THE LYMPHATIC DRAINAGE OF THE SPINAL NERVE ROOTS IN THE RABBIT

By E. J. FIELD and J. B. BRIERLEY

From the Department of Anatomy, University of Bristol

Attention has recently been drawn to the existence of a functional connexion between the spinal subarachnoid space and the prevertebral lymphatic system (Brierley & Field, 1948), though the detailed anatomical features of this communication could not be elucidated. The adoption of a new technique has now made it possible to outline completely the pathways in question and to make certain observations as to their character.

MATERIAL AND METHODS

The animal used was the rabbit, and all experiments were carried out on the living animal under nembutal anaesthesia. India ink, made by rubbing down a solid stick in physiological saline, was found to be unsuitable for direct introduction into the lymphatic system, since clumping of the particles prevented permeation of the finest vessels. Artificial stabilizing agents such as aerosol, fixanol, etc., were found to be worse than useless, as in many cases they actually brought about an immediate sedimentation of ink. The injection mass finally adopted utilized 4·5 % reconstituted human plasma protein solution as dispersion medium, this being an average value for the protein content of lymph (Drinker & Yoffey, 1941). Such a suspension, filtered through a no. 5 Whatman paper, was made up fresh for each experiment. In it the range of particle size extended from 0·4 to 1·5μ, but 90 % were about 0·5μ.

Three groups of experiments were carried out:

(1) In a preliminary series, ink was injected subcapsularly into the prevertebral and mesenteric lymph nodes. Such an injection gave good filling of the longitudinal channels on the front of the spine and of the small nodes along their course. The fine vessels leading dorsally from these channels were only filled, however, for 1 or 2 mm. beyond the point at which they disappeared from view by passing under the opposed medial margins of the psoas muscles. In these experiments no obstruction was offered to the free return of lymph from the abdomen. The thoracic duct quickly became filled with ink, whilst no perceptible increase in resistance to the injection was encountered.

(2) In order to introduce an element of stasis in the abdominal lymph return and thus facilitate retrograde filling of the small vessels leading dorsally from the prevertebral nodes, another series of experiments was undertaken in which the multiple longitudinal lymph collecting trunks grouped round the aorta and inferior vena cava were isolated and individually ligated. Into one

of their number a fine cannula, directed caudally, was introduced. The cannula was connected to a reservoir of ink at a height of about 200 mm.—a pressure well within the range recorded for this section of the lymphatic system (Drinker & Yoffey, 1941). In only one of a dozen experiments was successful retrograde filling of the fine dorsally directed vessels obtained by this procedure (Pl. 1, fig. 1). The failures were due to the presence of valves in the larger vessels easily capable of withstanding the pressure employed. The successful case was the result of a lucky circumstance in which no valves were interposed between the site chosen for cannulation and the nearest dorsally directed fine segmental lymphatic.

(3) In order to dilate these vessels and thereby render their valves incompetent, it was decided to ligate the thoracic duct before introducing India ink into the posterior abdominal lymphatic system. Ligation of the duct in the neck proved an uncertain procedure, and the operation was therefore carried out in the thorax. Lymphatico-venous communications do not appear to have been reported in the rabbit, but should they be of the same type as in the monkey (Silvester, 1910) and rat (Job, 1918), then ligation of the thoracic duct would not be expected to produce more than a temporary stagnation of the abdominal lymphatic return.

A preliminary tracheotomy was performed, and respiration was maintained mechanically throughout the experiment. The animal was placed on its left side over a pad on an electrically heated operating table, the right paw pulled well forward, and an oblique incision made parallel to the vertebral border of the scapula. The latter was retracted and a convenient rib, usually the seventh, resected. In order to obtain an adequate exposure of the posterior mediastinum the posterior limit of the resection was placed well back in the sacrospinalis musculature. In the lateral position the rabbit is very sensitive to mediastinal displacement and so, as soon as the pleura was opened, aeration of the lungs was adjusted to reduce this as far as possible. A swab was inserted and the right lung gently pulled forward. The operation field was illuminated by the small bulb of an electric auriscope introduced into the chest. In some of the earlier experiments a preliminary feed of olive oil by stomach tube was found to be of help in the identification of the thoracic duct, but with practice this was found to be unnecessary. The duct was usually easily seen, either at once or after minimal dissection, immediately to the left of the vena azygos. On two occasions, however, it could not be identified with certainty and the operation then became a 'blind' one, the success or failure of which was checked post-mortem. A fine braided black silk suture on a small eyeless needle was passed round the duct and tied. Whilst this procedure entailed little danger to the aorta, the azygos vein was occasionally injured. Bleeding, however, invariably ceased when the ligature was tied. Successful ligation of the duct resulted in immediate distension of its distal segment. The chest was then closed with large interrupted catgut sutures as rapidly as possible. It was found to be important to turn the animal half on to the operated side as soon as feasible.

The expedition with which the chest could be closed and the animal turned into this position seemed to determine materially the success of the operation as measured by the number of hours survival.

Laparotomy was undertaken forthwith in all but seven cases. In these latter an interval of 20–30 min. was allowed to elapse so that the general condition of the animal might improve before further operative trauma was inflicted. The prevertebral lymph nodes round the aortic bifurcation were cleared of overlying peritoneum and injected with India ink. The great lymphatic mass in the mesentery was similarly injected. In all cases injection was stopped as soon as resistance became at all appreciable. By repetition of this procedure at intervals of 2 or 3 hr. as much as 4 or 5 c.c. of ink could be introduced, though excellent results were obtained with as little as 3 c.c. or even less. Where a male animal was used, injection of the epididymis also was carried out. Altogether six out of twenty-two animals survived these formidable operative procedures for periods of 10–12 hr., during which time the pulse remained good and repeated doses of nembutal had to be administered to maintain an adequate depth of anaesthesia.

At the conclusion of an experiment the animal was perfused with saline followed by 10% formol saline. An adequate perfusion greatly facilitated post-mortem dissection.

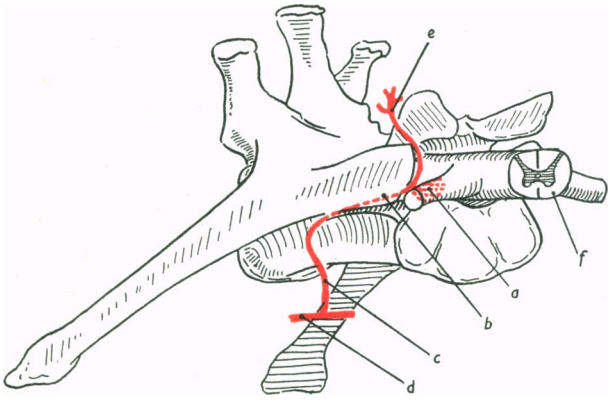
RESULTS

The full picture of retrograde flow of ink to the vicinity of the nerve roots was only met with in the six animals which survived operation for 10–12 hr. In those which were killed or died 2 or 3 hr. after operation, ink was never found to have extended more than a millimetre or two backwards in the fine 'segmental' lymphatics. The lapse of 10–12 hr. was essential to allow of backward movement of ink in the static lymph of these fine vessels.

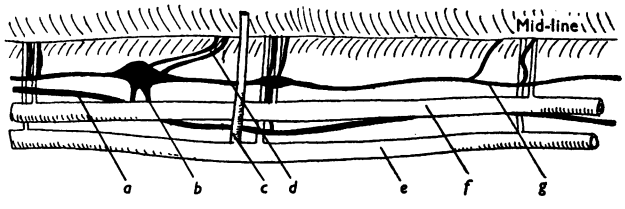
In the experiments of 10–12 hr. duration, laminectomy revealed that in the upper lumbar and lower thoracic regions varying amounts of ink reached the vicinity of the dorsal root ganglia. In two of these six cases fine lymphatic vessels were found to commence in the substance of the erector spinae musculature where their radicles could be seen under the binocular dissecting microscope coursing between small bundles of muscle fibres. The vessels, two or three in number, passed ventrally in company with the blood and nerve supply of the muscle towards the region of the intervertebral foramen. Here tributaries came in from the neighbourhood of the dorsal root ganglion and its related epidural fat (Text-fig. 1; Pl. 1, fig. 1). In these two cases India ink particles had actually reached the surface of the dorsal root ganglion and the adjoining cord membranes. The close relation between small ink-filled tributaries and the nerve just distal to the dorsal root ganglion is shown in Pl. 1, fig. 3. The lymphatics under consideration were wide channels lined with flattened endothelium (Pl. 1, fig. 4), and fine ink particles could be seen in all stages of passage through their walls. There was no swelling of the endothelial cells such as might have been expected if the particles had been actively phagocytosed. The

appearances suggested rather a purely passive movement of ink particles through the protoplasm of the endothelial cells. Outside the lymphatics many free ink particles could be seen lying in the meshes of the epidural fat of the intervertebral foramen.

Having received these tributaries from the dorsal root ganglion area the main lymph vessel passed ventrally and cranialward in company with blood vessels closely opposed to the vertebral body under cover of a wing-like bony



Text-fig. 1. Lumbar vertebra and spinal cord from the left and behind. (*a*) Nerve root with lymphatic radicles; (*b*) bony process hiding lymphatic afferents emerging from vertebral body; (*c*) main lymph vessel joining longitudinal trunk (*d*); (*e*) afferent vessels from dorsal musculature; (*f*) spinal cord.



Text-fig. 2. Dissection of the prevertebral lymphatic channels in the upper lumbar region. Two valveless longitudinal trunks (*a*, *g*) lie dorsal to the aorta and inferior vena cava (*e*, *f*) which have been displaced ventrally. Small prevertebral lymph nodules (*b*) are located along these trunks, and from them fine dorsally directed vessels (*d*) pass between the opposed medial margins of the psoas muscles in the mid-line. These are the vessels that constitute the 'segmental' drainage of the spinal subarachnoid space; (*c*) left renal artery.

plate (Text-fig. 1, *b*). A fibrous sheet passed from the edge of the plate to the vertebral body to complete an osteofascial tunnel for the vessels. Whilst in this situation the lymphatic received a tributary from the interior of the vertebral body. After emerging from under cover of the medial edge of the psoas it ended in one of the longitudinal ducts related to the front of the spine (Text-fig. 2). It was this terminal section of the vessel which had been noted previously as a 'segmental' lymphatic, following subarachnoid introduction of ink. Along

the course of the longitudinal collecting channel were disposed the small lymph nodes which are the 'regional' nodes of the spinal subarachnoid space (Brierley & Field, 1948). Neither the lymphatic, the course of which has been described above, nor the collecting trunk into which it emptied were possessed of valves, though the larger trunks more closely applied to the aorta and inferior vena cava were so provided.

DISCUSSION

Le Gros Clark (1929) has pointed out that many authors omit to demonstrate a true endothelial lining of so-called lymphatic vessels. The channels described above are lined with a single layer of endothelium (Pl. 1, fig. 4) and are not simply tissue spaces. Nevertheless, attention should not be directed towards endothelial lined channels to the exclusion of actual functional pathways if these latter should happen to possess no such lining. Thus there is a real functional pathway, through the membranes which form the subarachnoid cul-de-sac round the nerve roots, to the epidural fat and thence to the lymphatics which commence in the neighbourhood of the nerve roots as described above.

Evidence presented in a previous communication (Brierley & Field, 1948), suggested that free India ink particles (0·5–1·5μ) could make their way from the epidural fat into fine lymphatics arising in relation to the dorsal root ganglia, and that such migration could take place in the intact animal many hours after complete recovery from operation. In the present work ink particles could be traced in this process of migration albeit in the reverse direction. Thus in the successful cannulation experiment described above, where the pressure of introduction into the large posterior abdominal channel was about 200 mm. of ink, particles could be seen passing through the lymphatic wall into the closest relation to the nerve just beyond the dorsal root ganglion. There can be no doubt, then, that such lymphatics are permeable to particles 0·5–1·5μ in diameter. In the third series of experiments, where ink was injected into the prevertebral and mesenteric lymph nodes, no measurement of the injection pressure was made. In the absence of direct knowledge of the pressure in the lymphatics related to the dorsal root ganglia it would be unwise to draw conclusions from these experiments alone as to the permeability of lymphatic endothelium to particulate matter. Great care was, however, taken to discontinue the injection of the nodes as soon as the slightest increase in resistance was encountered. Furthermore, the puncture site in the lymph node acted as a safety valve through which any excess fluid not readily accommodated within the system injected might easily regurgitate. It is probable, therefore, that no inordinate pressure rise was transmitted to the distant fine lymphatics related to the root ganglia. The appearances presented by the lymphatic endothelium in these experiments (Pl. 1, fig. 4) are quite different from those given by Landis (1946) for the passage of India ink through endothelium as the result of an acute and excessive rise in pressure. On the other hand, they correspond closely with the description given by Clark &

Clark (1918–19) who, working with the transparent tails of tadpoles, injected small amounts of India ink directly into the lumen of a lymphatic capillary. On the following day ink particles were seen to be 'enclosed within the endothelial cells of that lymphatic which had been injected with ink. They were present as small black spots and as large clumps of granules within the areas surrounding the nuclei of the endothelial cells....' Field & Drinker (1936) found that graphite particles (approximately 1μ in diameter) may pass out of the circulation of the frog, apparently through blood-capillary endothelium which is physiologically intact. Moreover, phagocytosis did not seem to play a part in this migration. A similar passage of calcite particles (1–2μ) could also be followed through intact endothelium. Gillilan & Conklin (1938), working on the absorption of particulate matter from the lung of the snake, reported that the 'upper size limit for penetration of snake lymphatics must be placed at about 2μ'. These conclusions are in agreement with our own findings that particles below 1·5μ in diameter may readily pass through lymphatic capillary endothelium, and that such passage does not necessarily involve preliminary phagocytosis. It is probable that the path available for India ink particles is one available to any particulate matter of appropriate size.

It has been suggested that infective agents, particularly certain viruses, may primarily invade the lymphatics and later spread to the central nervous system. Such an extension has been visualized as occurring through the blood stream with the lymphocyte as virus carrier, as postulated by Yoffey & Sullivan (1939) for vaccinia, or it might take place directly along lymph channels. This latter possibility, hinted at by several authors, is placed on a sound anatomical basis by the demonstration of a system of valveless lymphatic vessels connecting the prevertebral and mesenteric lymph nodes with the spinal nerve roots. The circumstances under which retrograde flow in this system has been achieved are admittedly highly artificial. It is, however, possible that analogous conditions might arise in the presence of some physio-pathological hindrance to the free return of lymph from the abdomen. A temporary impairment of lymph return from the abdomen might thus produce conditions highly favourable to retrograde invasion of the nervous system. There can be no doubt that the larger lymphatic vessels, and the thoracic duct in particular, do possess the power of contraction, though the stimuli to which they respond are by no means clear. This problem is receiving further attention. It should be understood, however, that retrograde lymphatic invasion of the nervous system is not necessarily dependent upon an actual reversal of lymph flow. Diffusion of particulate matter will always take place from a region of higher to one of lower concentration. There must, however, be some limiting value of the speed of fluid stream against which such a process may take place (cf. Yoffey & Drinker, 1939; Landis, 1946). It is possible then that conditions favourable to the invasion of the nervous system may come about when the cerebrospinal fluid outflow falls below a certain limiting value. It is the difference between the pressure in the spinal subarachnoid space and the abdominal lymphatic

system which is the immediate factor in determining this outflow. Conditions which reduce this difference, either by lowering the subarachnoid pressure or raising the lymphatic pressure must therefore facilitate the diffusion of particulate matter against the centrifugal stream.*

The lymphatic connexions of the spinal subarachnoid space must be taken into account in evaluating the clinical and experimental phenomena met with in poliomyelitis. The subject is an extensive one and has been reviewed elsewhere (Field & Brierley, 1948).

In addition to the tributaries from the dorsal root ganglion region the vessel which passes ventrally round the side of the vertebral body receives a tributary from its interior. This arrangement, should it turn out to be similar in man, may be of significance as affording an anatomical pathway by which secondary deposits may reach the spine from the abdominal viscera and from the testicle. Rouvière (1932) has shown the existence in man of lymphatic vessels arising in the sacrospinalis musculature and passing ventrally to the para-aortic nodes. These vessels are no doubt analogous to those here described, but the author gives no details of their course, nor does he mention any tributaries that they receive.

SUMMARY

1. The technique of ligation of the thoracic duct in the mid-chest region is described.

2. The anatomical detail of the lymphatic connexions between the nerve root region and the prevertebral lymph nodes is described.

3. The permeability of lymphatic endothelium to particles below 1·5μ diameter is confirmed.

4. The possibility of retrograde spread of infective agencies to the central nervous system along direct lymphatic channels is briefly discussed, and the conditions favourable to such an occurrence considered.

It is a pleasure to acknowledge the sustained interest and encouragement of Prof. J. M. Yoffey. On general matters relating to the lymphatic system his never-failing advice and criticism have been invaluable. The authors are indebted to Dr G. H. Tovey, of the National Blood Transfusion Service, Southmead Hospital, Bristol, for the plasma used in their experiments, and to Messrs Abbott Laboratories for a supply of nembutal. Their thanks are due also to Dr D. D. Eley, University of Bristol, for his analysis of the factors involved in the diffusion of particulate matter. The histological preparations are the work of Mr Keith-Hunt whose help the authors gladly acknowledge. All photomicrographs were taken on 35 mm. film.

* Particulate matter is often used in biological problems as an indicator substance to determine fluid flow. Some knowledge of the physical factors which may influence the result obtained, e.g. particle size, viscosity of medium, temperature, etc. is very desirable. Dr D. D. Eley of the Department of Chemistry, University of Bristol, has kindly supplied the analysis set out as an appendix to this communication.

APPENDIX

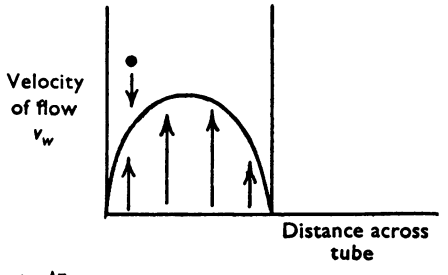
DIFFUSION OF PARTICLES AGAINST A FLUID STREAM

Theoretical considerations

Consider first the flow of water down a capillary. The velocity v_w will vary from zero at the sides to a maximum at the middle—such a liquid shows parabolic flow.

Consider a particle with a diffusion velocity v_D ($-v_D$ against stream). Its net velocity, v_p, will be the algebraic sum of v_D and v_w,

$$v_p = v_D + v_w.$$



For many purposes one might assume an average velocity across tube, $v_w^{\text{Av.}}$,

$$v_p = v_D + v_w^{\text{Av.}}.$$

For $v_p = 0$, zero net velocity,

$$-v_D = v_w^{\text{Av.}} - \text{the velocity of streaming.}$$

The factors influencing v_D are determined by the Stokes-Einstein equation for the diffusion coefficient D, and we may assume $v_D \propto D$,

$$D = \frac{RT}{6\pi a \eta N},$$

N = Avogadro's number, T = absolute temperature, η = viscosity of liquid, R = gas constant, a = radius of particle.

This equation holds for a *dilute* suspension of spherical particles of radius a, preferably considerably greater than the molecular radius of the liquid, i.e. it would hold for particles such as India ink or virus.

We may then derive the conditions for a particle just not to diffuse against a certain fluid flow, $v_w^{\text{Av.}}$. It is

$$\frac{T}{a\eta} = \text{constant.} \qquad (1)$$

To appreciate the effect of temperature increase, we must allow for its effect on η. Then for water,

37° C. $\eta = 0{\cdot}6947$ centipoise.

38° C. $\eta = 0{\cdot}6814$ centipoise.

Thus a rise of 1° C. affects T by only 1 part in 310 (310–311° K.). η is affected by 1·3 parts in 69. In fact, for most purposes we express η as

$$\eta = \eta_0 e^{b/T},$$

where η_0 and b are constants for the liquid concerned. Then condition (1) becomes

$$\frac{T}{a\eta_0 e^{b/T}} = \text{constant.}$$

Thus, treating the lymph as pure water to a first approximation, there is a logarithmic relation between a (the radius of particle which is just stopped by a certain average flow of fluid) and temperature, of the form

$$\log a = \text{constant} + \log T - \frac{b}{T}.$$

Hence an increase in T will considerably increase the size of particle which will be able to diffuse against a constant fluid stream. Similarly, at constant temperature, any change in η of the lymph, due, say, to varying protein content, will affect inversely the limiting size of particle which will just be able to diffuse against a constant stream.

D. D. ELEY

REFERENCES

BRIERLEY, J. B. & FIELD, E. J. (1948). The connexions of the spinal subarachnoid space with the lymphatic system. *J. Anat., Lond.* (in the Press).

CLARK, R. E. & CLARK, E. L. (1918–19). Phagocytosis of carbon and carmine granules in the transparent tails of tadpoles. *Anat. Rec.* **15**, 151–163.

CLARK, W. E. LE GROS (1929). Report to the Committee on vaccination on an anatomical investigation into the routes by which infections may pass from the nasal cavities into the brain. *Rep. publ. Hlth. med. Subj., Lond.*, no. 54.

DRINKER, C. K. & YOFFEY, J. M. (1941). *Lymphatics, Lymph and Lymphoid Tissue.* Harvard Univ. Press.

FIELD, E. J. & BRIERLEY, J. B. (1948). The lymphatic connexions of the subarachnoid space; an experimental study of the dispersion of particulate matter in the cerebrospinal fluid with special reference to the pathogenesis of poliomyelitis. *Brit. med. J.* **1**, 1167–1171.

FIELD, M. E. & DRINKER, C. K. (1936). The passage of visible ink particles through the walls of blood capillaries and into the lymph stream. *Amer. J. Physiol.* **116**, 597–603.

GILLILAN, L. A. & CONKLIN, R. E. (1938). Removal of foreign substances by the lymphatics of the snake lung. *Amer. J. Physiol.* **123**, 598–607.

JOB, T. T. (1918). Lymphatico-venous communications in the common rat and their significance. *Amer. J. Anat.* **24**, 467–485.

LANDIS, E. M. (1946). Capillary permeability and the factors affecting the composition of the capillary filtrate. *Ann. N.Y. Acad. Sci.* **46**, 713–731.

ROUVIÈRE, H. (1932). *Anatomie des lymphatiques de l'homme.* Paris: Masson et cie.

SILVESTER, C. F. (1910). On the presence of permanent lymphatico-venous communications at the level of the renal veins in adult South American monkeys. *Anat. Anz.* **37** (Ergänzungsheft), 111–113.

YOFFEY, J. M. & DRINKER, C. K. (1939). Some observations on the lymphatics of the nasal mucous membrane in the cat and monkey. *J. Anat., Lond.*, **74**, 45–52.

YOFFEY, J. M. & SULLIVAN, E. R. (1939). The lymphatic pathway from the nose and pharynx. *J. exp. Med.* **69**, 133–141.

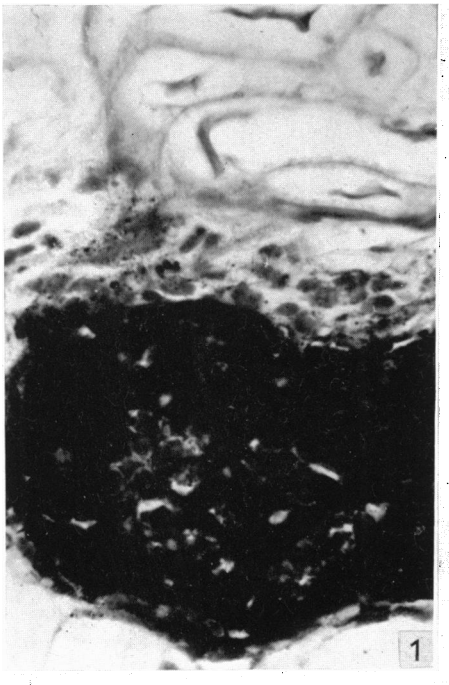
EXPLANATION OF PLATE 1

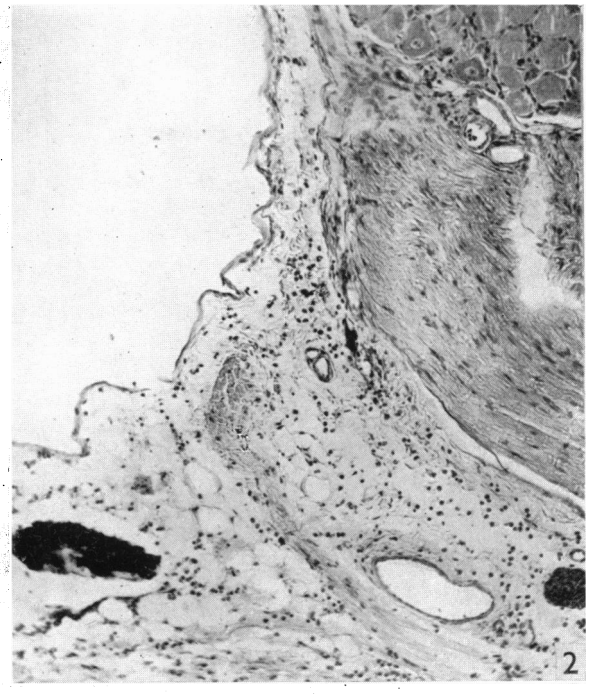
Fig. 1. A lymphatic vessel filled with India ink, in close relation to the spinal nerve just beyond the dorsal root ganglion. Fine particles of free ink have migrated through the lymphatic wall and have come into intimate relationship with the nerve. × 1300.

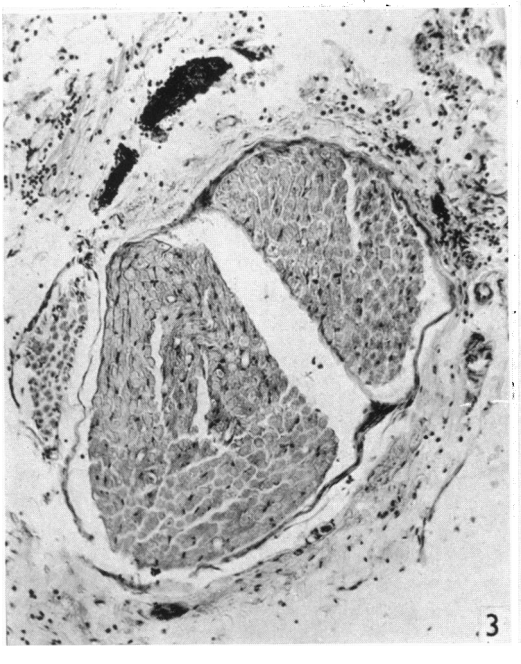
Fig. 2. Ink-filled lymphatics in the epidural fat close to the dorsal root ganglion. × 105.

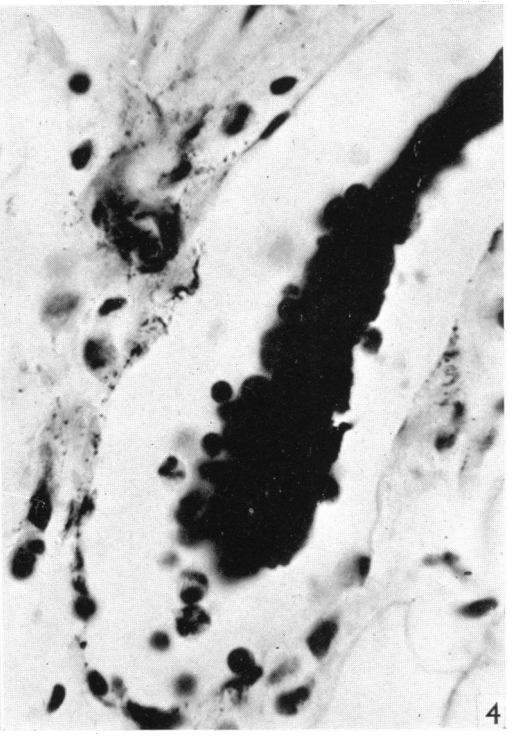
Fig. 3. Lymphatics grouped round the nerve just distal to the dorsal root ganglion. × 105.

Fig. 4. Ink-filled lymphatic close to dorsal root ganglion. The endothelial lining of the vessel is apparent and particles of India ink can be seen passing through it. The central dark mass is an accumulation of ink together with inflammatory cells. × 800.









FIELD AND BRIERLEY—LYMPHATIC DRAINAGE OF THE SPINAL NERVE ROOTS IN THE RABBIT