ON THE RELATIONS OF THE LYMPHATICS OF THE SPINAL CORD.¹

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(PLATES XXIII.—XXVII.)

It is becoming more and more evident that an accurate knowledge of the lymphatic system of the spinal cord (and the same holds equally true with regard to the brain) is essential for the proper interpretation of the pathological processes which take place within it. Much has hitherto been attributed to the blood vessels which there is increasing reason to think is in reality to be associated rather with the lymphatic system. The latter is admitted to be the main channel for the removal of waste products and products of degeneration of the constituent elements of the central nervous system, more especially of the myelin. Attention is now being more and more drawn to the effects of disorders of the lymphatic circulation—such as stasis and dilatation of the spaces—upon the nervous elements, but little definite information is as yet available. Arndt (1870¹) was one of the first to recognise the influence of a hyperlymphosis or lymphostasis upon the specific elements of the central nervous system, and since his time various important contributions have been made to this subject, some of them quite recently. There is an accumulating body of evidence that the lymphatics play a part probably not subordinate to that of the blood vessels as channels by which infective agents (toxins and organisms) are conveyed to the cord or distributed within it. Among other papers we may refer to the work of Meyer on tetanus, of Orr and Rows (1907²) on the flow of the lymph stream in the spinal roots of the cord, and to the classical work of Wickman on poliomyelitis.

LYMPH CHANNELS.

It is generally admitted that the brain and spinal cord do not possess lymph vessels closed in by walls of their own. The lymph circulates in spaces around the blood vessels, and these spaces communicate with the lymph sacs

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in the membranes. Our present knowledge of these channels is largely due to the early researches of His (1865⁵), Robin, and Obersteiner (1900⁷), and later of Axel Key and Retzius. In the text-books of Obersteiner and Schmaus-Sacki (1901¹²) we find it stated that within the spinal canal from without inwards are found: the epidural space, filled with fat and venous plexuses; the dura covered on both sides with endothelium, and composed of dense connective tissue in which are numerous narrow lymph spaces communicating with the subdural space; the subdural space, normally a capillary space; the arachnoid, a non-vascular membrane covered on both surfaces with endothelium; the sub-arachnoid space, in which circulates the cerebro-spinal fluid, and crossing which are numerous connective-tissue threads dividing it into larger and smaller lymph sacs (this space communicates with the lymph spaces in the pia and also with the lymph spaces within the perineural sheaths); the pia, in which are found the blood vessels which penetrate the cord, also numerous lymph spaces which communicate with the sub-arachnoid space; the epispinal space or space of His, a fissured space often permeated by radial fibres; dense glia forming the cortical surface of the cord (Lenhossek's membrane or *membrana limitans gliae superficialis*).

In early development the central nervous system has no blood vessels, and these first enter as buds from the pia into the up till now solely ectodermal tissue, carrying before them the *membrana limitans superficialis* as a *membrana limitans perivascularis*. With the buds there also enters loose connective tissue from the pia, which plays the rôle of adventitia, and the spaces of which are continuous with the spaces of the pia and through them with the sub-arachnoid space. Therefore, between the adventitia and the *membrana limitans perivascularis* we have a space—the perivascular space of His—continuous with the epispinal space of His, and a continuation of the sub-arachnoid space in the loose tissue of the adventitia—the adventitial lymph space or Virchow-Robin space. These two lymph spaces are said to exist everywhere independently of each other around the vessels of the central nervous system, one within the adventitia (intravascularis), and one outside the adventitia (extra- or perivascularis). Where the adventitia is absent or hardly perceptible, as in the finest capillaries, the two spaces blend into one, and there is only one space between the limiting membrane and the capillary endothelium. Further, each ganglion cell is surrounded by a wide space—the space of Obersteiner—through which it sends its processes and within which two or three lymphoid cells are frequently found. Obersteiner's diagram, copied into most text-books, has familiarised us with his statement that there is a direct transition of the perivascular space into the pericellular space, and that the ganglion cells are thus embedded in the perivascular lymph system.

The tissue lymph spaces discharge everywhere into the perivascular space, either through the interstices of the glia tissue, or by the protoplasmic processes of the glia reticulum, and the lymph flows out in the direction of the periphery of the cord. A lymph flow outwards is also assumed in the adventitial lymph sheaths.

The lymph channels of the anterior and posterior roots are also in communication with the lymph system. Orr and Rows (1907⁹) have pointed out the evidence of a continuous flow of lymph upwards along the peripheral nerves and spinal roots. Their researches prove that the main current lies at the periphery of the nerve bundles in the inner meshes or lymph spaces of the fibrous perineural sheaths. The current, as it reaches the cord, passes partly to the lymph spaces of the pia-arachnoid, but mainly along the nerve roots into the substance of the cord.

According to this account, therefore, we have two independent lymph spaces, namely, the inner, known as the Virchow-Robin or

adventitial space, continuous with the sub-arachnoid space, and the outer, known as the perivascular space of His, continuous on the one hand with the epispinal space of His, and on the other hand with the pericellular space of Obersteiner. Regarding the adventitial lymph space all is clear and definite; regarding the perivascular and pericellular lymph spaces, however, many false views have been promulgated, and these spaces are often confounded with another space which is regarded by many observers emphatically as an artefact.

In transverse sections of the cord fixed in formalin or chromic salts and hardened in alcohol, obvious clefts are found around the vessels and cells. His (1865⁴) stated that these clefts were smooth, and that the outer wall was formed of a condensed layer of glia substance covered with endothelial cells. His further succeeded in completely filling up the perivascular and pericellular spaces by injecting from the epispinal space. Obersteiner (1900⁷) found, in the presence of small cells, indistinguishable morphologically from lymphoid cells, within the pericellular spaces, a proof of their being lymph spaces. Friedmann (⁸) also came to the conclusion that the perivascular and pericellular spaces were preformed and lined by endothelium analogous to lymphatic endothelium.

More recent investigations have proved that the occurrence of the spaces of His and Obersteiner can be avoided, and that they are due to shrinkage through the methods of fixation. A careful consideration of the clefts shows that they rarely possess the smooth walls ascribed to them by His, and that where such is the case, the endothelial lining of His and Friedmann is undoubtedly a layer of glia cells which have so folded themselves round a space through the tissue retraction. More usually, fine threads of glia fibrils connect the adventitia of the vessel or membrane of the ganglion cell to the ragged outer wall of the space (Plate XXIV. Figs. 5 and 6). Obersteiner's picture of "a rose-branch beset with thorns" can thus be traced to fine glia fibrils which have remained existent between the vessel and the retracted tissue (Plate XXIV. Fig. 5). Obersteiner admits that the perivascular and pericellular spaces may increase in size through tissue retraction, but maintains that the normal existence of such spaces is proved by the presence of the free round cells already mentioned.

Many advocates of the perivascular space admit the presence of these fine threads connecting the outer wall with the adventitia of the vessel and with the ganglion cell, and amongst these Schroeder (1908¹³) maintains that the true perivascular space of His is still within the *membrana limitans perivascularis*, which remains adherent to the ganglion cell or adventitia, and that the shrinkage space is external to this limiting membrane.

The usual treatment with alcohol in the process of hardening gives rise to considerable shrinkage on account of the varying consistence of

the individual elements of the tissue, and so we get rupture at certain parts. One of the parts of predilection is in the neighbourhood of the vessels. The tear here is directly external to the *membrana limitans perivascularis*, and as this is a condensed layer it remains adherent to the vessel adventitia, while the glia tissue outside retracts. Such artificial shrinkage spaces are constantly found in preparations, and the vessel as a rule lies somewhere on the wall of the space—not in the middle (Plate XXIII. Fig. 3). The space is traversed by individual small fibres radiating from the perivascular glial ring round the vessel. In oedema of the cord and in arterio-sclerosis we find that these shrinkage spaces are specially large.

Amongst those who have most strenuously opposed the teaching regarding the perivascular and pericellular lymph spaces is Nissl. Nissl has followed Degenkolb and Weigert in looking upon the adventitia as a biological limiting layer, separating mesodermal from ectodermal constituents of the nervous system. He finds in this biological limiting layer the key to the understanding of the histo-pathology of the nervous system. Nissl (1903⁶) claims that there is no penetration of mesodermal elements into the nervous substance so long as this limiting layer remains intact, and that were the perivascular and pericellular spaces really lymph channels with endothelial nuclei in their walls, the parenchyma of the nerve tissue would be over-run with lymphoid (mesodermal) elements. To grant the existence of preformed perivascular and pericellular spaces would be, he asserts, to mistake and underestimate the part played in the histo-pathology of the cord by the non-nervous cells of ectodermal origin. In inflammatory conditions in the cord and in slow degenerations the reparatory processes within and without this limiting layer are altogether independent of each other, but if ectodermal or mesodermal elements are so injured that they could no longer form checks to the growth of uninjured elements, then the destroyed biological balance would be restored by progressive processes on the part of constituents of either origin. These views are too theoretical to be of service in the solution of the problem.

ARTIFICIAL INJECTION OF THE LYMPHATICS.

His and Obersteiner injected the perivascular and pericellular spaces from the epispinal space, but there are many objections to such a method, the chief being that the very delicate nature of the nerve tissue causes it to give way at the points of least resistance under the pressure. Key and Retzius found it impossible to inject the perivascular and pericellular spaces from the sub-arachnoid space, from which they inferred that these spaces are not in communication with the large lymph spaces of the central nervous system.

Many observers see in the epispinal space a result of tissue retraction. This would indeed almost follow from the absence of the perivascular space, for if the adventitia is closely applied to the *membrana limitans perivascularis*, then the *pia intima*, which is carried in as the outermost layer of the adventitia, must be closely applied to the *membrana limitans superficialis* without any epispinal space. In

tissues fixed in Zenker's solution, and very gradually carried through the different hardening fluids, we find that this *pia intima* is closely adherent to the surface of the cord and that no perivascular or pericellular spaces are present. Ranke (1908¹⁰) has also found this to be the case in frozen sections from fresh material and in tissues fixed in Cox's solution.

It will be seen from what has just been said that artificial injection of the lymph paths of the cord gives unreliable results. Fortunately, however, there is a natural process of injection of the lymphatics which can be depended upon as being absolutely selective, and which is therefore eminently suitable for the study of the lymph paths. This process occurs in every case in which a slow degeneration of the myelin is taking place, and it is seen specially well in cases of long-continued compression of the cord, or in the sub-acute combined degenerations such as are so often seen in pernicious anaemia. Here we get the most marked distension of the *adventitial lymph spaces* by cell elements filled with fat—the so-called compound granular cells. The course of all the vessels, arterial, venous, and capillary, can be traced by the dense layer of these cells which surrounds the vessels. *In every case it is significant that the cells have pushed apart the connective-tissue fibrils of the adventitia and that an outer layer of adventitia, pink with Van Gieson's stain, bounds the dilated lymph spaces and is closely applied to the glial membrane, leaving no perivascular space.*

Van Gieson's method of staining is particularly well adapted to demonstrate the boundary of the lymphatic space and its relation to the surrounding glial tissue, as it stains the connective tissue a more or less deep pink colour, and the glial tissue an orange-yellow colour. As will be seen from Plate XXIII. Figs. 1 and 2, the space surrounding the small arterioles, venules, and capillaries is bounded by the pink line of connective tissue which represents the outer layer of the adventitial lymphatic sheath.

COMPOUND GRANULAR CELLS.

When the degeneration of myelin has reached a certain stage, then resorption of the degenerated material sets in by means of granular cells. The space occupied by the degenerated nerve fibres becomes replaced by granular cells. These may remain a shorter or longer time, but finally they reach the lymphatic spaces in the adventitia, and are thence carried to the inner layers of the pia. (Nissl named these compound granular cells *Gitterzellen*, or lattice cells, because he considers that the lattice structure of the protoplasm—found after the fat is extracted—is the chief characteristic of these elements (Plate XXIII. Figs. 1 and 2).) The fat may be absorbed by the cell in the form of clumps of degenerating myelin, or in a dissolved form as saponified fat and, by synthesis within the cell, be transformed

again into neutral fat when it appears as fine droplets (Plate XXV. Figs. 9 and 10). Simultaneous with the work of phagocytosis of the fat the solution of the degenerated myelin takes place. This process is helped by the lymph stasis through its power of distending and dissolving the nerve fibre. The fluid components may become directly absorbed by the cells as just stated, or be absorbed directly into the lymph spaces; and one finds in the lymph sheaths, in addition to granular cells, free granular, fatty detritus. The granular cells at first fill up the space of the degenerated fibre, and on longitudinal sections of the cord are seen in rows in the tubular space originally occupied by the nerve fibres. On transverse section they appear as large, rounded cells with faintly staining, finely vacuolated protoplasm (Plate XXIII. Figs. 1 and 2). As the granular cells become laden with fat, the adventitial lymph spaces are opened up, and the peripherally streaming lymph, acting as a suction pump, draws the cells into the spaces. The number of the compound granular cells varies with the duration of the process and the nature of the degeneration. When all the decayed material has been absorbed by the cells, and these have been drawn into the lymph spaces of the adventitia, a dense glia tissue, with narrow tissue spaces, takes the place of the degenerated fibres and compound granular cells. The latter may still be found isolated in the tissue and immediately around the vessels. Through a further resorption of the fat from the cell—the fat may be absorbed into the lumen of the vessel (see Plate XXV. Fig. 9) or into the lymph spaces and become free—the granular cell undergoes atrophy, and vessels may be found in densely sclerosed areas where the granular cells in the adventitial lymph spaces have given up their fat. Such cells remain in the adventitia as “ghost cells,” with shrunken nuclei and the remains of cell body and membrane, to increase the nuclear content of the adventitia. Through this process comparatively few of the large numbers of compound granular cells reach the surface of the cord, and the course of the vessels always shows a marked diminution of the dilated adventitial spaces near the periphery of the cord (Plates XXVI. and XXVII. Figs. 11 and 13). The cells as they reach the pia spread into the spaces of its inner layers—the spaces of the adventitia being continuous with the pial lymph spaces. It has been stated that no perivascular space can be recognised in transverse sections of the cord. The cells fill out the spaces of the adventitia and separate its fibrils to so great an extent that the outermost layer of the adventitia is closely pressed against the *membrana limitans perivascularis*. Likewise, on the surface of the cord *no epispinal space can be recognised*, for the innermost layer of the pia, stained pink by Van Gieson's stain, can be found closely applied to the *membrana limitans superficialis* and distinctly within the layer of granular cells, filling the lymph spaces of the *pia intima*. Even in Marchi preparations it can be distinctly noted that the compound granular cells are spreading in the inner pial layers, not in an epispinal space. Such

natural injections of the lymph spaces therefore lend no support to the view of the existence of perivascular and epispinal spaces. It should also be noticed, as a further important evidence of the non-lymphatic character of the so-called space of His, that in all the preparations in which there was natural injection of the lymphatics, where the process of fixation and hardening had produced a shrinkage space between the vessel and the surrounding nervous tissue, this space was invariably devoid of compound granular corpuscles. It is inconceivable that this could have been the case had His's space been really a lymphatic space.

With regard to the pericellular spaces, there is no corresponding natural injection by compound granular corpuscles. It is quite true that they may have in their neighbourhood one or more lymphoid corpuscles when the cells are in a state of degeneration, but it is not clear that these are lying in lymphatic spaces. There is no indication of any endothelial lining to the space outside the cell, and if the tissue has been carefully hardened by Zenker's method, no fissure will be seen. If the process of fixation has been imperfect, a space may appear either round the cell and its processes or in relation to part of it, but in such cases careful examination will show, in many instances, that the process of fissure formation has begun by rupture of the glial tissue immediately surrounding the nerve cell, and that a portion of its tissue has been left adherent to the nerve cell, with ragged threads projecting either into or across the fissure (Plate XXIV. Fig. 6). In our opinion the process of pericellular fissure formation is exactly analogous to that of the perivascular one. There is no more a space of Obersteiner than there is a space of His. Both are artefacts due to imperfect fixation of tissue, aided frequently, no doubt, by pathological changes which render the glial tissue less resistant. In both instances the rupture is through the glial tissue, part of which remains attached either to the vessel or the cell, as the case may be. We are of opinion that there is but one set of lymphatic vessels, namely, the adventitial, which, obtaining its lymph supply either from the vessels or absorbing it from the nerve tissue, conveys it into the deep layers of the pia.

An examination of various cases of degeneration of the white matter shows that the main course of the lymphatic current goes towards the periphery of the cord. In the case of pernicious anæmia, which was the subject of most of the illustrations of this paper, sections stained by Marchi's method showed no evidence that the compound granular corpuscles were making their way either towards the central canal or the grey matter of the cord. The latter fact appears to us to be an additional argument in favour of the intimate relationship of lymphatic and blood vessel, as the vessels of the white and grey matter of the cord are almost entirely independent of each other. Therefore, in an involvement of the distribution of the

vessels of the white matter, the lymphatics of the other area would remain unaltered.

In conclusion, briefly: Within the spinal cord there is no lymphatic epispinal space of His; there is no perivascular space of His; there is no pericellular space of Obersteiner. The lymphatic channels, as far as we know, follow the adventitia of the capillaries, veins, and arterioles towards the surface of the cord, where they enter into the deep layer of the pia mater, through which they probably communicate with the sub-arachnoid space. The lymphatic path has in the main an outward direction, but there is no doubt that it permits of a current inwards or of an invasion by cellular elements, micro-organisms and toxic substances.

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DESCRIPTION OF PLATES XXIII.-XXVII.

PLATE XXIII.

FIG. 1.—Transverse section of three small vessels; distension of their adventitial sheaths, with compound granular corpuscles. Zenker fixation. Van Gieson's stain. ($\times 400$.)

Three capillaries are seen surrounded by a closely compacted number of compound granular corpuscles. These are enclosed by the outer layer, stained pink, of the connective-tissue wall of the adventitia. No perivascular space is seen anywhere. Within the adventitia connective-tissue cells are seen between the granular corpuscles.

FIG. 2.—Minute capillary transversely divided: adventitial sheath distended by a single ring of compound granular corpuscles. Note pink outline of adventitia immediately adjoining glia tissue, leaving no evidence of perivascular space.

Note the glia cells and compound granular cells in the adjacent tissue. Van Gieson's stain. Zenker fixation. ($\times 500$.)

FIG. 3.—Transverse section of a slightly larger capillary surrounded by ring of compound granular corpuscles enclosed within the adventitia, which is limited by a pink line of connective tissue. On the left of the vessel is seen a shrinkage space formed by the artificial separation of adventitia and glia. This is the space commonly described as the perivascular lymphatic. Van Gieson's stain. Zenker fixation. ($\times 500$.)

PLATE XXIV.

FIG. 4.—Small venule cut longitudinally, showing adventitial sheath distended with numerous nucleated elements. Note the complete absence of these from the space (shrinkage space) lying outside the vessel. Van Gieson's stain. Formalin fixation. ($\times 200$.)

FIG. 5.—Longitudinal section of venule showing the formation of the perivascular shrinkage space. Tags and filaments of glial tissue are seen adhering to the outer layer of the adventitia of the vessel. The outer wall of the shrinkage space is similarly found. Van Gieson's stain. Formalin fixation. ($\times 320$.)

FIG. 6.—Shows early stage of development of a pericellular shrinkage space. Fine fibrillæ of glia are seen passing over the space to the body and processes of the cell. Formalin fixation. ($\times 500$.)

FIG. 7.—Section of pia mater, surface of cord, and entering vessel, showing the close adhesion of the inner layer of the pia to the condensed layer of the glia—membrana limitans superficialis—and the complete absence of any epispinal space. Van Gieson's stain. Zenker fixation. ($\times 320$.)

PLATE XXV.

FIGS. 8-14.—Marchi sections from case of pernicious anæmia.

FIG. 8.—Longitudinal section of lateral column of cord. The compound granular corpuscles, stained black, form interrupted longitudinal lines leading towards, and surrounding, the capillaries. ($\times 40$.)

FIGS. 9 and 10, corresponding respectively to $\times$ and $\times \times$ of Fig. 8, but magnified 250 and 200 times, show the extensive infiltration of the adventitia with the compound granular cells and free granules of fat.

PLATE XXVI.

- FIG. 11.—Margin of cord with pia showing a peripheral vessel carrying compound granular corpuscles in its adventitial wall towards the deep layer of the pia mater, and within which they spread in both directions. Note the narrowing of the stream of corpuscles as the surface of the cord is approached. ($\times 70$.)
- FIG. 12.—Longitudinal section showing how the compound granular corpuscles approach and enter the adventitia of the vessels, and spread in the deep layers of the pia. ($\times 70$.)

PLATE XXVII.

- FIG. 13.—Transverse section of posterior columns of cord showing early degeneration in the outer zone, and more advanced change in the inner zone, where the black staining is restricted to the periphery of the vessel wall. The paler appearance of the inner zone is due to the compound granular corpuscles having been drained from the tissue along the adventitial lymphatics. ($\times 30$.)
- FIG. 14.—Transverse section of capillary showing a ring of compound granular corpuscles in its adventitia, and the approach of the cells in the tissue towards the vessel. ($\times 250$.)

FIG. 1.

FIG. 2.

FIG. 3.

FIG. 4.

FIG. 5.

FIG. 6.

FIG. 7.

Fig. 8

Fig. 9

Fig. 10