J. Anat. (1989), **166**, pp. 43–54
With 13 *figures*
Printed in Great Britain

Fine structure of lymph pathways in nodes from the superficial inguinal lymph centre in the pig

HUGH J. SPALDING AND TREVOR J. HEATH

Department of Anatomy, University of Queensland, St Lucia, Queensland 4067, *Australia*

(*Accepted* 24 *January* 1989)

INTRODUCTION

The lymph nodes of the pig differ significantly from those of most other mammals. Dense nodular tissue, which corresponds to the cortical tissue in other species, is located mainly towards the centre of the node, whilst diffuse tissue, which contains relatively few lymphocytes and is the counterpart of medullary tissue, is located mainly at the periphery (Hoshi *et al.* 1986: Spalding & Heath, 1987).

In a previous paper we described the overall arrangement of lymph pathways through the pig lymph node (Spalding & Heath, 1987). The aim of this study was to describe the fine structure of these pathways. This was done using light microscopy and transmission and scanning electron microscopy.

MATERIALS AND METHODS

Nine Landrace/Large White-cross pigs weighing between 50 and 70 kg were obtained from a minimal disease piggery.

Anaesthesia was induced with either sodium thiopentone (Pentothal, Ceva Chemicals Australia Pty Ltd, Hornsby, N.S.W.) or sodium pentobarbitone (Nembutal, Ceva Chemicals Australia Pty Ltd), and was maintained with halothane (Fluothane, ICI Australia Operations Pty Ltd, Villawood, N.S.W.) and oxygen in a closed circuit. The pigs did not regain consciousness, and were killed with an overdose of anaesthetic.

All of the nodes studied were from the superficial inguinal lymph group of nodes. Nine nodes were fixed by a perfusion through one of their afferent lymphatics. Surgical methods similar to those described by Spalding & Heath (1987) were used to cannulate an afferent lymph vessel. Normal saline (0·5 ml) was perfused into the vessel and this was followed by 0·3, 0·5, or 2 ml of a mixture of 2·5% glutaraldehyde and 4% paraformaldehyde at pH 7·2, over 10–15 minutes. A few drops of Evans' Blue dye were included with the fixative to mark the perfused area in the node.

Two nodes were preserved by an infusion through the blood vessels. Normal saline (600 ml) followed by 600 ml of fixative was perfused through a cannula in the abdominal aorta.

The lymph nodes were removed immediately and cut into 2–3 mm slices and these were placed in fixative for four hours. In addition, eight nodes were fixed only by immersion.

Tissue was prepared for scanning and transmission electron microscopy using methods described by Spalding & Heath (1987).

Tissue studied with the light microscope included 2 μm thick Araldite sections

which were stained with toluidine blue and paraffin sections stained with either haematoxylin and eosin, or orcein (Drury & Wallington, 1967) with light green as a counterstain.

RESULTS

Afferent pathways

The afferent lymphatics penetrated into the lymph node where the capsule was invaginated to form large trabeculae. These trabeculae, containing the afferent lymphatics, ramified through the dense nodular tissue located centrally in the node (Fig. 1). The afferent lymphatics gave rise to numerous branches and these opened into sinuses surrounding the trabeculae. These trabecular sinuses were continuous with a subcapsular sinus which overlay a small amount of dense nodular tissue located peripherally in the node. Occasionally afferent lymphatics entered directly into this subcapsular sinus (see Spalding & Heath, 1987). The peripheral dense nodular tissue was penetrated by trabeculae, although these were much smaller than those which contained the afferent lymphatics.

Pathways through dense nodular tissue

Trabecular sinuses

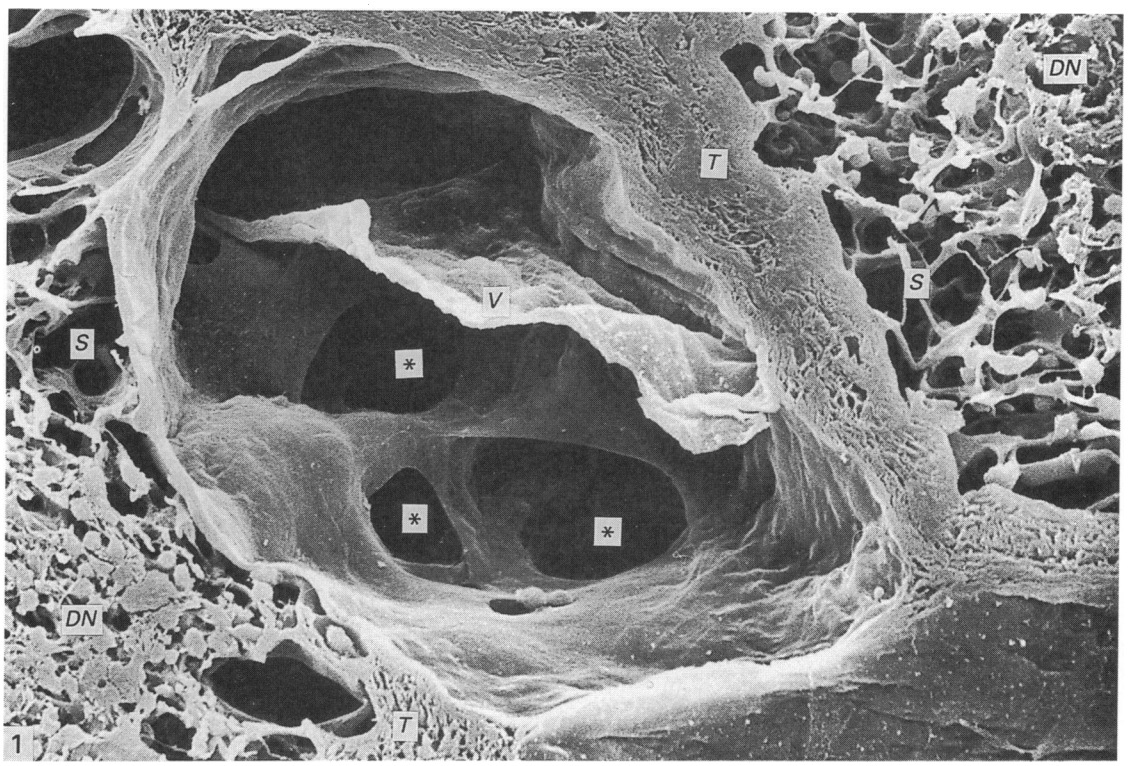
Trabecular sinuses were difficult to detect in many areas in tissue fixed by immersion, or by perfusion through blood vessels. However, when the fixative was perfused through the lymphatics, a sinus was seen around nearly all parts of each trabecula. It was a general finding that the structure of lymph pathways was poorly displayed in tissue not fixed by a perfusion through the lymphatics, and this was particularly evident when the scanning electron microscope was used.

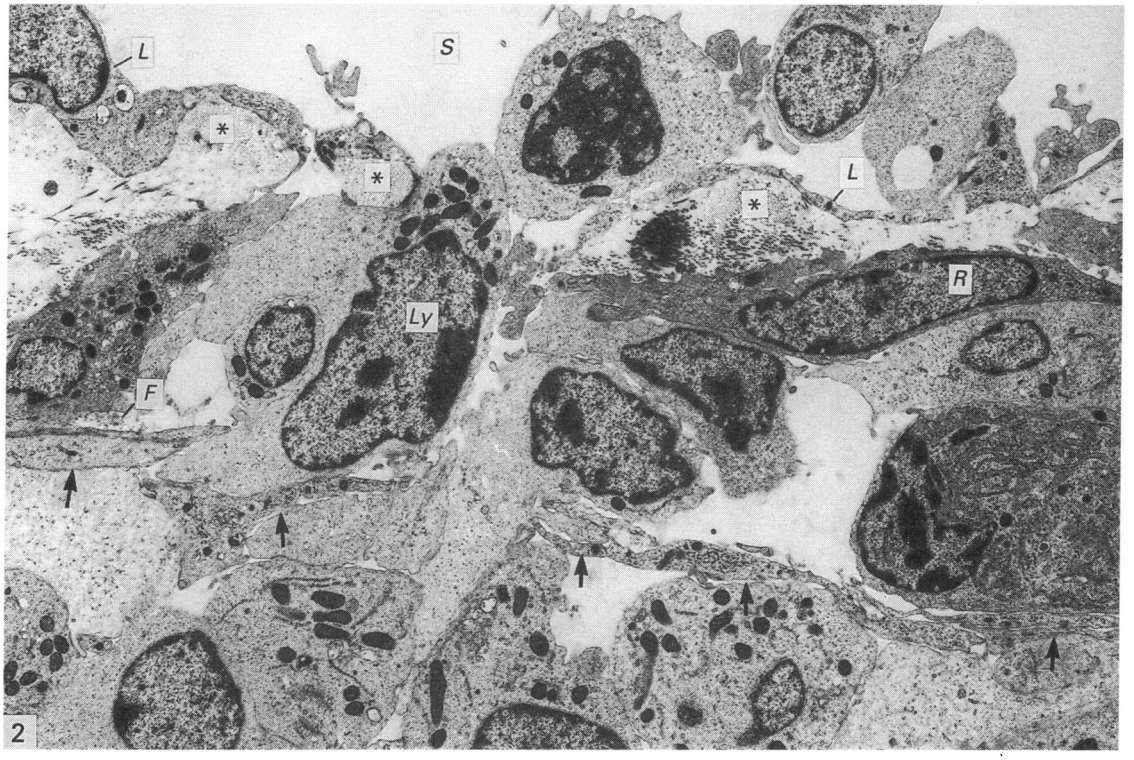
The sinus lining adjacent to each trabecula consisted of a distinct, single layer of sinus lining cells in which no gaps were seen. However, the lining on the parenchymal side of the sinus was more complex. It consisted of lining cells and bundles of collagenous fibres (Fig. 2). A basal lamina adjacent to the lining cells was not seen. The fibre bundles were up to 3 or 4 μm thick and they were often lined by reticular cells on their abluminal side. Many gaps up to 15 μm across were present in the sinus lining and cells were often seen which appeared to have been migrating through these gaps. In some areas a second incomplete layer of reticular cells and fibres was present 5–10 μm from the sinus lining (Fig. 2). The sinus lining cells were usually spindle-like in cross-section and their nuclei contained little heterochromatin. The cytoplasm often contained lysosome- and phagosome-like bodies and it formed a thin layer over the collagenous fibres but this was often less than 0·5 μm thick (Fig. 2). In places there were gaps in this cellular layer and the fibres were exposed to the sinus space.

It was often difficult to trace the lining of each trabecular sinus and in some areas it appeared that a distinct lining was absent. The difficulty in tracing the lining was due

Fig. 1. Scanning electron micrograph of a lymph node which was fixed by a perfusion through an afferent lymphatic. A terminal branch of the afferent lymphatic is contained within a trabeculum (*T*). It communicates with trabecular sinuses (*S*) adjacent to dense nodular tissue (*DN*) through several apertures (*) and contains what is probably a valve cusp (*V*). × 730.

Fig. 2. Transmission electron micrograph of the lining of a trabecular sinus (*S*) in dense nodular tissue. It consists of lining cells (*L*) and collagen fibres (*), which are covered on their abluminal side by a reticular cell (*R*). A gap is present in the lining and a lymphocyte (*Ly*) appears to have been migrating through this. An incomplete layer of reticular cell processes (arrows) and collagen fibres (*F*) is present about 10 μm from the sinus lumen. × 4800.


DN
T
S
V
S
*
*
*
DN
T
1


L
S
*
*
L
*
R
Ly
F
2

in part to the presence of many gaps, and also because the outline of the sinus was often very irregular. Many of the irregularities occurred where reticular processes projected from the lining and traversed the sinus lumen. These processes consisted of a core of collagen fibres and an outer layer of cells which were similar to, and continuous with, those of the sinus lining. The reticular fibres were usually fully enveloped by the processes of the reticular cells, but gaps were occasionally seen in this lining.

Pathways through diffuse tissue

Trabecular sinuses

Most of the trabeculae which ramified through the central dense nodular tissue terminated near the margin between this tissue and the diffuse tissue. Some extended part way into the diffuse tissue but only rarely did they traverse it and join the capsule. The trabeculae usually ended as short branches that gave rise to numerous slender processes which were less than 3 μm in diameter, but often projected more than 250 μm into the diffuse tissue. They apparently contained elastic fibres as they were stained red-brown with orcein. However, they were not prominent in sections stained with haematoxylin and eosin. Where each trabecula terminated, the lining of the trabecular sinus appeared to be absent and the trabecular sinus merged with the interstices of the diffuse tissue (Fig. 3).

In addition to the trabeculae emanating from the central dense nodular tissue, the diffuse tissue also contained a few small trabeculae which projected from the adjacent capsule. These were surrounded by sinuses which were continuous with the subcapsular sinus. Trabeculae which appeared to be remnants of capsular tissue were seen along the apparent plane of fusion of node *anlagen*.

Structure of the parenchyma

Most of the diffuse tissue showed little evidence of structural organisation and did not contain lymph sinuses with a distinct lining. However, many interstitial spaces of 5–10 μm were seen (Figs. 3, 4), and these were most obvious in tissue which was fixed by a perfusion through the afferent lymph vessel. Cells in the diffuse tissue were supported by a loose framework of reticular fibres. Some fibres were in bundles which were enveloped by cell processes but many were lying free in the interstices (Fig. 4).

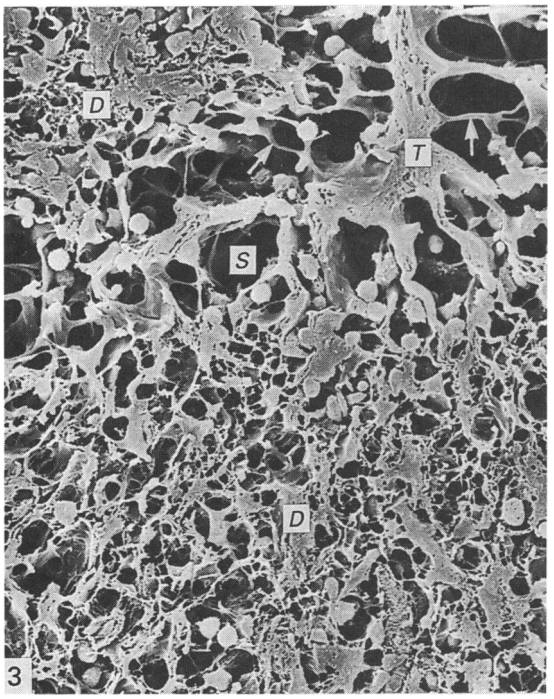
The cells in the diffuse tissue included occasional lymphocytes, plasma cells and mast cells, and numerous reticular cells. In addition there were many cells with numerous slender cytoplasmic processes and, often, many clear vacuoles, which gave them a frilled or frothy appearance (Fig. 4). These cells were variable in shape but

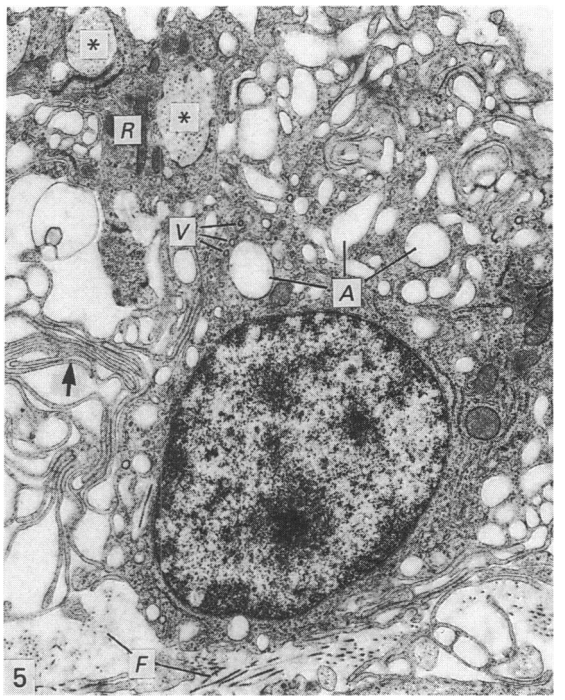
---

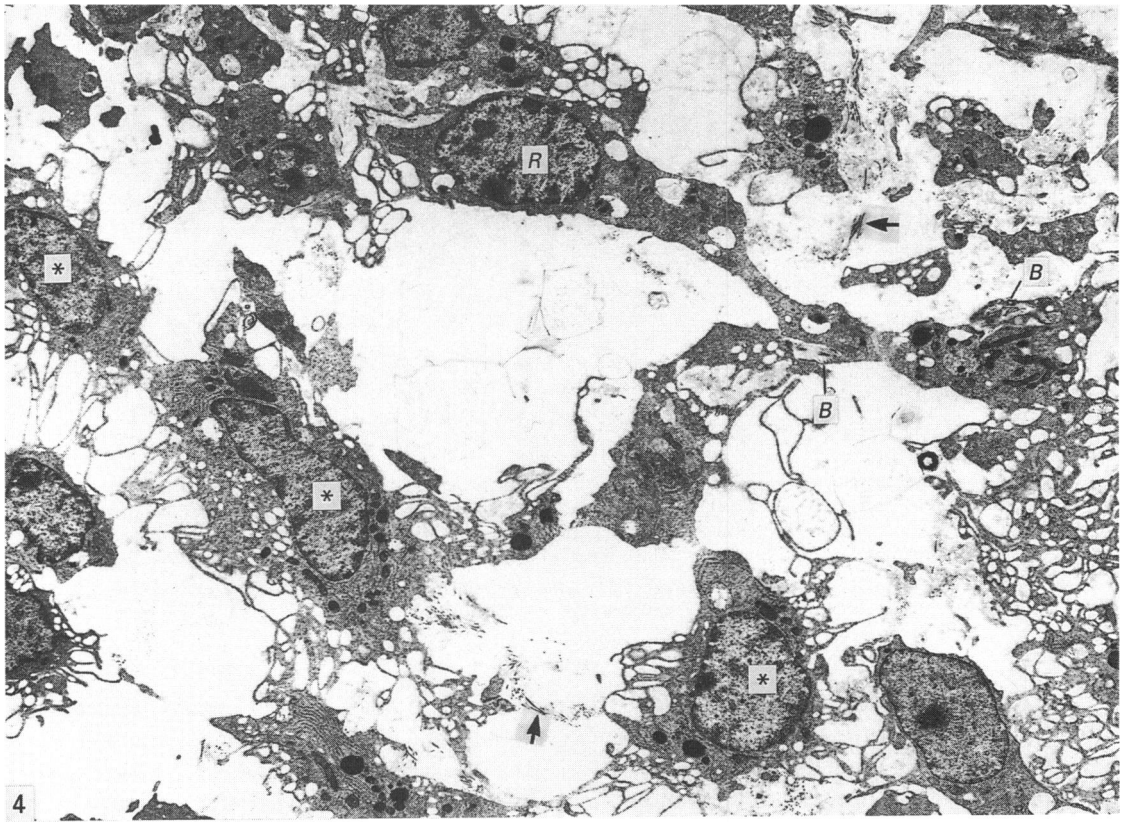
Fig. 3. Scanning electron micrograph of a trabecula (*T*) which terminates in diffuse tissue (*D*) in a node which was fixed by a perfusion through an afferent lymphatic. Numerous reticular processes (arrows) traverse the lumen of the trabecular sinus but the sinus does not have a distinct lining and its lumen merges with the interstices of the diffuse tissue. × 650.

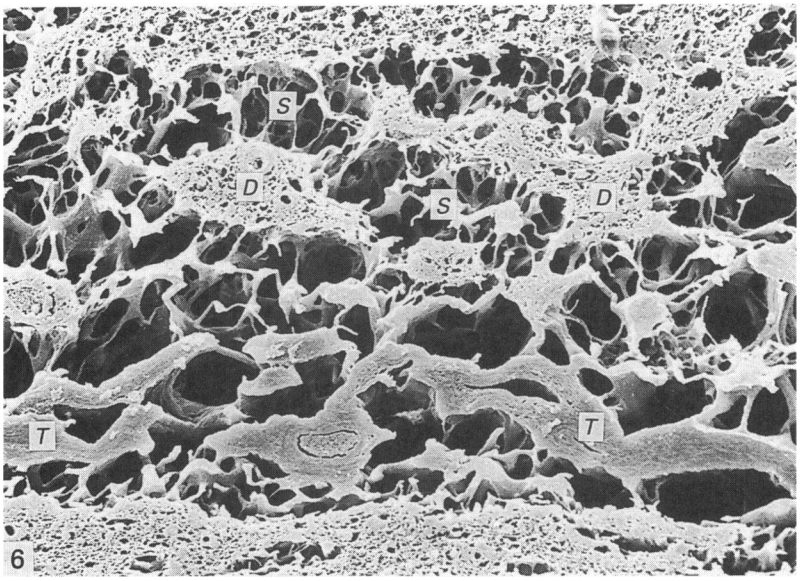
Fig. 4. Transmission electron micrograph of diffuse tissue which was fixed by immersion. There is a substantial amount of space between cells which include frilled macrophages (*) and a reticular cell (*R*). Collagen fibres are present, both free within the interstices (arrows) and in bundles (*B*) enveloped by reticular cell processes. × 3300.

Fig. 5. Transmission electron micrograph of a frilled cell in diffuse tissue. This cell lined the wall of a sinus similar to those shown in Figures 6–8 and it is in close association with collagen fibres (*F*) of the wall. It contains numerous clear vacuoles (*A*) and small vesicles (*V*) and has elaborate cytoplasmic processes. Some of the processes (arrow) were from a neighbouring cell not in view. Collagen fibres (*) in the sinus lumen are enveloped by part of a reticular cell (*R*). × 8000.


D
T
S
D
3


*
R
*
V
A
F
5


R
*
B
B
*
*
4



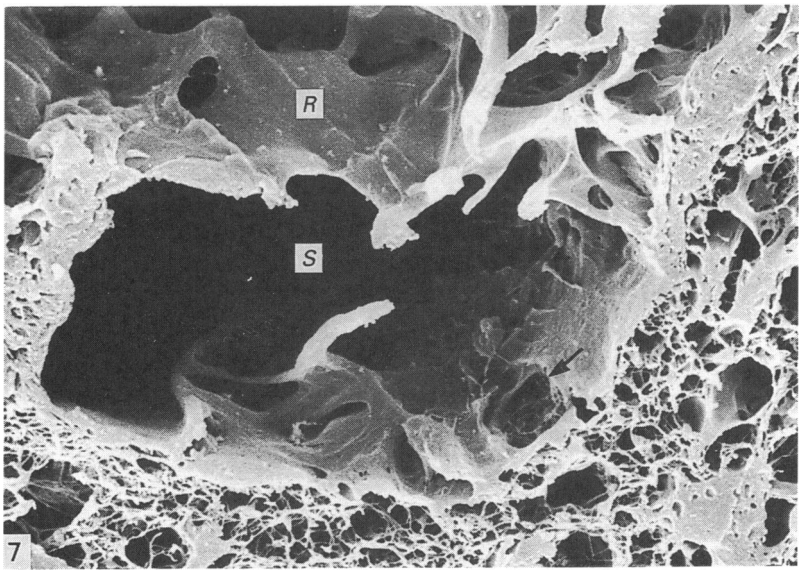


Fig. 6. Scanning electron micrograph of diffuse tissue which was fixed by a perfusion through afferent lymphatics. This tissue was taken from a region which appeared to be along the plane of fusion of adjacent node *anlagen*. The diffuse tissue (*D*) is arranged into cords which are surrounded by sinuses (*S*) in a manner very similar to medullary tissue in other species. *T*, trabeculae. × 300.

Fig. 7. Detail of a sinus (*S*) similar to those in Figure 6; it includes a reticular cell (*R*) which traverses the lumen and a hole (arrow) in the sinus lining. × 2100.

tended to be rounded or ovoid, and they were up to 15 μm across. They also varied considerably in the number of apparent lysosomes and phagosomes they contained. Some cells contained a dozen or more, and these cells appeared to be active macrophages. Others contained only a few lysosomes, but these cells often had more elaborate processes (Fig. 5).

Parts of the diffuse tissue contained sinuses which were not associated with

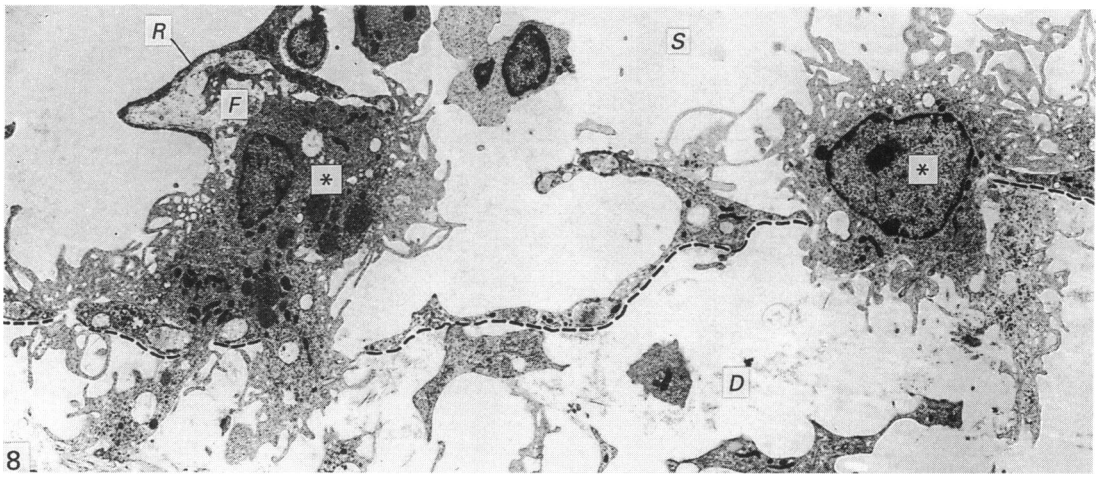


Fig. 8. Transmission electron micrograph of the lining of a sinus (*S*) in diffuse tissue (*D*). Numerous gaps are present in the cell lining of the sinus (which is indicated by the dotted line) and these gaps are bridged by two frilled macrophages (*). With greater magnification the macrophage on the left was seen to be in contact with collagen fibres (*F*) of a reticular process in the lumen of the sinus. These fibres were only partly enveloped by a reticular cell (*R*). × 3000.

trabeculae or the capsule, but were bordered completely by parenchyma (Figs. 6, 7). These usually occurred near the periphery of the node, or near the apparent plane of fusion of node *anlagen*. The tissue here often resembled the typical arrangement of parenchymal cords and sinuses which occurs in the medulla of lymph nodes in other species.

Structure of sinuses associated with diffuse tissue

The various sinuses of the diffuse tissue – including sinuses bordered partly by trabeculae and the capsule, and those bordered only by parenchyma – had many similarities in their structure. Sinus walls adjacent to the parenchyma consisted of reticular lining cells and a few collagen fibres but contained many gaps up to 10 μm across. The sinus lumen was continuous with the interstices of the diffuse tissue through some of these gaps (Fig. 7) but others were bridged by frilled cells (Fig. 8). Frilled cells also seemed to adhere to the reticular lining cells and some were in direct contact with collagenous fibres of either the sinus wall or reticular processes which traversed the lumen (Fig. 8). Dense areas were seen along the cytoplasmic membranes of frilled cells in areas adjacent to both reticular lining cells and collagenous fibres.

The trabeculae and capsule adjacent to sinuses were lined by a single continuous layer of lining cells.

Efferent pathways

Subcapsular sinus

A subcapsular sinus occurred over virtually all of the diffuse tissue. It was traversed by numerous reticular processes and its lining was generally similar to that of other sinuses adjacent to diffuse tissue. However, in some places a layer of connective tissue with a course parallel to the capsule formed the inner wall of the subcapsular sinus and gave the impression of a second, but incomplete, capsule (Fig. 9).

The subcapsular sinus was interrupted by a barrier of connective tissue, near areas of peripherally located dense nodular tissue, which was up to about 200 μm wide (Figs.

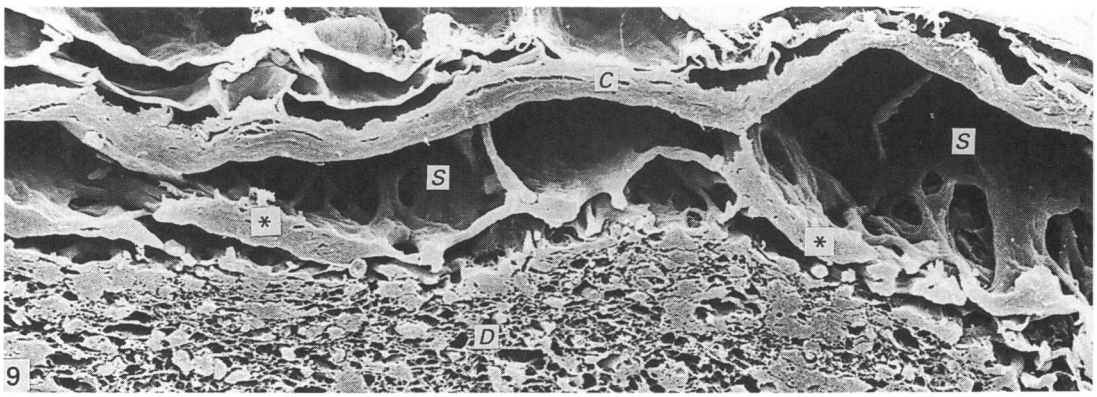


Fig. 9. Scanning electron micrograph of a subcapsular sinus (*S*) adjacent to diffuse tissue (*D*). An incomplete layer of connective tissue (*) forms part of the inner wall of the sinus and gives the impression there of a second capsule (*C*). × 560.

10, 11). This barrier was continuous with the capsule, but unlike a trabecula it did not penetrate through the parenchyma. Instead, it merged directly with the adjacent diffuse tissue (Fig. 10).

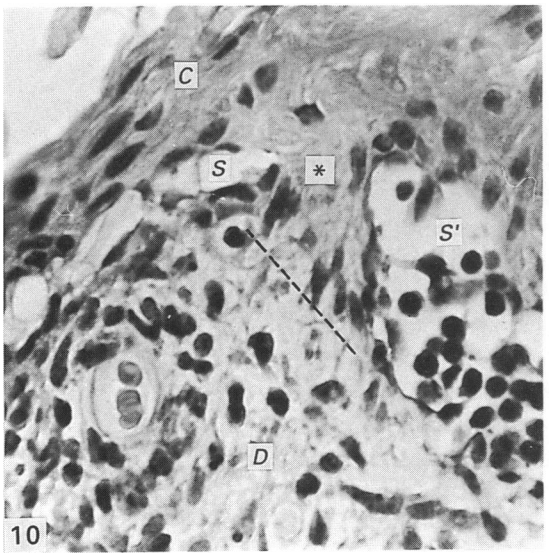
Efferent lymph vessels

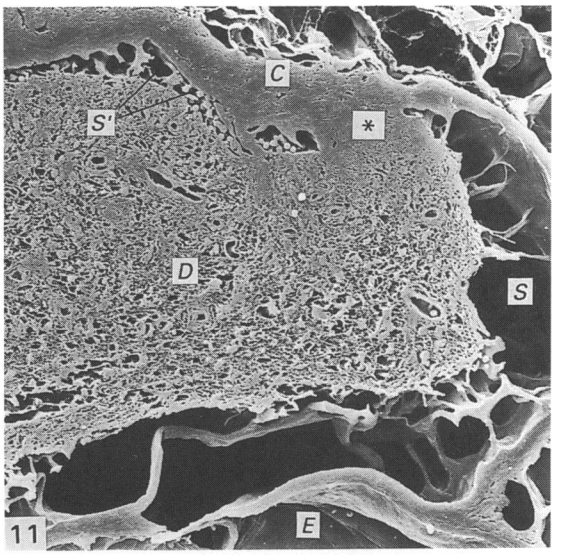
Large numbers of lymph vessels coursed over the surface of the node in areas where diffuse tissue was in a subcapsular position. Most of the initial efferent vessels – or collecting ducts (Spalding & Heath, 1987) – did not contain valves and these generally lay within the substance of the capsule. They communicated with the subcapsular sinus through holes 80 μm or more across in their walls (Figs. 12, 13). Others occurred within the diffuse tissue and they were often present in trabeculae which appeared to be remnants of capsule along the plane of fusion of node *anlagen*. The walls of the collecting ducts consisted of an endothelial lining and dense connective tissue similar to that in the capsule or trabeculae. Occasionally efferent lymphatics containing valves joined, more or less directly, with sinuses in the diffuse tissue near the capsule.

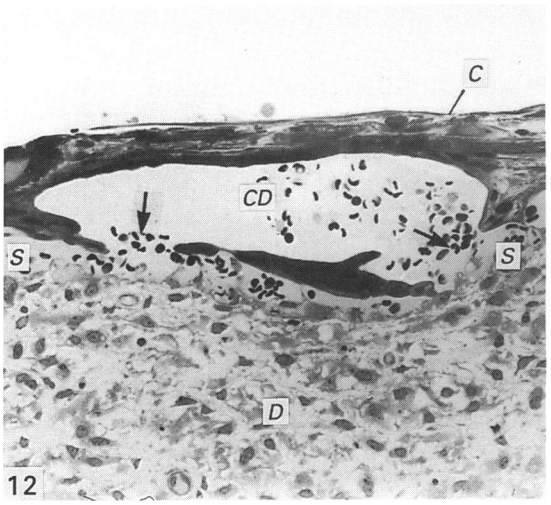
DISCUSSION

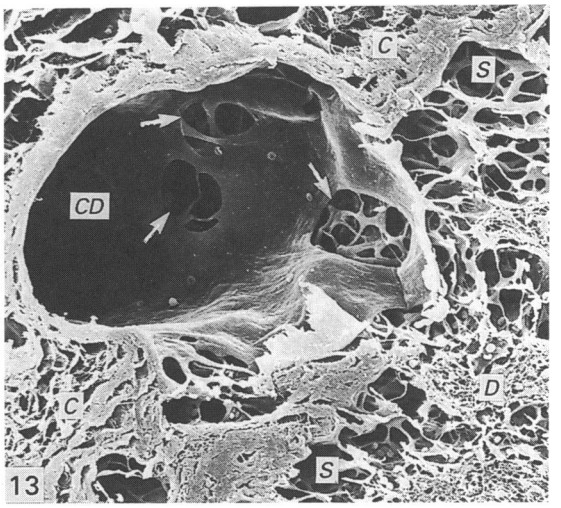
In the pig, most of the terminal afferent lymphatics penetrate the node within trabeculae and deliver lymph directly to trabecular sinuses in the dense nodular tissue. However, occasionally branches may arise from the afferent lymphatics outside the lymph node and join with a subcapsular sinus where dense nodular tissue is located peripherally (Spalding & Heath, 1987; Hoshi *et al.* 1988*a*). The trabecular and subcapsular sinuses appear basically similar in structure to their counterparts in other species (Clark, 1962; Moe, 1963; Luk, Nopajaroonsri & Simon, 1973; Fossum, 1980; Heath & Spalding, 1987). However, counterparts to the tubular sinuses in the cortex of sheep (Heath & Spalding, 1987) and similar sinuses in rodents (Soderström & Stenström, 1969; He, 1985) and rabbits (Kurokawa & Ogat, 1980; Kelly, 1975) were not found.

The trabecular and subcapsular sinuses are traversed by numerous reticular processes which are continuous with the sinus walls. Many gaps are present in the sinus lining over the dense nodular tissue and cells apparently migrate through these. Gaps are also present in the sinuses of lymph nodes in man (Forket, Thliveris &









Figs. 10–11. Light micrograph (Fig. 10) and scanning electron micrograph (Fig. 11) showing connective tissue (*) which projects from the capsule (*C*) into the node. This projection is unlike a trabecula in that it does not penetrate far into the parenchyma (not beyond the dotted line in Fig. 10) and is not accompanied by a sinus. It occurs near the margin of diffuse tissue (*D*) and peripherally located dense nodular tissue and it divides the subcapsular sinus into one part (*S*) which is continuous with efferent lymph vessels (*E* in Fig. 11) and another part (*S'*) which extends over dense nodular tissue (which is not in view). Fig. 10 ×700; Fig. 11 ×200.

Figs. 12–13. Light micrograph (Fig. 12) and scanning electron micrograph (Fig. 13) of collecting ducts (*CD*). These ducts lie partly within the capsule (*C*) and they communicate with the subcapsular sinus (*S*) through apertures (arrows) in their walls. *D*, Diffuse tissue. Fig. 12 ×270; Fig. 13 ×240.

Bertalanffy, 1980), rodents (Clark, 1962; Moe, 1963; Luk *et al.* 1973; Fossum, 1980) and sheep (Heath, Kerlin & Spalding, 1986; Heath & Spalding, 1987) particularly in the subcapsular sinus over cortical tissue. However, in the pig there were places where a distinct sinus lining appeared to be absent. Discontinuities in the sinus lining would allow lymph to percolate to some extent through the dense nodular tissue and the studies of the passage of carbon particles through the node support this (Spalding & Heath, 1987).

The large trabeculae in the centrally located dense nodular tissue send branches into the diffuse tissue where they terminate and give rise to long slender fibres. The trabecular sinuses are continuous with the many interstitial spaces up to 10 μm across in the diffuse tissue. It appears that most, if not all, of the lymph passing through the node must percolate through these interstices. However, an alternative route may occasionally exist along sinuses associated with trabeculae which project from the dense nodular tissue, through the diffuse tissue, to join with the capsule.

A feature of the diffuse tissue is the large number of cells with elaborate cytoplasmic processes which give the tissue a somewhat frothy appearance in light and transmission electron microscopy. It is not possible to classify these cells rigidly, but many contain large numbers of dense bodies thought to be lysosomes and phagosomes and can be identified as macrophages. Others have only a few dense bodies and resemble the dendritic cells described by Hoshi *et al.* (1988*b*). However, it is not clear if these also represent macrophages in a different state of activity.

Large numbers of reticular fibres in the diffuse tissue are not ensheathed by cell processes and are apparently directly exposed to lymph passing through the region. This also appears to occur in the sinuses of the dense nodular tissue where there are gaps in the cellular lining of reticular processes and the sinus wall.

Reticular fibres traversing the sinus lumen are apparently involved in antigen trapping in lymph nodes in rats (Sainte-Marie & Peng, 1986). However, in this species the fibres only rarely, if ever, appear to be exposed to the sinus space (Fossum, 1980; Han, 1961) and the exact mechanisms by which antigen would make contact with the fibres was not described (Sainte-Marie & Peng, 1986). Antigen trapping in the pig lymph node has not been examined but the many reticular fibres, that appear to be exposed to the lymph, may play an important role in this process.

Towards the capsule and near the plane of fusion of adjacent node *anlagen*, the diffuse tissue is often penetrated by lymph sinuses. These have a distinct cell lining which includes gaps that would allow lymph to pass into them from the interstices of the parenchyma.

Hoshi *et al.* (1988*b*) concluded, however, that no lymph sinuses or spaces are present in the diffuse tissue. It seems likely that their failure to identify these features may be related to the method used for tissue fixation, although this method was not described. In our experiments, immersion and arterial perfusion were much less effective for displaying the sinuses than perfusion of fixative through an afferent lymphatic.

Hoshi *et al.* (1988*b*) did identify numerous structures similar to sinuses in the 'medulla-like' (diffuse) tissue, but concluded that these were minute efferent lymph vessels. We also found efferent lymph vessels (collecting ducts) within the diffuse tissue, but these were readily distinguished from sinuses on the basis of the structure of their walls. Collecting ducts and efferent lymph vessels are lined by endothelial cells and their walls are formed by dense connective tissue whereas the walls of sinuses in the diffuse tissue consist of lining cells and frilled macrophages with relatively few collagenous fibres. The sinus lumen is traversed by numerous reticular processes.

The collecting ducts are very similar in appearance to some of the initial efferent lymph vessels found in horse lymph nodes (Heath & Perkins, 1989).

The subcapsular sinus adjacent to peripherally located dense nodular tissue occasionally receives lymph directly from the afferent lymphatics. It is also continuous with the trabecular sinuses of the central dense nodular tissue and receives lymph from these (Spalding & Heath, 1987). However, the subcapsular sinus is interrupted by a connective tissue barrier situated near the margin of peripheral dense nodular tissue

and the diffuse tissue. This barrier was always present in the nodes we studied but the degree to which it was continuous in each node was not determined. It is likely that this barrier is the cause of distinct interruptions in the casts of the subcapsular sinus made with Mercox by Hoshi *et al.* (1988*a*). Furthermore, it may well play a role in preventing afferent lymph from flowing round the subcapsular sinus to efferent lymph vessels. A similar barrier is present at the hilar margin of sheep lymph nodes (Heath & Spalding, 1987).

SUMMARY

In the pig lymph node most lymph passes from afferent lymphatics to trabecular sinuses in centrally located dense nodular tissue. The lining of these sinuses is continuous adjacent to the trabecula but it is interrupted by numerous gaps adjacent to the parenchyma. Where the trabeculae end, their associated sinuses are continuous with the many interstitial spaces, up to 10 μm across, in the diffuse tissue. Lymph percolates through these spaces and is directly exposed to large numbers of macrophages with elaborate cytoplasmic veils and to reticular fibres which could be involved in antigen retention. Parts of the diffuse tissue are arranged into sinuses and cords in a manner similar to the medullary tissue in other species and a subcapsular sinus is also present over the diffuse tissue. There are gaps in the lining of these sinuses through which they communicate with the interstices of the parenchyma. Lymph flows from the sinuses in the diffuse tissue into efferent lymph vessels; these are usually in the capsule or along the plane of fusion of adjacent node *anlagen*.

We thank the Pig Research Council of Australia for financial support, and the veterinary students Diana McPhee and Stephen Nikles for help with experiments.

REFERENCES

CLARK, S. L. (1962). The reticulum of lymph nodes in mice studied with the electron microscope. *American Journal of Anatomy* **110**, 217–258.

DRURY, R. A. B. & WALLINGTON, E. A. (1967). *Carleton's Histological Technique*, 4th ed. London: Oxford University Press.

FORKET, P., THLIVERIS, J. A. & BERTALANFFY, F. D. (1977). Structure of sinuses in the human lymph node. *Cell and Tissue Research* **183**, 115–130.

FOSSUM, S. (1980). The architecture of rat lymph nodes. II. Lymph node compartments. *Scandinavian Journal of Immunology* **12**, 411–420.

HAN, S. S. (1961). The ultrastructure of the mesenteric lymph node of the rat. *American Journal of Anatomy* **109**, 183–225.

HE, Y. (1985). Scanning electron microscope studies of the rat mesenteric lymph node with special reference to high-endothelial venules and hitherto unknown lymphatic labyrinth. *Archivum histologicum japonicum* **48**, 1–15.

HEATH, T. J., KERLIN, R. L. & SPALDING, H. J. (1986). Afferent pathways of lymph flow within the popliteal node in sheep. *Journal of Anatomy* **149**, 65–75.

HEATH, T. J. & PERKINS, N. R. (1989). Pathways between lymph vessels and sinuses in lymph nodes: a study in horses. *Anatomical Record* (In Press).

HEATH, T. J. & SPALDING, H. J. (1987). Pathways of lymph flow to and from the medulla of lymph nodes in sheep. *Journal of Anatomy* **155**, 177–188.

HOSHI, N., HASHIMOTO, Y., KITAGAWA, H., KON, Y. & KUDO, N. (1986). Histological and immunohistochemical studies on the architecture of lymph nodes in pig. *Japanese Journal of Veterinary Science* **48**, 1097–1107.

HOSHI, N., HASHIMOTO, Y., KITIGAWA, H., KON, Y. & KUDO, N. (1988*a*). A scanning electron microscopic study on the architecture of lymph vessels and intranodal lymph pathways of lymph nodes in pigs. *Japanese Journal of Veterinary Research* **36**, 1–14.

HOSHI, N., HASHIMOTO, Y., KITIGAWA, H., KON, Y. & KUDO, N. (1988*b*). Fine structures of medulla-like tissues and the lymph sinuses in the lymph nodes of pig. *Japanese Journal of Veterinary Science* **50**, 83–92.

KELLY, R. H. (1975). Functional anatomy of lymph nodes. I. The paracortical cords. *International Archives of Allergy and Applied Immunology* **48**, 836–849.

KUROKAWA, T. & OGATA, T. (1980). A scanning electron microscopic study on the lymphatic microcirculation of the rabbit mesenteric lymph node. A corrosion cast study. *Acta anatomica* **107**, 439–466.

LUK, S. C., NOPAJAROONSRI, C. & SIMON, G. T. (1973). The architecture of the normal lymph node and haemolymph node. A scanning and transmission electron microscopic study. *Laboratory Investigation* **29**, 258–265.

MOE, R. E. (1963). Fine structure of the reticulum and sinuses of lymph nodes. *American Journal of Anatomy* **112**, 311–335.

SAINTE-MARIE, G. & PENG, F.-S. (1986). Diffusion of a lymph-carried antigen in the fibre network of the lymph node of the rat. *Cell and Tissue Research* **245**, 481–486.

SODERSTRÖM, N. & STENSTRÖM, A. (1969). Outflow paths of cells from the lymph node parenchyma to the efferent lymphatics. *Scandinavian Journal of Haematology* **6**, 186–196.

SPALDING, H. J. & HEATH, T. J. (1987). Pathways of lymph flow through superficial inguinal lymph nodes in the pig. *Anatomical Record* **217**, 188–195.