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Lymph drainage from the mammary gland in sheep

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INTRODUCTION

The ovine mammary gland provides a valuable model for the study of mastitis, and the lymph nodes which receive lymph from this gland play an important role in immunity to mastitis (Outteridge, Rock & Lascelles, 1965; Lee & Lascelles, 1970; Colditz & Watson, 1982; Watson, 1980, 1984).

The routes by which lymph drains from the mammary parenchyma have been described (Lee & Lascelles, 1969), but little precise information is available on the pathways involving the secondary and tertiary lymph nodes (Schummer, Wilkens, Vollmerhaus & Habermehl, 1981). These nodes are likely to respond to cells and soluble factors carried in lymph from the mammary nodes, and to help in the development and propagation of the immune response (Fahy *et al.* 1980; Fahy, 1980).

The path taken by lymph within lymph nodes has been studied extensively in rats. Sainte-Marie, Peng & Belisle (1982) concluded that these nodes are arranged in physiological compartments, each of which is based on a single afferent lymphatic vessel. The afferent lymphatic vessels of sheep lymph nodes are much more complex (Heath & Brandon, 1983) and preliminary evidence indicates that the distribution of lymph within these nodes is considerably less precise than in rats or in rabbits (Kett, Illényi, Lukács & Nyárády, 1978; Sainte-Marie *et al.* 1982; Heath, Brandon & Norman, 1984).

The aim of this study is to describe the pathways taken by lymph from the mammary gland to the lumbar lymphatic trunk in ewes, and in particular to (*a*) describe the lymphatic vessels and lymph nodes that are involved, and (*b*) determine whether soluble and particulate components of mammary lymph are distributed to all, or only part of, these lymph nodes.

MATERIALS AND METHODS

Thirty lactating merino ewes were used. They were kept indoors in pens with free access to food and water. The lambs remained with the ewes, and appeared to be suckling normally.

Anaesthesia was induced with pentobarbitone (Nembutal; Ceva Chemicals Australia Pty Ltd, Hornsby, N.S.W.) and maintained with halothane (Fluothane; I.C.I. Australia Limited, Villawood, N.S.W.) while vinyl cannulae (Dural Plastics and Engineering Pty Ltd, Dural, N.S.W.) were placed in blood or lymph vessels associated with lymph nodes of the hindquarters. The anaesthetised ewes were killed by exsanguination, or with pentobarbitone. The hindquarters were removed and

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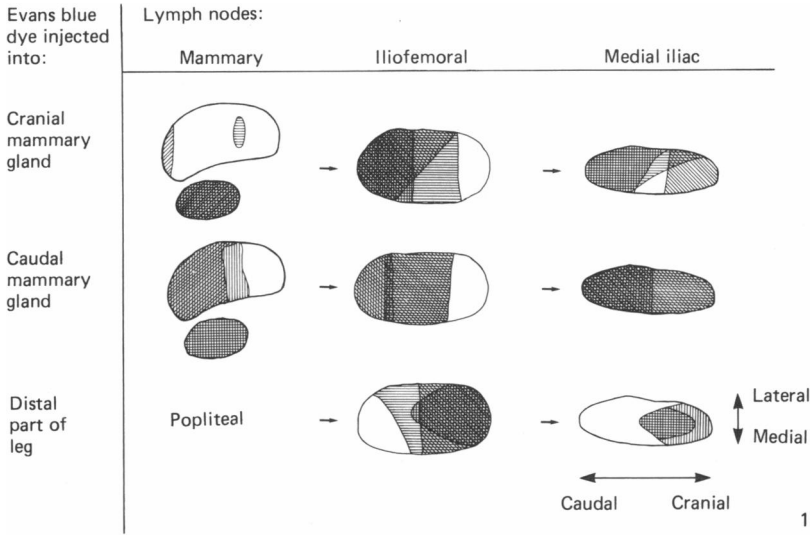


Fig. 1. Diagrams of the mammary, iliofemoral and medial iliac lymph nodes, showing the distribution of Evans blue 30 minutes after 2 ml of a 1 % solution was injected into the mammary gland either cranial or caudal to the teat. The bottom panel shows, for comparison, the distribution of dye after it has been injected into the distal part of the leg; this would have passed through the popliteal lymph node. For each series, vertical, horizontal and diagonal hatching each represent the distribution in one ewe. The orientation of each node is shown at bottom right.

positioned with the ventral surface uppermost, so that the lymphatics and lymph nodes could be identified and their locations recorded.

Pathways taken by efferent lymphatics

Microfil Rubber Injection Compound (Canton Bio-medical Products Inc, Boulder, Colorado) was injected through a cannula into lymphatics from the mammary lymph nodes on 12 sides in nine ewes. In some cases, Microfil of a different colour was also injected into lymphatics from the popliteal and subiliac lymph nodes. Microfil was injected at 0·05–0·1 ml/minute, until it had filled the lymphatics and nodes to the desired level. In some ewes, the arteries to the lymph nodes were filled with latex (Rubber Latex Australia, Kilsyth, Victoria), which was allowed to set in the cold-room. The casts were dissected out and cleared in methyl salicylate (Heath & Brandon, 1983).

Distribution of lymph constituents within lymph nodes

The protein marker Evans blue (2 ml of a 1 % solution; Hopkin & Williams, Chadwell Heath, Essex, England) or a suspension of carbon (China ink; 2 ml; Rotring, Rotring-Werke) was injected into the mammary parenchyma either 10–20 mm cranial, or 20–40 mm caudal, to the teat. Other ewes received subcutaneous injections of China ink or Evans blue dorsolateral to the hoof.

The ewes were killed 30 minutes after the injections of Evans blue and the lymph nodes were removed immediately to prevent diffusion of the dye into adjacent parts of the node (Heath *et al.* 1984). China ink was given either 30 minutes or 24 hours before the ewes were killed.

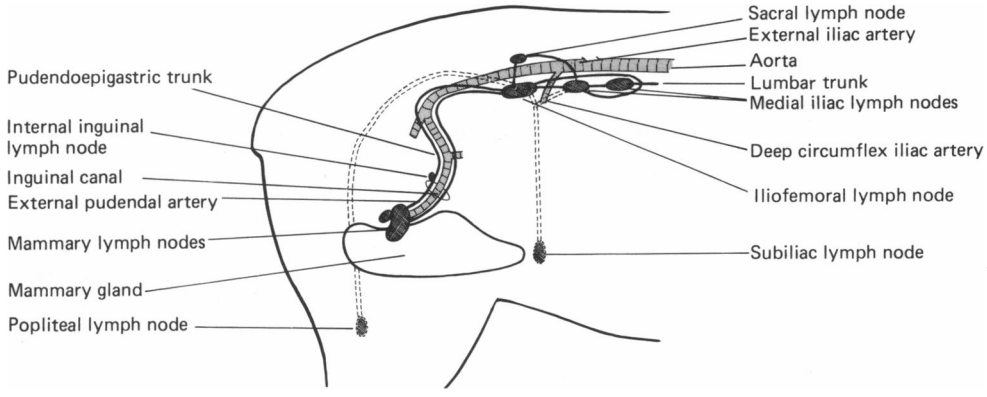


Fig. 2. Diagram of the hindquarters of a sheep, showing the pathways of lymph drainage from the mammary gland. Efferent lymph from the mammary lymph nodes flows through the inguinal canal, the iliofemoral lymph node and the medial iliac nodes to the lumbar trunk. Some also passes through the internal iliac node, and some through a sacral node. The iliofemoral node also receives lymph from the subiliac and popliteal lymph nodes.

The nodes were labelled to maintain their orientation, then removed. Each mammary gland was then cut into slices 20 mm thick, and the distribution of markers within the glands was noted. Most lymph nodes were packed individually in containers on dry ice until dissected, 1–2 hours after death. However, some carbon-containing lymph nodes were frozen *in situ* and were dissected immediately they had thawed. The dissections were done under × 3 magnification, and the nodes were cut transversely at 2 mm intervals. The distribution of marker was recorded diagrammatically (Fig. 1).

The microscopic distribution of carbon was studied in 10 μm sections stained with haematoxylin and eosin, from lymph nodes which had been fixed in Bouins' fluid.

RESULTS

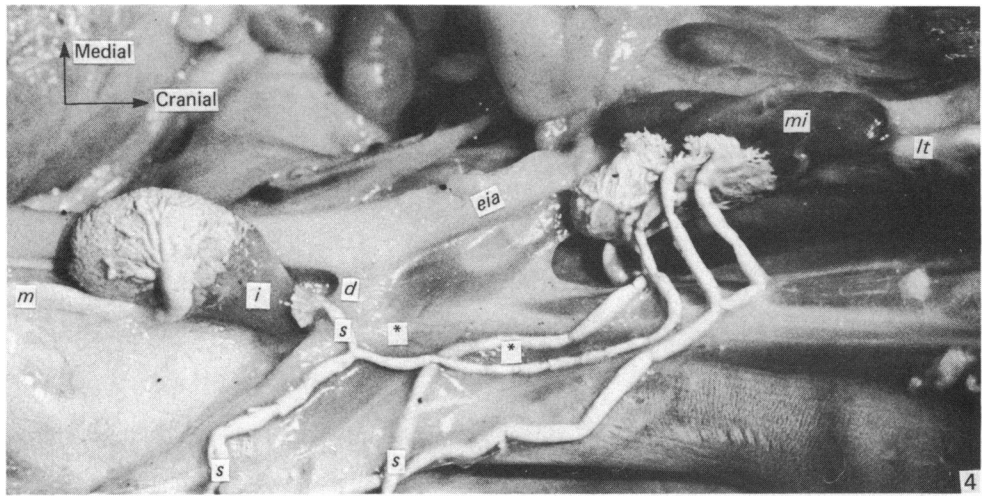
Pathways taken by efferent lymphatic vessels

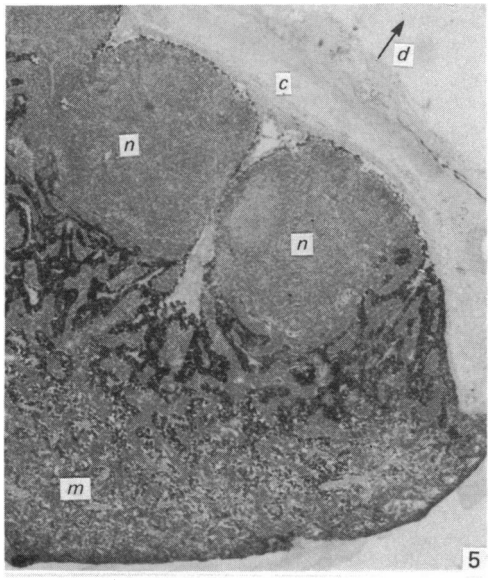
Studies with Microfil revealed that mammary lymph normally passed through the mammary, iliofemoral and medial iliac lymph nodes, and that it might also traverse up to three other nodes, before passing through the lumbar trunk and thoracic duct to the bloodstream (Fig. 2).

The mammary nodes were dorsocaudal to the mammary gland, and immediately adjacent to the external pudendal vessels (Figs. 2, 3). In some cases the artery passed through one of the nodes (Heath, Brandon & Fogarty, 1985). There were 1–4 nodes but most commonly there were two: one node was 5–10 mm long located medially to a larger node which was 10–40 mm long (Figs. 1–3).

Two or more efferent lymphatic vessels passed through the inguinal canal with the external pudendal vessels. In eight out of twelve cases, one of these vessels entered an internal inguinal node, 2–4 mm in diameter, at the deep inguinal ring (Fig. 2).

In each case the other mammary lymphatic(s), with an efferent lymphatic from the internal inguinal node, passed craniodorsally across the pelvic wall to the iliofemoral node. This node was on the lateral border of the external iliac artery immediately caudal to the deep circumflex iliac artery (Figs. 2, 4). In two out of





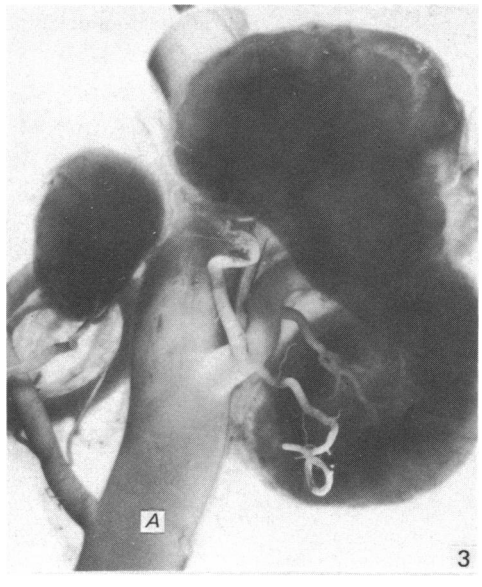


Fig. 3. Photograph of two mammary lymph nodes, showing their relationship to the external pudendal artery (*A*) and its branches; these are filled with latex. × 3.

Fig. 4. Photograph of the dorsolateral wall of the pelvis after Microfil had been injected into efferent lymphatic vessels from the mammary and subiliac lymph nodes. The mammary (*m*) and one subiliac (*s*) lymphatic terminate on the iliofemoral lymph node (*i*), at the lateral border of the external iliac artery (*eia*) adjacent to the deep circumflex iliac artery (*d*). Other Microfil-filled efferents from the subiliac lymph node terminate on the medial iliac lymph node (*mi*), and some Microfil has reached the lumbar trunk (*lt*). One lymph-filled lymphatic can be seen passing from the iliofemoral to the medial iliac lymph node (asterisks).

Fig. 5. Photomicrograph of an iliofemoral lymph node showing the distribution of carbon in paracortical and outer medullary regions, 24 hours after 2 ml China ink was injected into the mammary gland. *c*, capsule; *d*, dorsal; *n*, lymph nodules; *m*, medulla. × 35.

twelve cases a second, smaller, node was found adjacent to the lateral border of the main iliofemoral node.

The iliofemoral node was 20–40 mm long and flattened dorsoventrally. The dorsal layer of cortical tissue was penetrated by terminal afferent lymphatic vessels, trabeculae and trabecular sinuses, and a loosely branched system of cortical sinuses (Figs. 4–8). Studies in which Microfil was injected into lymphatic vessels from mammary lymph nodes (12/12 cases) and from the popliteal (4/4 cases) and subiliac (3/3 cases) nodes, demonstrated that lymph from each of these nodes flowed to the iliofemoral node (Figs. 2, 4). In one case, efferent lymphatics from the subiliac node also terminated on a medial iliac node (Fig. 4). Although the vessels from the different nodes generally remained separate, studies with Microfil revealed that anastomotic connections might occur (Fig. 8).

Each vessel divided at or near the surface of the node and 10–40 or more terminal afferent vessels entered the node in a fairly well defined area on its dorsal surface (Figs. 4, 7). Many terminal afferents entered the subcapsular sinus, but in each node 20–50 or more penetrated the dorsal surface of the node at right angles and terminated more deeply within the node (Figs. 6–8). At least some of these penetrating afferents were surrounded by tubular sinuses (Fig. 8).

The terminal lymphatic vessels carrying mammary lymph showed a predilection for the caudal part of the dorsal surface of the iliofemoral node whereas those from the popliteal or subiliac nodes terminated more cranially (Fig. 4). When Microfil of different colours was injected into lymphatics from the popliteal as well as the mammary nodes, some sinuses contained Microfil of both colours.

Efferent lymphatics emerged from the iliofemoral node over most of its ventral surface. When Microfil of different colours entered the iliofemoral node from the mammary, popliteal and subiliac nodes, some mixing of Microfil was evident in the efferent lymphatics. These lymphatic vessels either terminated on a medial iliac node, or became continuous with a lumbar trunk (Fig. 2).

Three variations in the drainage from the iliofemoral node were seen. A single example was seen of the first two variations, and the third variation was seen in four out of twelve cases. These were, first, an efferent lymphatic accompanied a nodal artery through the *dorsal* surface of the node, near the afferent lymphatics; second, an efferent lymphatic crossed the midline to the contralateral iliofemoral node, and third, an efferent lymphatic passed medially to a sacral node, located near the medial border of the external iliac vessels (Fig. 2).

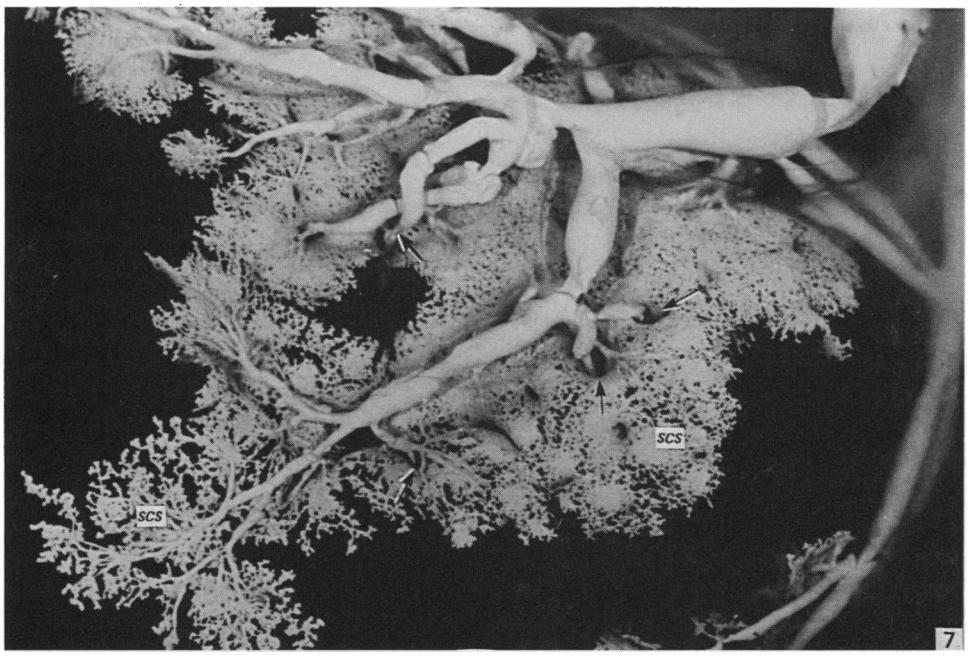
One or two medial iliac nodes, 10–50 mm by 10–20 mm, were parallel to the lateral border of the external iliac artery near its origin. Their efferent lymphatic vessels were continuous with the lumbar trunk(s) (Figs. 2, 4).

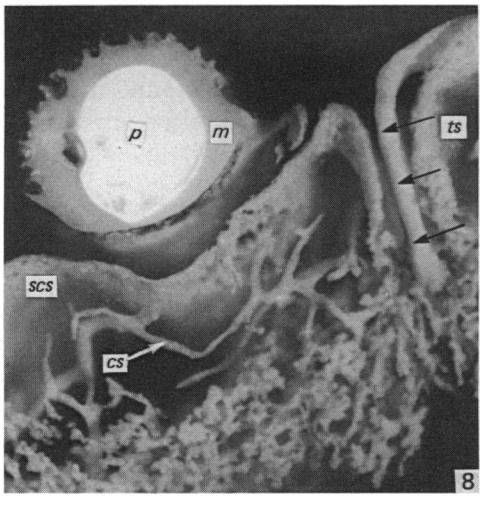
Distribution of markers within lymph nodes

Soluble protein marker

When Evans Blue dye was injected into the mammary gland and the ewe killed 30 minutes later, the dye showed a fairly constant distribution within the mammary and iliofemoral lymph nodes (Fig. 1).

In mammary nodes, dye was preferentially distributed over the smaller, medial node. When the dye was injected caudal to the teat it was distributed over the caudal half of the larger node(s). However, when the dye was injected cranially, the larger





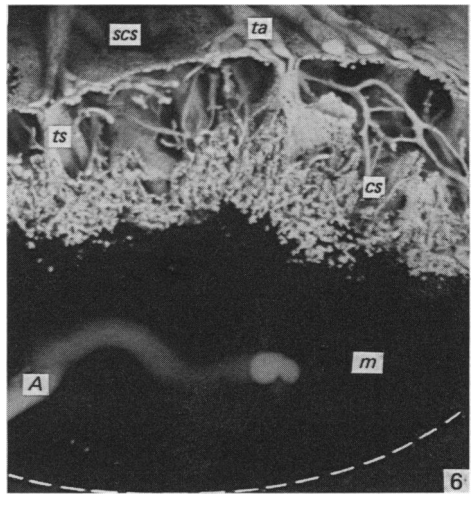


Fig. 6. Photograph of a slice through an iliofemoral lymph node which received Microfil through a mammary lymphatic vessel, showing subcapsular (*scs*), cortical (*cs*), and trabecular (*ts*) sinuses and terminal lymphatic vessels (*ta*). Broken line, ventral surface of node; *A*, artery filled with latex; *m*, medulla. × 12.

Fig. 7. Photograph of the dorsal surface on an iliofemoral lymph node showing Microfil-filled afferent lymphatic vessels entering the subcapsular sinus (*scs*), and penetrating more deeply into the node (arrows). × 20.

Fig. 8. Photograph of a slice through the dorsal part of an iliofemoral node showing an afferent lymphatic which provides evidence of anastomotic connections between lymphatics draining from two different primary nodes. The white Microfil (*p*) was injected into a popliteal lymphatic, and this is surrounded by maroon Microfil (*m*) which was injected into a mammary lymphatic vessel. The photograph also shows cortical sinuses (*cs*) ramifying in the lymphoid parenchyma beneath the subcapsular sinus (*scs*) and the terminal branch of an afferent lymphatic (arrows) penetrating into the node, surrounded by a tubular invagination of the subcapsular sinus (*ts*). × 15.

mammary node(s) contained either no dye, or it was localised to a small part of that node (Fig. 1).

The dye showed some preference for the caudal half of each iliofemoral node, but the medial iliac nodes did not show any preferred distribution (Fig. 1). Internal inguinal nodes, when they were present, contained dye throughout their substance, but sacral nodes contained it mainly in the caudal half.

When Evans blue dye was injected into the distal part of the leg and flowed through the popliteal node (Heath & Brandon, 1983), it was found mainly in the cranial part of both iliofemoral and medial iliac lymph nodes (Fig. 1).

Particulate marker

China ink was distributed fairly densely across each mammary lymph node after it was injected into the mammary gland. Some variation in density did occur between different nodes, but it was not possible to quantify these variations. Furthermore, no consistent differences could be detected between cranial and caudal poles of each node, or between nodes taken after 30 minutes and 24 hours, or between nodes taken after ink was injected into the different parts of the gland.

Ink was injected into 20 mammary glands, and carbon was detectable in iliofemoral nodes receiving lymph from 15 of these, and in 10 medial iliac nodes. When present, the carbon was distributed, albeit not in uniform concentration, over the whole length of the node. A similar result was obtained when the ink was given into the distal part of the leg.

After 30 minutes the carbon was almost all within sinuses. In the mammary nodes, most of the carbon was free although some was within macrophages, and it was in highest concentration in the paracortical region. The distribution was similar in the iliofemoral (Fig. 5) and medial iliac nodes, although the total amount of carbon was less. Macrophages had taken up most of the carbon in the iliofemoral nodes, and virtually all of that in the medial iliac nodes. After 24 hours, the amount of carbon within macrophages had increased still further, and some of these cells had migrated out into the lymphoid parenchyma.

Carbon was evident in subiliac nodes adjacent to four out of twenty mammary glands. Two of these had received ink cranial, and two caudal, to the teat.

No evidence was found that either Evans blue or carbon crossed over from one mammary gland to the other, and none was found in the contralateral mammary lymph nodes.

DISCUSSION

Lymph from the mammary gland of the ewe passes through at least three, and sometimes up to six, lymph nodes before reaching the bloodstream. This lymph carries cells and soluble factors between the lymph nodes, and in this way, immune reactions which originate in the mammary gland are likely to be propagated and amplified (Fahy *et al.* 1980; Fahy, 1980).

The primary nodes are usually in the mammary (superficial inguinal) group on the ipsilateral side. The present observation that neither Evans blue nor China ink crossed the midline from one gland to another is consistent with that of Barnwal & Dhingra (1978) in buffaloes.

In 20% of cases, carbon particles injected into the mammary gland were found in the subiliac node. This node, like the mammary, forms part of the superficial inguinal lymph centre, which also receives lymph from the skin and superficial layers of the

thigh and abdominal wall (Schummer *et al.* 1981). The subiliac node has been mentioned as a recipient of lymph from the mammary gland in the cow (Schummer *et al.* 1981), but no evidence is available on its precise role in relation to mammary lymph in any animal.

The second node traversed by most mammary lymph is immediately caudal to the origin of the deep circumflex iliac artery. It is not clear whether this node should be regarded as one of the medial iliac group, or as a separate node. According to Schummer *et al.* (1981), the medial iliac nodes are lateral to the external iliac artery near the end of the aorta, whereas the iliofemoral nodes are inconstant in sheep, and lie more distally in species in which they occur.

This node is always large and in a constant position, and it has an unusual flattened shape. Also, it occupies a key position as the secondary node to three primary nodes: the popliteal and subiliac as well as the mammary. We assume that it represents a fusion of the most caudal of the medial iliac nodes with the iliofemoral node of other animals (Schummer *et al.* 1981). For these reasons and for clarity, this node is termed here the iliofemoral, while the term medial iliac is used for nodes which are cranial to the deep circumflex iliac artery.

The distribution of lymph constituents and especially particulate material within these nodes seems less circumscribed than in rabbits (Kett *et al.* 1978) and rats (Belisle & Sainte-Marie, 1981; Sainte-Marie *et al.* 1982). In the case of the iliofemoral node, most afferent lymphatics carrying mammary lymph enter the caudal part of the node whereas those from the popliteal and subiliac nodes enter more cranially. Furthermore, when Evans blue entered the iliofemoral node in mammary lymph it stained more of the caudal part, but when it entered in lymph from the popliteal node it stained more of the cranial part of the node. This appears to indicate that lymph from the mammary gland has a *predilection* for the caudal part of the node.

However, when carbon particles reached the iliofemoral node in the mammary lymph and were retained largely within macrophages, they were present over a much larger area of the node than that covered by the relevant afferent lymphatic vessels. This was consistent with the observations made after Microfil was injected into a mammary lymphatic vessel. It may be argued that the distribution of Microfil was influenced by the pressure at which it was injected. However, since the Microfil was injected at less than the rate of mammary lymph flow (Lascelles & Morris, 1961) and then flowed for 15–20 cm through a fragile and distensible lymphatic vessel, it is unlikely that the pressure at which it entered the node would have exceeded the normal range of lymph pressures (Campbell & Heath, 1973).

It is not likely that the widespread distribution of carbon in the iliofemoral node was affected by injection pressure because, in this case, the carbon particles would have entered lymphatic capillaries from the tissue spaces in the mammary gland, and then passed through the mammary lymph nodes before being carried to the iliofemoral node.

It is probable that lymph constituents from the popliteal and subiliac nodes may mix with those from mammary nodes. These constituents, including cells and cytokines, could play an important role in the propagation of immune responses initiated in any of the three primary nodes (Fahy, 1980; Fahy *et al.* 1980).

SUMMARY

Lymph from the mammary gland passes through at least three lymph nodes. These are usually the mammary, iliofemoral and medial iliac, although the subiliac, internal inguinal and sacral lymph nodes may also be involved.

The iliofemoral, a large, flattened node in the dorsolateral wall of the pelvis, receives lymph from subiliac and popliteal as well as mammary nodes. Afferent lymphatic vessels either enter the subcapsular sinus on the dorsal surface of this node, or penetrate more deeply into the node. Lymphatics carrying mammary lymph enter more caudally than those from the popliteal or subiliac lymph nodes.

Evans blue dye, injected into the mammary gland, showed a characteristic distribution within the mammary and iliofemoral nodes. Within the iliofemoral nodes it was present mainly in the caudal part. However, when China ink was used, carbon particles were distributed throughout most of the ipsilateral mammary nodes. In the iliofemoral node the carbon was distributed more widely than the Evans blue; it was found, mostly within macrophages, over the whole length of the node. A similar distribution of carbon was seen when it entered from the popliteal node.

It appears likely that, although lymph from the mammary gland shows some preference for the caudal part of the iliofemoral node, constituents of this lymph may mix within the node with lymph from other primary nodes.

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