THE CONNEXIONS OF THE SPINAL SUB-ARACHNOID SPACE WITH THE LYMPHATIC SYSTEM

By J. B. BRIERLEY and E. J. FIELD
Department of Anatomy, University of Bristol

The first clear evidence of a connexion between the cranial sub-arachnoid space and the cervical lymphatic system was provided by Schwalbe (1869), and the existence of such a connexion is now generally accepted although detailed knowledge of the precise pathway is still incomplete. In general, it is believed that the route is from the sub-arachnoid space along the perineural spaces of the olfactory nerves to the tissue spaces of the nasal mucosa and thence, via fine lymph vessels, to the deep cervical lymph nodes.

The normal direction of flow in this sub-arachnoid-lymphatic communication is moreover not yet established. Thus, whilst the cervical lymph nodes can be filled from the cranial sub-arachnoid space under physiological pressures suggesting a centrifugal flow (Quincke, 1872; Weed, 1914; Ivanow, 1927; Galkin, 1930) substances introduced into the nose may later be demonstrated in the sub-arachnoid space (Le Gros Clark, 1929). Yoffey & Drinker (1939) discuss the factors involved in determining the direction of flow, but the significance of such factors under physiological conditions is difficult to assess.

That the spinal sub-arachnoid space has a similar connexion with the lymph vessels and nodes of the thorax, abdomen and pelvis has been amply demonstrated by Speransky and his co-workers in a series of papers which have appeared since 1927.

Review of Literature

Schwalbe (1869) introduced Berlin blue under constant but unspecified pressure into the cranial sub-arachnoid space of rabbits and dogs recently killed by exsanguination. As a uniform result he obtained filling of the lymph vessels and nodes of the neck and claimed that the latter were filled through vessels issuing from the jugular foramen in the base of the skull and forming a plexus on the anterior cervical muscles. In one case lymph nodes became injected along the entire length of the vertebral column, and Schwalbe took this to indicate that the spinal sub-arachnoid space had a close connexion with the lymphatic system.

Quincke (1872), using only dogs, injected 1 c.c. of cinnabar into the spinal sub-arachnoid space through a laminectomy opening in the upper lumbar region. The animals survived from 2 to 4 days. Quincke concluded that:

(1) A part of the cerebro-spinal fluid leaves the sub-arachnoid space along the nerves.

(2) There is a sac of arachnoid around the issuing nerve roots in which cinnabar collects and is not usually carried further except in the optic nerve.

(3) The cerebro-spinal fluid passes into the cervical and sub-maxillary lymph nodes.

As a serious contribution to this problem, the much quoted injection work of Key & Retzius (1875) is of little value on account of the high pressure used (60 mm. of mercury).

The same objection applies even more forcibly to the work of Spina (1900–1), for all his injections, with one exception, were carried out at pressures from 120 to 400 mm. of mercury.

Sub-arachnoid injection of a 1% solution of trypan blue was used by Goldmann (1913), who effected this both through a parietal trephine hole in the skulls of dogs and a type of lumbar puncture into the spinal sub-arachnoid space of rabbits. A small amount of cerebro-spinal fluid was first withdrawn, and the quantity of trypan blue injected varied from 1·0 to 2·5 c.c. Only when the latter volume was injected could staining of the deep cervical nodes be seen. Once again one must comment upon failure to control the pressure of injection.

Weed (1914) introduced into the sub-arachnoid space of the dog an isotonic mixture of potassium ferrocyanide and ferric ammonium citrate, and demonstrated granules of Prussian blue in the cervical lymph nodes when the tissues were subsequently treated with formalin, acidified with hydrochloric acid. Serial sections of the nasal region showed granules in the peri-neural spaces of the olfactory nerves, extending into the nasal mucosa and staining it blue. Here the granules lay beneath the epithelial cells in the meshes of loose connective tissue. In this tissue were thin-walled granule-containing vessels which Weed believed to be lymphatics. He concluded that a tissue space always lies between the peri-neural sheath containing cerebro-spinal fluid and the fine terminal vessels of the lymphatic system.

Certain aspects of Weed's work deserve special attention.

(*a*) He was careful to employ pressures 'only slightly in excess of physiological'. (Details not given.)

(*b*) The site of introduction of the solution was always in the lower thoracic or upper lumbar region. This focused attention exclusively on the cranial section of the sub-arachnoid space and made it impossible to investigate any outflow into lymphatics from the lumbar part of the sub-arachnoid space. It is just this region which Ivanow (1927) believed to possess a particularly rich lymphatic connexion.

(*c*) The Weed double salt mixture has been criticized by Spirov (quoted by Ivanow, 1927), who repeated Weed's technique on living dogs and dead human embryos. He observed that the salts impregnated the cranial bones, the bones and ligaments of the vertebral column and the cellular tissues around the blood vessels and nerves of the neck. This led Spirov to conclude that this crystalloid mixture could diffuse very rapidly through tissues and was no true indicator of preformed anatomical pathways.

(*d*) Weed carried out a large series of injections of suspensions of carbon granules into the spinal sub-arachnoid space, but always '. . . no granules could

be found on microscopic study in the cervical lymph glands and their channels' (1914, p. 80). This conflicts with the results of many subsequent workers and the disparity is probably due to the differences in the size of the particles used.

Woollard (1924) found trypan blue in the cervical lymph nodes of cats following its introduction into the cisterna magna, but dismissed this finding as being secondary to leakage from the puncture site.

Speransky and his co-workers began their investigation of the sub-arachnoid outflow in 1927, when Spirov repeated the work of Weed as already described. As a result of Spirov's findings, Ivanow (1927) employed India ink as the indicator substance. The introduction of the ink into the sub-arachnoid space via the lumbar route was commenced in the living dog and continued after its death for from 1 to 3 days. This led to a filling of the deep cervical, posterior abdominal and posterior thoracic lymph nodes, although the actual outflow channels were not evident. An exactly similar picture was obtained in the living animal after the introduction of 5·0 c.c. of ink suspension by the lumbar route.

A more detailed account of these channels was given by Ivanow & Romodanowsky (1928), who worked on the corpses of dogs and on living animals, the India ink being introduced by cisternal puncture or by laminectomy in the lumbo-sacral region. These workers concluded that:

(*a*) After cisternal injection, India ink was seldom seen to move farther caudally than the mid-thoracic region.

(*b*) Peri-neural spread in the spinal nerves only occurred as far as the intervertebral ganglia. This was true for the fifth and seventh cranial nerves also. Only in the olfactory, optic and acoustic nerves did ink spread throughout the whole extent of the nerve.

(*c*) Ink was seen to pass into 'segmental' lymph vessels which arose from the surface of the dura at points where digitations of the ligamenta denticulata were attached.

(*d*) These lymph vessels filled only sharply demarcated portions of the deep lymph nodes.

Moreover, in all the above experiments it was the nodes at the bifurcation of the aorta which showed maximal filling, commencing soon after the cervical filling. After small injections and brief periods of survival, it was these two groups, the cervical and aortic, which alone contained ink.

Galkin (1930), again using India ink, extended Ivanow's results by investigating the relative outflows from various isolated segments of the sub-arachnoid space under a perfusion pressure of 30 cm. of water. Cervical and lumbo-sacral outflows were found to be considerably in excess of that obtaining in the thoracic region.

Confirmation of a connexion between the sub-arachnoid space and the lymphatic system at all spinal levels was obtained by Oschkaderow (1936), who used India ink, Gerota mixture and thorium dioxide as injection masses. He also demonstrated lymph vessels passing from the arachnoid through the

atlanto-occipital membrane to lymph nodes below and behind the ear, and thence to the deep cervical lymph nodes.

Since 1936 work has been concerned exclusively with the outflow from the cranial sub-arachnoid space and its relation to the nasal mucosa and the cervical lymph nodes. Such literature falls outside the scope of the present review as far as regional distribution is concerned, although it may possibly throw light on the mechanism of the outflow presently to be considered.

Considerations of technique

(1) *The nature of the indicator substance*

This must be non-irritant and non-toxic. Estimation of the particle size is essential and in the India ink employed in the present work the particle sizes range from 0·4 to 1·5μ. The majority (more than 90 %) are under 0·5μ. The form of the smaller particles is roughly spherical, while the larger ones are aggregates of more than one particle.

(2) *Site of introduction of the indicator substance*

The indicator substance must have access to the whole of the sub-arachnoid space. Moreover, there should be as little leakage of ink as possible from the introduction site. With these two provisos in view, the lumbar introduction as practised by Weed and the sub-occipital method of Woollard (1924) were rejected. Instead ink was introduced directly into the ventricular system or into the cranial sub-arachnoid space through a small burr hole which could be effectively plugged with bone wax.

(3) *Control of injection pressure*

The ink suspension was allowed to run in at a pressure not exceeding 120 mm. of ink, and was only introduced after withdrawing an equal or rather greater volume of cerebrospinal fluid.

(4) *Quantity of ink injected*

By making repeated injections, as much as 4·5 c.c. of ink could be introduced into one animal.

MATERIAL AND METHODS

Animals

Adult rabbits of weights from 1·5 to 3·0 kg. were used.

Anaesthesia

This would appear to influence materially the result of the experiment. Thus, in the earlier experiments of this series when urethane was employed and the animals did not recover consciousness, it was found that very little ink left the sub-arachnoid space. Later, with the use of sodium nembutal (2·5 % intravenously; something less than 2·0 c.c./kg. body weight) allowing of the recovery of the animal in about 2–2½ hr., the outflow of ink was markedly

increased. It would seem important then, that the animal should return to full activity as soon as possible after the operation, presumably in order that a normal level of cerebrospinal fluid pressure may be restored.

Preparation of the India ink

It is important to use good-quality India or Chinese ink in stick form. The stick of ink was rubbed down in double strength Ringer solution until a concentrated suspension was produced, then filtered through a no. 5 Whatman paper and sterilized immediately before use.

Technique

Approach to the lateral ventricle in the first five animals of the series was made by removing a temporal bone flap. The underlying ventricle was then approached through the area of brain thus exposed. This technique was abandoned because it was felt that the presence of a decompression caused by the removal of a bone flap might prevent the return of cerebrospinal pressure to normal and also because complete control of leakage of ink was found to be unattainable.

In later experiments a vertical incision was made half-way between the posterior border of the supraorbital ridge and the nuchal ridge of the occiput, and the temporalis muscle divided in the line of the incision after separating it from the bone with an eye spud. The bone was drilled with a dental drill of about 1 mm. diameter, directed backwards at 20° to the coronal plane and downwards at 20–25° to the horizontal plane. To puncture the dura, to fuse effectively dura and arachnoid and to prevent bleeding from the dural vessels, a blunted needle at dull red heat was passed down the drill hole and just through the dura. A fresh sterile drill was placed in the hole to serve as a guide for the needle. The latter—a children's type lumbar puncture needle—was then swung into place over the hole and slowly introduced until clear cerebrospinal fluid could be obtained on slight suction. A 2·0 c.c. syringe containing that amount of ink was then attached to the needle and supported in a clamp. The ink ran in briskly at first but slowed up as the volume entering approached that of the cerebrospinal fluid withdrawn. The introduction usually took about 5 min. This method was particularly suitable for repeated injections as the same hole could be entered two or three times before changing to the other side. As the needle was withdrawn the burr hole was filled with bone wax. The animals were usually killed by exsanguination through the thoracic aorta under paraldehyde anaesthesia.

Subsequent examination showed that in many animals the needle had passed through the whole thickness of the occipital lobe and had entered the sub-arachnoid space on the dorsum of the brain stem. In these cases the amount of cerebrospinal fluid withdrawn was in excess of that which was normally obtained from true ventricular punctures. In so far as the pressure of introduction of the ink did not exceed 120 mm. of ink, and that the possibility of

leakage had been eliminated, such introductions were perfectly valid for the purposes of the present investigation.

OBSERVATIONS

On laminectomy the most striking feature is the accumulation of India ink around the nerve roots of the lumbo-sacral and cervical regions. These accumulations are the 'ink-cuffs' ('Tuschenmanchetten' of Ivanow). They are less marked in the thoracic region.

On the dorsal nerve root the typical cuff takes the form of a truncated cone with base directed medially—up against the cord—and apex falling just short of the dorsal root ganglion. Whilst the position of the apex is constant in all

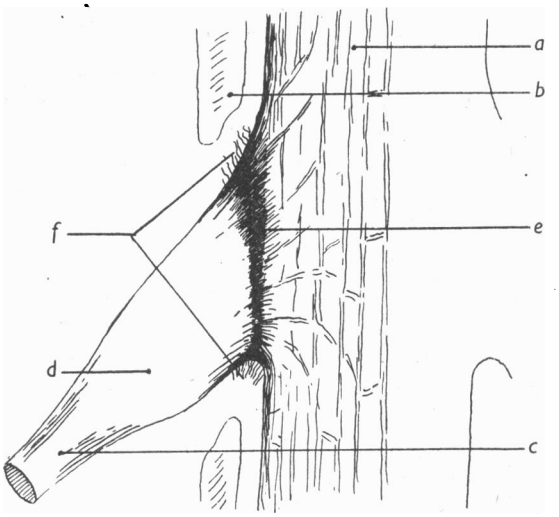

Fig. 1. Ink-cuff of upper sacral nerve. *a*, dura mater; *b*, pedicle of vertebra; *c*, spinal nerve; *d*, dorsal root ganglion; *e*, ink-cuff; *f*, fine black lines arising from ink-cuff.

cases, the exact position of the base is dependent upon the amount of accumulated ink—which in turn depends upon the amount introduced. From the base, thin black lines pass on to the cord apparently due to the presence of ink between diverging fibre bundles. In this way a fan-like effect is produced which may bear a superficial resemblance to a lymphatic network (Fig. 1). Along the anterior nerve roots the accumulation of ink is less intense. Its distal limit is less defined, but is as a rule some 2–3 mm. farther out along the nerve than is the rule in the dorsal nerve root.

Observation under the low-power binocular dissecting microscope showed a leash of fine black lines (? lymph vessels) passing from the region of the nerve root culs-de-sac ventrally towards the paravertebral lymph nodes. This appearance was again only well developed in the lumbo-sacral region.

In those animals that had received larger quantities of ink (2–4 c.c.) it was possible to see a few slender black lines passing from the ventral surface of the

dural tube to either side of the posterior longitudinal ligament at the point where it spans the large foramen in the posterior surface of the vertebral body. On the posterior abdominal wall, definite fine lymphatic vessels could be traced backwards from the ink-filled nodes to the mid-point of the vertebral bodies between the two psoas muscle masses. It seems evident that these vessels collect ink both from the ink-cuff and the ventral surface of the dural tube.

In certain animals it was noticed that the epidural fat was stained grey, particularly in the sacral region. Histological examination of the fresh material revealed India ink particles lying, not in vessels but free in the meshes of the fat.

The presence of ink in one or more of the cervical, posterior thoracic, posterior abdominal and pelvic lymph nodes was a constant finding in all animals of the series. The accompanying table indicates the distribution and intensity of the ink in the various lymph nodes, the animals being arranged in order of their survival times. With adequate filling it can be seen that ink accumulates most markedly in the nodes of the neck, those around the bifurcation of the aorta and in the nodes of the hollow of the sacrum.

The deep cervical nodes are the first to fill and are followed by a pair of nodes in front of the upper part of the sacrum. Ink is visible in the superficial cervical lymph nodes at approximately the same time as in those of the aortic bifurcation. The typical picture of the widely separated ink-containing nodes of the cervical and aortic regions is now complete. After an interval of 30–36 hr. certain small nodes of the posterior body wall begin to exhibit ink. The first of these lies in front of the bodies of the lumbar vertebrae in the groove between the two psoas muscles. These nodes are always incompletely filled, such filling commencing in the posterior part of the node and being due to the arrival of ink particles along fine vessels which can be traced backwards to the mid-point of the body of each lumbar vertebra. Such vessels are strictly segmental, whereas the small nodes just described do not always qualify for this description as their number may not correspond to that of the related vertebrae. Nevertheless, 'segmental node' has been used as a term of convenience in this work.

In the thoracic region the first nodes to contain ink are those grouped together in the posterior mediastinum. The last nodes of the body wall to exhibit ink are two or three lying on the bodies of the thoracic vertebrae between the bifurcation of the trachea and the crura of the diaphragm.

Beyond the segmental vessels already mentioned, it was not possible to identify any other vessels that might be conveying ink particles from the sub-arachnoid space to the lymph nodes. As it was, segmental vessels never showed more than a moderate greyness even in those animals that received the largest quantities of ink.

Histological examination of a lymph node in an early stage of filling showed much of the ink lying in the peripheral lymph sinus. At first it appears to be largely free, but later the number of macrophages containing ink granules increases.

Table 1. *Analysis of experimental results*

Animal	Weight (kg.)	Anaesthetic	Approach	Vol. of Ink (c.c.)	Injections	Survival (hr.)	Lymph nodes: Cervical: Superficial	Cervical: Deep	Thoracic: Mediastinal	Thoracic: 'Segmental'	Lumbo-sacral: 'Segmental'	Lumbo-sacral: Aortic	Lumbo-sacral: Sacral
1.1 24	2·6	Paraldehyde	Bone flap	0·8	1	1	○	+	○	○	○	○	○
1.1 18	2·5	Paraldehyde	Bone flap	0·5	1	12	+	+ +	○	○	○	○	+
1.1 23	1·5	Paraldehyde	Bone flap	0·5	1	17	+ +	+ + +	○	○	○	+ +	+
1.1 13	2·5	Paraldehyde	Bone flap	0·8	1	18	+ +	+ + +	○	○	○	+ + +	+ +
1.1 14	2·4	Paraldehyde	Bone flap	0·5	1	22	+ +	+ + +	+	○	○	+ + +	+ +
1.1 26	3·0	Paraldehyde	Drill hole	0·5	1	24	+ +	+ + +	+	○	○	○	+
1.1 27	3·5	Paraldehyde	Drill hole	0·8	1	25	○	+ +	○	○	○	○	○
1.1 31	2·1	Nembutal	Drill hole	1·0	1	29	+ +	+ + +	○	○	+	+ +	+ + +
1.1 30	2·1	Nembutal	Drill hole	1·0	1	36	+	+ +	+	+	+ +	+ +	+ + +
1.1 34	3·5	Nembutal	Drill hole	2·4	3	56	+ +	+ + +	+	+	+	+ +	+ + +
1.1 28	2·4	Ether	Drill hole	1·0	1	57	+ +	+ + +	+ +	+	+	+ +	+ + +
1.1 32	2·1	Nembutal	Drill hole	2·3	3	84	+ +	+ + +	+	+	+	+ +	+ + +
1.1 29	3·0	Ether	Drill hole	1·0	1	98	+ +	+ + +	○	○	+ +	+ +	+ +
1.1 33	2·6	Nembutal	Drill hole	2·6	4	101	+ +	+ + +	+	+	+	+ + +	+ + +
1.1 36	2·4	Nembutal	Drill hole	4·4	5	108	+ + +	+ + +	+ +	+	+ +	+ + +	+ + +

Animals arranged in order of survival times.
○ = no ink in lymph node.
+ = ink just recognizable macroscopically.
+ + = enough ink present to give node dark grey colour.
+ + + = enough ink present to give node black or almost black colour.

DISCUSSION

The choice of India ink as the indicator substance in this work was made after careful consideration of the arguments levelled against it. Thus Weed believed that the channels used by the body for the removal of particulate material from the sub-arachnoid space might differ from those normally used for the absorption of the cerebrospinal fluid itself in so far as the former might involve a process of phagocytosis. Weed quoted his own expermental results in support of this argument, showing that sub-arachnoid introduction of a carbon suspension (particle size not stated) never resulted in the appearance of carbon in the cervical lymph nodes unless excessive pressure (100 mm. Hg) was used. On the other hand, similar introduction of the ferrocyanide solution always allowed Prussian blue granules to be demonstrated in that site. The present investigation has failed to confirm this distinction, and with India ink of particle size 0·4–1·5μ, a marked and rapid filling of the cervical lymphatic system was constantly obtained. It follows then that the rigid differentiation maintained by Weed cannot be upheld and that both particulate and solute indicators may reach the lymphatic system.

In view of the apparent unimportance of phagocytosis as a means of transport, it must be concluded that the particulate suspension will collect at points of outflow of cerebrospinal fluid from the sub-arachnoid space. Thus marked aggregations of ink are always found around the olfactory bulbs and it is believed that the 'ink-cuffs' of the spinal nerves have a similar significance. Nevertheless, it must be recognized that the various solutes of the cerebrospinal fluid stand closer in the matter of diffusibility to 'crystalloids' than to particulate matter. Thus the routes taken by Weed's double salt mixture may in some respects indicate more accurately the physiological potentialities of solute migration.

The normal pigment of rabbit lymph nodes may cause some confusion with India ink, particularly to the naked eye. Microscopically, however, the distinction between pigment and ink is easily made as the former appears as golden yellow or yellow brown (intra-cellular) granules, whilst ink particles are jet black and are both intra- and extra-cellular.

The employment of injection pressure not exceeding 120 mm. of ink cannot be in any way regarded as unphysiological, particularly as the ink merely replaces a corresponding volume of cerebrospinal fluid. It is to be noted, however, that the pressures used by Ivanow (1927), though well below those used by preceding workers other than Weed, ranged from 30 to 50 cm. of water and it might be argued that, as such, they were excessive and likely to produce a false picture through damage to tissues. Nevertheless, his results are substantially similar to those obtained in the present series of much more 'physiological' experiments.

The more striking accumulation of ink in the 'ink-cuffs' of the cervical and lumbo-sacral nerve roots is probably related to their relatively larger size. The

precise anatomy of the region is still disputed. A detailed but somewhat schematic description of the nerve roots between their emergence from the spinal cord and the level of the posterior root ganglion is given by Sicard & Cestan (1904), who termed this zone the 'nerf de conjugaison'. They investigated its structure by means of Chinese ink injected into the sub-arachnoid space through the atlanto-occipital membrane and found culs-de-sac of both sub-dural and sub-arachnoid spaces. This latter they found to have no connexion with the sub-epineural space of the peripheral nerve. Ballooning of the sub-arachnoid cul-de-sac was found to be the initial result of increased cerebrospinal fluid pressure.

Two distinct mechanisms may be invoked to explain the high concentration of ink in the cul-de-sac:

(i) A partial 'filtration' of the cerebrospinal fluid at these points, the majority of the particles being retained as by a strainer or filter, whilst fluid itself passes on into the lymphatic system carrying with it passively a certain amount of ink which eventually accumulates in the regional lymph nodes.

(ii) Phagocytosis of ink particles by macrophages normally stationed in the neighbourhood of the culs-de-sac and subsequent migration of these cells to the lymph nodes. Little or no fluid transudation may be involved in such a process.

The observations already recorded show that the description of the pathway taken by ink particles from the outer surface of the dura to the 'segmental' lymph nodes is still incomplete. The important and most elusive link in the anatomical chain lies between the sub-arachnoid space and the exterior of the dural tube in the region of the 'ink-cuff'. To explain the passage of India ink through this 'dura-arachnoid' membrane, either in the region of the 'ink-cuff' or elsewhere, one or more of the following mechanisms may be invoked:

(i) The dura-arachnoid might possess stomata permitting the passage of particles into the epidural connective tissue whence they pass into fine lymph vessels. Such stomata have often been postulated but never satisfactorily demonstrated.

(ii) The dura-arachnoid might exhibit a special permeability in respect of particles in the region of the 'ink-cuff'.

Either of these two mechanisms would explain the presence of free ink in the epidural fat of the lower sacral region.

(iii) Particulate matter might be taken up by phagocytic cells of the dura-arachnoid and actively transferred to lymphatics on the outside of the dura. Such cells would therefore be present at the sites of maximal ink concentration, notably in the 'ink-cuffs'.

The relative unimportance of phagocytosis in this region is shown by the histological examination of the 'ink-cuff', where only a minority of particles are seen to lie within macrophages. Thus it would seem unlikely that phagocytosis is responsible either for the concentration of ink in the 'ink-cuff' as suggested above, or for its transport to the epidural tissue and beyond. It is to be noted, moreover, that microscopic examination of the lymph nodes

indicates that in the early stages of filling most particles lie free in the peripheral lymph sinus and only later become ingested by macrophages.

Quincke (1872, pp. 161–162), using cinnabar, also concluded that transport could not be due to carriage by macrophages, as in many experiments masses of fine cinnabar particles could be found at a considerable distance from the point of introduction.

More recently much the same problem has arisen in connexion with the transport of foreign particles from the alveoli of the lungs to the regional lymph nodes. The balance of evidence suggests that movement of such particulate matter is not brought about—at any rate exclusively—as a result of preliminary phagocytosis (Drinker & Yoffey, 1941, p. 87). The rapidity with which transference takes place militates against the latter view, and the same point may be urged with regard to our findings as to the rapidity of appearance of ink in the cervical nodes after introduction into the lateral ventricle. It is to be noted also that the size of particles employed in the present work was well below the limit of 2μ given by Gillilan & Conklin (1938) as capable of direct passage into lymphatic channels.

It appears probable then, that the cuff is an area in which both fluid and particulate matter may leave the sub-arachnoid space through the thickness of the dura-arachnoid and so become epidural. The local epidural fat or connective tissue becomes ink-stained, and it is in this epidural layer that blindly ending lymphatics arise and conduct fluid and particles to the regional lymph nodes. This postulated pathway is summarized in Fig. 2.

The mechanism here outlined is comparable in its main features with Weed's (1914) reconstruction of the passage of the Prussian blue solution from the sub-arachnoid space into the nasal mucosa and cervical lymph system (Fig. 3). The tissue space which, this author maintains, always intervenes between the sub-epineural space (sub-arachnoid prolongation) and the small blind lymph vessels, is represented in the above spinal scheme by the epidural space and its contained fatty connective tissue.

Weed (1914) from the results of the ferrocyanide method observes that '...there is an obvious perineural deposit of precipitated granules which can be followed a short distance outward along the anterior and posterior nerve roots' (p. 90); and again, 'From the perineural space about the spinal nerve roots absorption takes place along lymphatic channels'. He concludes that this is the sole pathway for fluid escape from the spinal meninges (pp. 90–91). Unfortunately, he presents no adequate anatomical basis for this important statement.

The volume of fluid which may leave the sub-arachnoid space by lymphatic channels probably depends upon several factors. Of these, the pressure of the cerebrospinal fluid is probably predominant, but this itself can be influenced in a number of ways—e.g. by the type of anaesthetic, the depth and duration of anaesthesia, variations in respiration and blood pressure, and possibly the functional conditions of the absorbing channels.

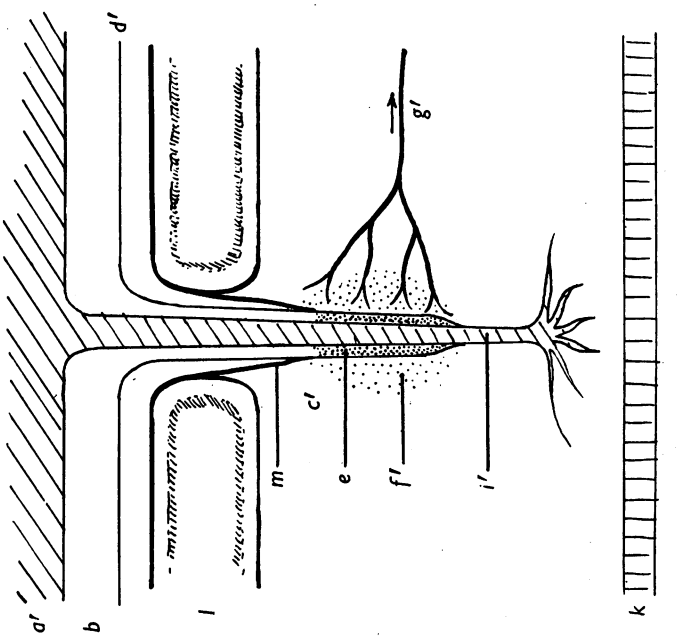

Fig. 3. Diagram of olfactory nerve and its ink-containing arachnoid sheath (rabbit).

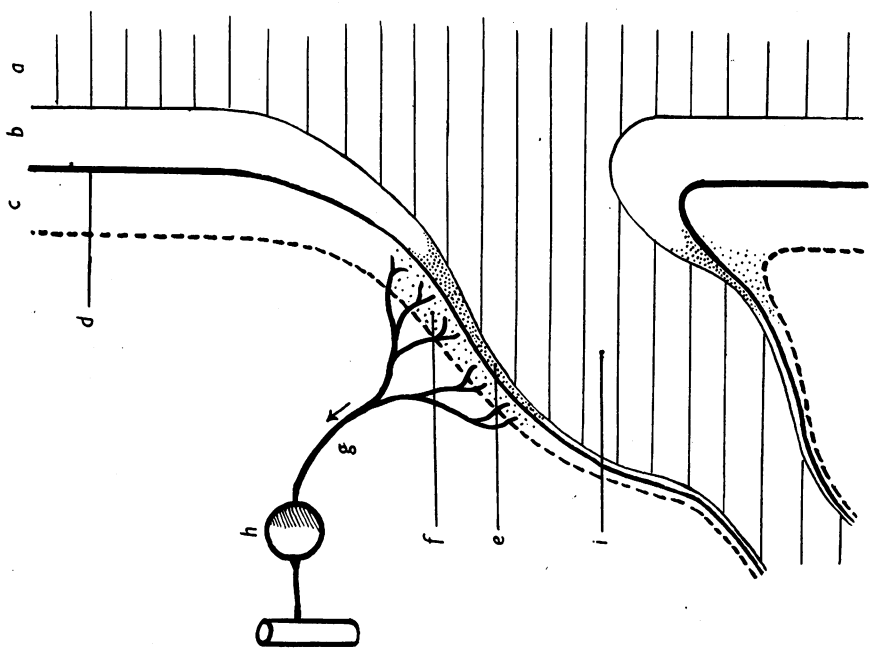

Fig. 2. Diagram of spinal nerve root (rabbit) and its surrounding 'ink-cuff'.

The present communication has thus demonstrated that under conditions as nearly physiological as possible, particles not over 1·5μ can pass readily from the sub-arachnoid space not only into the cervical lymph nodes but into those lying in front of the vertebral column. These latter indeed may be regarded as the regional lymph nodes of the spinal sub-arachnoid space.

SUMMARY

1. The literature relating to the connexions between the spinal sub-arachnoid space and the lymphatic system is reviewed.

2. A technique for introducing India ink into the cranial sub-arachnoid space of the rabbit without resultant rise in intracranial tension is described.

3. Following such introduction ink appears within 4 hr. in the nasal mucosa and cervical lymph nodes.

4. Ink introduced into the cranial sub-arachnoid space can be found as low as the mid-thoracic level at the end of 1 hr. and throughout the spinal sub-arachnoid space in 6 hr.

5. Concentration of ink is marked around the lumbo-sacral nerve roots and in the terminal part of the spinal sub-arachnoid sac.

6. Lymph nodes around the aortic bifurcation and in the hollow of the sacrum show ink filling in 6 hr.

7. Repeated ink injection produces filling of the lymph nodes arranged along the front of the thoracic and lumbar vertebrae in a more or less 'segmental' manner.

8. The pathway of this outflow is discussed.

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Lettering to Figs. 2 and 3

a, spinal cord; *a'*, olfactory bulb; *b*, sub-arachnoid space; *c*, epi-dural connective tissue; *c'*, nasal sub-mucosa; *d*, dura-arachnoid (rabbit); *d'*, arachnoid; *e*, ink particles in sub-arachnoid cul-de-sac; *f*, ink particles in epi-dural connective tissue; *f'*, ink particles in nasal sub-mucosa; *g* and *g'*, fine lymph channels draining epi-dural tissue and nasal sub-mucosa; *h*, lymph nodes of body wall; *i*, spinal nerve; *i'*, olfactory nerve; *j*, longitudinal collecting channel; *k*, nasal mucous membrane; *l*, cribriform plate; *m*, dura mater.

REFERENCES

CLARK, W. E. LE GROS (1929). *Rep. publ. Hlth med. Subj., Lond.*, No. 54.

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

GALKIN, W. S. (1930). Zur Methodik der Injektion des Lymph-systems von Sub-arachnoidalraum aus. (Bedeutung der Injektionstelle.) *Z. ges. exp. Med.* **74**, 482–489.

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

GOLDMANN, E. E. (1913). *Vitalfarbung am Zentralnervensytems. Beitrag zur Physio-Pathologie des Plexus chorioideus und der Hirnhäute.* Verlag von Königl. Akademie der Wissenschafte, Berlin.

IVANOW, G. (1927). Über die Abflusswege aus den submeningealen Räumen des Rückenmarks. *Z. ges. exp. Med.* **58**, 1–21.

IVANOW, G. (1929). Über die Abflusswege aus den Sub-arachnoidalräumen des Gehirns und Rückenmarks und über die Methodik ihrer intravitalen Untersuchung. *Z. ges. exp. Med.* **64**, 357–375.

IVANOW, G. & ROMODANOWSKY, K. (1928). Über den anatomischen Zusammenhang der cerebralen und spinalen submeningealen Räume mit den Lymphsystem. *Z. ges. exp. Med.* **58**, 596–607.

KEY, A. & RETZIUS, G. (1875). *Studien in der Anatomie des Nervensystems und des Bindegewebes.* Stockholm.

LEWANDOWSKY, M. (1900). Zur Lehre von der Cerebrospinalflüssigkeit. *Z. klin. Med.* **40**, 480–494.

OSCHKADEROW, W. I. (1936). Beiträge zur Frage der Abflusswege der Cerebrospinalflüssigkeit des Gehirns und des Rückenmarks. *Anat. Anz.* **82**, 441–472.

QUINCKE, H. (1872). Zur Physiologie der Cerebrospinalflüssigkeit. *Arch. Anat. Physiol., Lpz.*, 153–177.

SCHWALBE, G. (1869). Die Arachnoidalraum ein Lymphraum und sein Zusammenhang mit den Perichorioidalraum. *Zbl. med. Wiss.*, **7**, 465–467.

SICARD, J. A. & CESTAN, R. (1904). Étude de la traversée meningoradiculaire au niveau du trou de conjugaison. — Le nerf de conjugaison (nerf radiculaire spinal). Quelques déductions cliniques. *Bull. Soc. méd. Hôp. Paris*, no. 21. 3e serie, 715–725.

SPERANSKY, A. D. (1934). *A basis for the theory of medicine.* New York: International Publishers.

SPINA (1900, 1901). Untersuchungen über die Resorption des Liquors bei normalen und erhöhtem intracraniellen Drucke. *Pflüg. Arch. ges. Physiol.* **83**, 120–151, 415–440.

SPIROV, M. S. (1927). The paths of distribution of cerebrospinal fluid and of injection masses from the sub-arachnoid space of the cerebrum and spinal cord. *Russ. Arch. Anat. Histol. Embryol.* **6**, no. 2 (quoted by Ivanow—not obtainable).

WEED, L. H. (1914). Studies on the cerebro-spinal fluid. *J. med. Res.* **31**, 21–117.

WOOLLARD, H. H. (1924). Vital staining of the leptomeninges. *J. Anat., Lond.*, **58**, 89–100.

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.