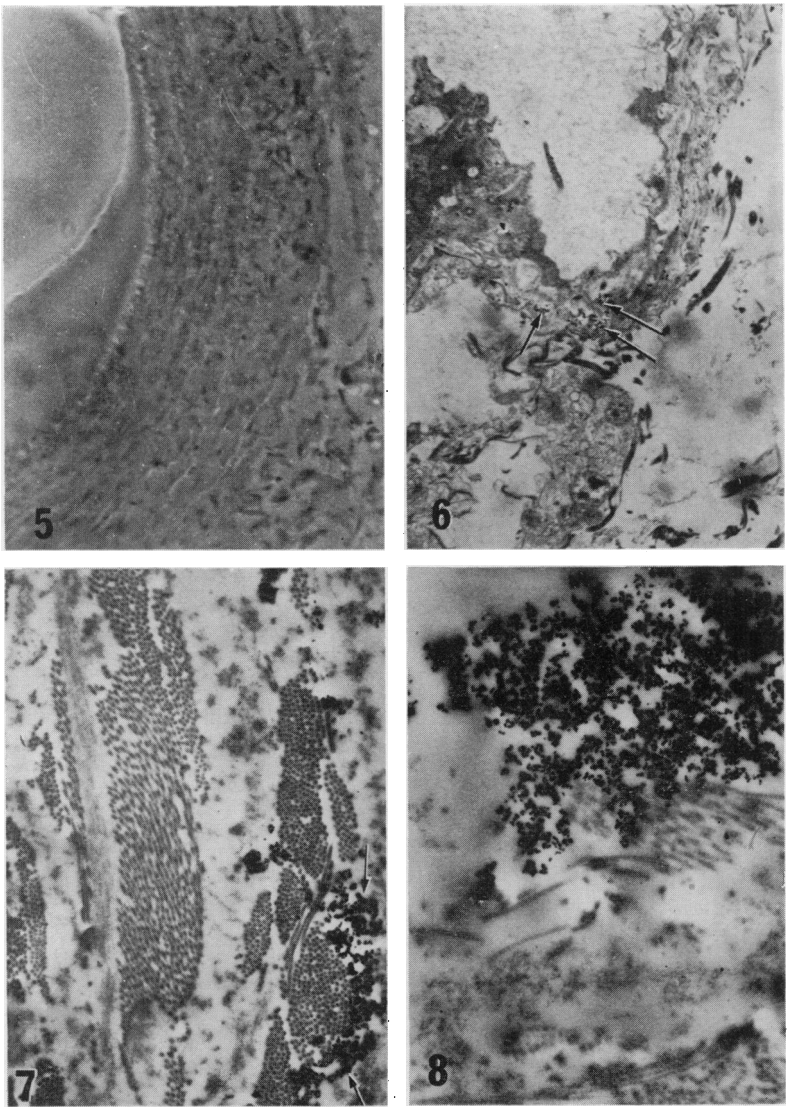

FIG. 5.—A light micrograph of the internal carotid artery of a cat whose cervical lymphatics had been ligated 3 weeks previously. There is a large irregular space in the adventitia. × 417.

FIG. 6.—An electron micrograph of the cerebral cortex of a rabbit, as in Fig. 2. There is much oedema and carbon (arrows) can just be seen, at this magnification, in a space at the periphery of the vessel's wall. × 4170.

FIG. 7.—As for Fig. 6. Carbon (arrows) is present in the adventitia of part of the circle of Willis, together with enlarged spaces between the collagen fibres. The typical structure of Indian ink is visible, showing that it is indeed some that was injected into the cerebral cortex. × 8350.

FIG. 8.—As for Fig. 6. A considerable amount of carbon is present in spaces in the adventitia of the internal carotid artery outside the skull. × 16,700.

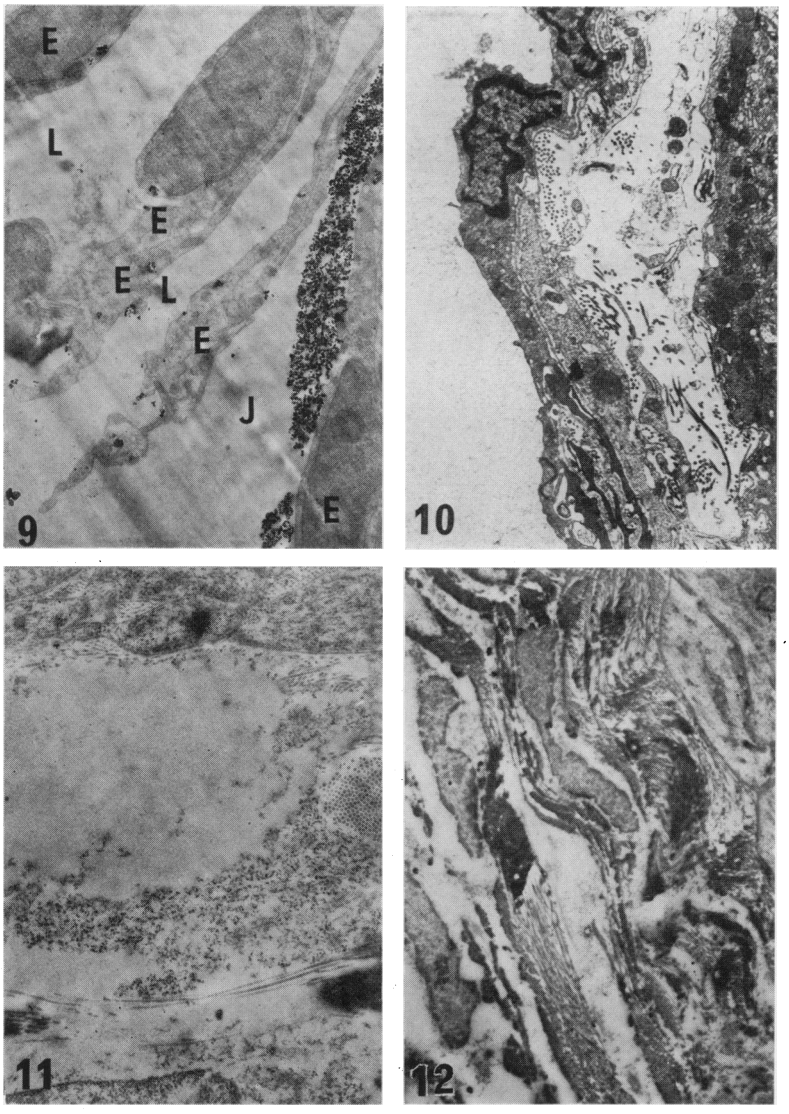

FIG. 9.—As for Fig. 6. In the adventitia of an internal carotid artery outside the skull, a considerable amount of carbon can be seen passing via a junction (J) between 2 lymphatic endothelial cells (E) into part of a complex of lymphatics (L). × 2505.

FIG. 10.—An electron micrograph of the hypothalamus of a cat, whose cervical lymphatics had been ligated 3 weeks earlier. There is a large oedematous space external to a vessel. × 4175.

FIG. 11.—As for Fig. 10. There is a large space and a collection of protein in the adventitia of part of the circle of Willis. × 6680.

FIG. 12.—As for Fig. 10. There are irregular spaces containing protein in the adventitia of the internal carotid artery, outside the skull. × 3340.

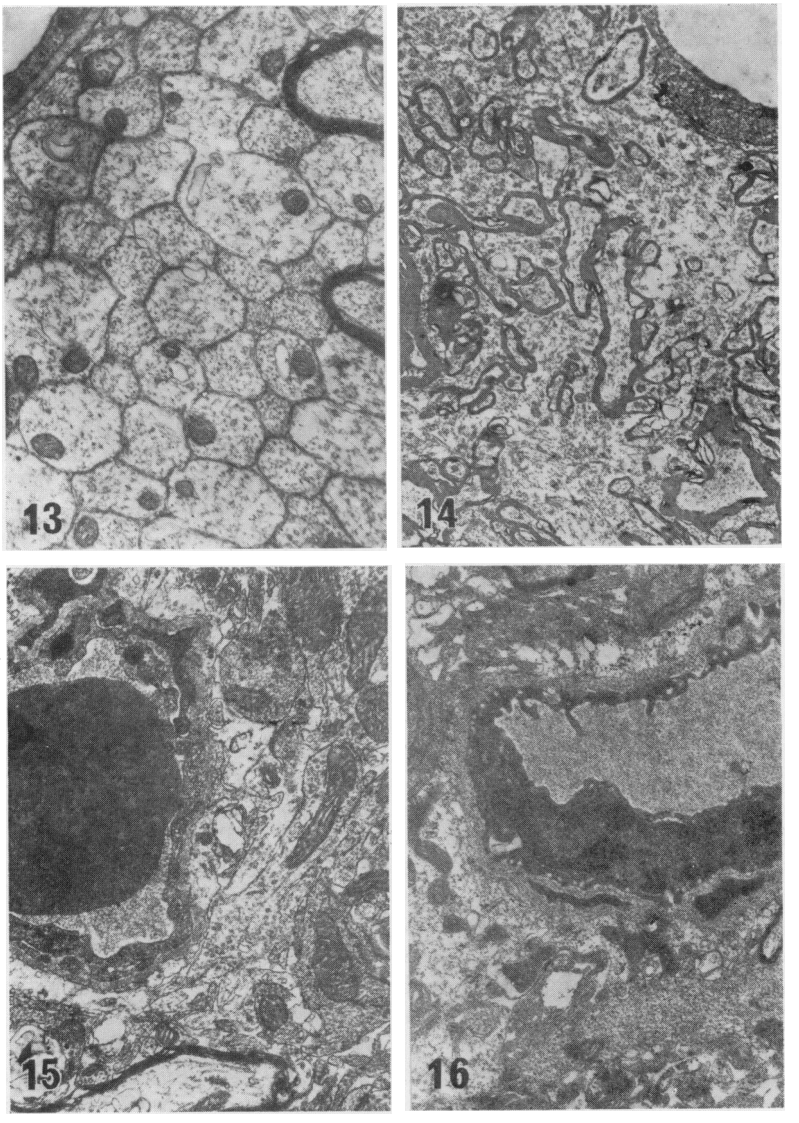

FIG. 13.—An electron micrograph of sham-operated rabbit cerebral cortex. × 5845.

FIG. 14.—As for Fig. 13, but 24 h after the ligation of the cervical lymphatics. There is considerable oedema, with proteinaceous material present, and with the spaces being continuous with that external to the vessel. × 4175.

FIG. 15.—An electron micrograph of normal cat hypothalamus. There is no oedema, although the pale glial cells are a little dilated. × 4175.

FIG. 16.—As for Fig. 15, but 3 weeks after the ligation of the cervical lymphatics. There is a large amount of proteinacious material filling the spaces in the tissue, which are continuous with those peripheral to the vessel. × 4175.

protein, and to be more severe in the more chronically affected animals (Fig. 14, 16). Again the normal and sham operated animals did not appear oedematous (Fig. 13, 15), nor did the anterior or posterior pituitaries from any of the animals.

DISCUSSION

The present findings confirm the previous ones mentioned earlier. They show that the brain is made oedematous by the ligation of the cervical lymphatics which must therefore play an important role in its functioning. (The fact that this does not seem to apply to the pituitary may be due to its being particularly securely encased and adjacent to well-drained tissue, or to the presence of fenestrated blood capillaries (Casley-Smith, 1976). Thus, the lymphatic system is important for the drainage of the brain, and presumably also of the retina, *etc.* It would appear from the present and previous experiments that this drainage occurs via a continuous series of spaces, the prelymphatics. These have been demonstrated by lymphatic ligation. Their importance, the reality of their continuity, and the drainage by them, has been demonstrated by the carbon injections. In addition these injections showed that the prelymphatic system connects with the cervical lymphatic vessels adjacent to the internal carotid artery, and that these transport the material which has drained from the brain into the cervical lymph nodes.

It is of more than passing interest that pyridoxine, pantothenic acid and the benzo-pyrone group of drugs have been shown to be very effective in reducing the extent of the brain, and other, oedema caused by the ligation of the cervical lymphatics (Casley-Smith *et al.*, 1974). This is similar to the effect of benzo-pyrones on high-protein oedemas in general which is probably due to their increasing the normal proteolysis by the cells of the tissues (Casley-Smith and Piller, 1974). This is probably largely an effect on the macrophages, and perhaps other cells, which has also been demonstrated *in vitro* (Bolton and Casley-Smith, 1975). Since one of the main roles of the lymphatic system is the removal of protein from the tissue, it is likely that this is similarly one of the main roles of the prelymphatics. This suggestion is strengthened by our present finding of more proteinaceous material in these vessels after the longer period of lymphoedema. Hence these drugs, in causing the cells largely to take over this function, can greatly reduce all high-protein oedemas, including that produced by an obstructed lymphatic, or prelymphatic, system. In clinical medicine they have proved effective in the therapy of lymphogenous encephalopathy (Földi-Börcsök and Földi, 1972).

While it is normally held that the protein content of the interstitial spaces of the brain is low, it has recently been shown that at least some cerebral arterioles normally permit its passage (Westergaard and Brightman, 1973). While such vessels are not numerous, the few vesicles in the other vessels must surely permit some passage, to which will be added that from the regions where the blood-brain barrier is absent and the capillaries are fenestrated. This protein must be removed else it will accumulate and its colloid osmotic pressure will cause oedema. Certainly some will pass to the cerebrospinal fluid and leave via the arachnoid villi and the cribriform plate (Courtice and Simmonds, 1951); however, as mentioned earlier, the passage from deep within the brain to the subarachnoid space is likely to be very slow. Westergaard and Brightman (1973) made the significant suggestion that some of the protein is likely to be lysed by the proteases normally present in the CSF (Riekkinen and Rinne, 1968). Such proteases may well be the ones enhanced by the benzo-pyrones, but at present we do not know their origin.

At all events, it is evident that protein does accumulate when the cervical lymphatics are ligated and the prelymphatics

thus rendered non-functional. We conclude, therefore, that not only does the prelymphatic system exist in this region, but that it normally plays a significant role.

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