

Lymphatic Obstruction and Lymph Node Changes - — a study of the rabbit popliteal node

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Summary

Dense cortical lymph node fibrosis is associated with primary lymphedema and proximal obstructive hypoplasia. The fibrosis is not related to the duration of disease nor to episodes of clinical cellulitis. This suggests that the disease may start in the nodes but the effects of obstruction in the adjacent lymph vessel must be elucidated before the assumption is made.

The popliteal nodes of 49 rabbits were studied following the ligation of either afferent or efferent lymphatics, and compared with nodes following a sham operation. The efficacy and late effects of ligation were assessed in half the rabbits by cine-lymphangiography. In the rest Patent Blue Violet and not Lipiodol was used to minimize any spurious effect on node histology. The rabbits were sacrificed between 6–84 days. Node area was measured on serial x-rays and node volume after removal of the node.

Afferent ligation resulted in a significant and permanent decrease in size ($p < 0.005$) and lymphocytic congestion; efferent ligation resulted in an increase in size ($p < 0.02$) and large lymph spaces.

The dense node fibrosis of primary lymphedema was not seen.

Introduction

In a previous study it has been established that there is an abnormal amount of fibrosis in the lymph nodes of patients with primary lymphedema (1). The fibrosis was neither related to previous episodes of clinical cellulitis nor to the duration of the lymphedema.

Ischemia of the lymph nodes might lead to fibrosis but there is evidence that the vascular supply to a lymph node rapidly recovers following ligation of the hilar vessels (2, 3, 4).

Herman, Lyonnet et al. (5) found that the blood supply increased with lymph node hyperplasia and we found that lymph nodes were more vascular than normal in primary lymphedema (1).

It is well known that lymph stasis produces fibrosis, and it was therefore somewhat surprising that there was no correlation between the duration of the clinical lymphedema and the lymph node fibrosis. This led us to the conclusion that the fibrosis might precede the distal lymphatic vessel disease.

It was therefore decided to study the effects of efferent and afferent lymphatic ligation on a lymph node. In this way it was hoped to discern whether the fibrosis was a primary process within the node or secondary to lymphatic vessel obstruction.

Methods

Rabbits and experimental plan

Forty nine rabbits were studied and were divided into four groups. Each rabbit had either the afferent or efferent lymphatic of one popliteal node ligated and a sham operation performed on the contralateral limb.

Half of these rabbits had lymphography to assess the efficacy of ligation, alterations in lymphatic drainage and any changes in lymph node area. The rest had no Lipiodol injected since this oil has an acute effect on the lymph node (6, 7, 8) which resolves with time (9, 10).

No attempt was made to produce lymphedema in view of the considerable difficulties that

others had encountered. It was fully realized that lymph would drain via other pathways following the ligation of the vessels to the popliteal node, but it was hoped that the collateral circulation would allow a longstanding obstruction in the popliteal node to be maintained, and that the lymphatic channels through the lymph node would not regenerate. These effects were monitored by serial lymph node area measurements, lymph node volumes, cinelymphangiography and lymph node histology. In the rabbits that had no lymphography with Lipiodol subcutaneous Patent Blue Violet was used to ensure that there was complete lymphatic ligation.

The lymphatic anatomy of the hind limb and the blood supply of the rabbit popliteal node are well documented (3, 11). A schematic representation of the lymphatic drainage of the rabbit hind limb is shown in Fig. 1.

Most of the lymphatic drainage of the superficial and deep tissues of the foot and ankle runs with the deep blood vessels (the anterior and posterior tibial arteries and the peroneal artery), and then enters the popliteal node.

The posterior plantar region of the foot and the dorsum of the ankle are drained by lymphatics which later run with the long saphenous vein and then along a branch that runs superficially across the popliteal fossa; here the lymphatics leave the vein and enter the popliteal node.

The popliteal node of this animal has an advantage over more proximal nodes because it can be explored as a relatively minor procedure.

Cinelymphangiography

The terminal 5 cm of polyvinyl tubing was filled with normal saline in order that the meniscus of Lipiodol could be seen radiologically as it left the needle. Without this radio-lucent fluid accurate timing of flow from the needle would not have been possible. 0.5–1.0 ml of Ultrafluid Lipiodol was infused on each investigation. A radio-opaque graduated centimeter scale was placed alongside the animal so that direct measurements from the monitor could be calibrated and the flow of Lipiodol recorded in millimeters per second.

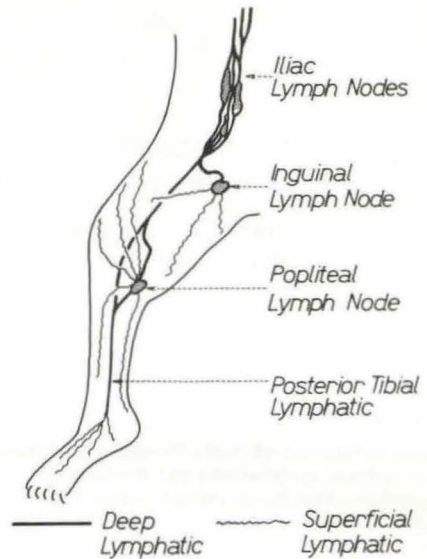


Fig. 1 Schematic representation of the lymphatic drainage of the rabbit hind limb

The rabbits were sacrificed between two and twelve weeks after the ligation of lymphatics. At this stage a second lymphangiogram was performed and the transit time of Lipiodol as it passed through the lymphatics measured. A xerogram or radiograph was also taken to record the changes in lymphatic anatomy.

Lymph Node Areas

The rabbits that had lymphangiograms were followed with serial lymphadenograms every two weeks. Supine radiographs of the animal were performed with the head of the x-ray tube and the table height at a constant level for all films. A radio-opaque centimeter scale was also placed alongside the animal on each occasion to ensure that the magnification remained constant. The lymphadenograms were then traced onto millimeter graph paper, giving a result of lymph node area in square millimeters.

Lymph Node Volumes

The popliteal lymph nodes were removed and the volume estimated by measuring length,

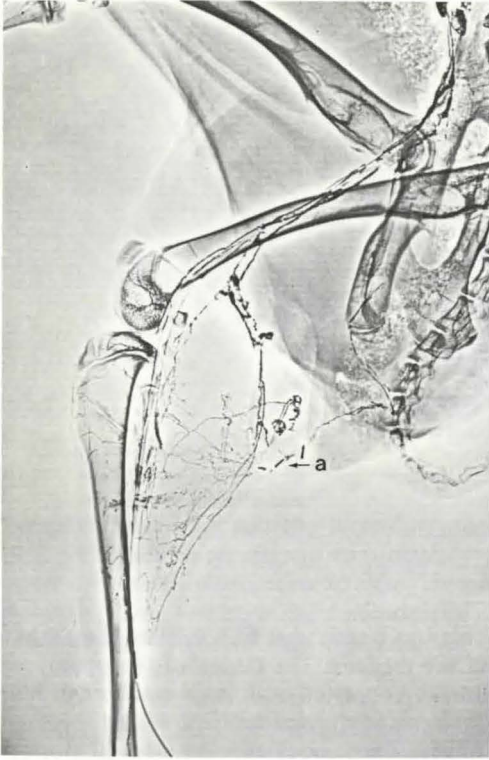


Fig. 2 Lymphangiogram 6 weeks after afferent lymphatic ligation (a) resulting in lymphatic bypass of popliteal node

width and depth with Venier calipers and applying the formula for an ellipsoid to these measurements ($\frac{1}{3} \times \text{longest length} \times \text{longest width} \times \frac{1}{2} \text{ height}$). This method was chosen since it allowed us to measure lymph node volumes at the initial popliteal fossa dissection without removal of the node.

Lymph Node Histology

The lymph nodes were transected through the hilum before being placed in a 10% solution of formal saline; they were then sectioned and stained with hematoxylin and eosin, Weigert's elastic stain, van Gieson's stain, picro Mallory violet for fibrin, and Foot's stain for reticulum.

Results

Experimental Problems

One rabbit died three weeks after surgery and a second rabbit was excluded from consideration due to technical failure of the procedure.

Three rabbits developed lymphoceles following afferent lymphatic ligation, but none following an efferent lymphatic ligation.

Wounds healed well by primary intention in all the rabbits, and no wound became infected and discharged pus. The wounds usually healed with little induration so that a second lymphangiogram at the same site was possible, but occasionally the lymphatics were encased in fibrous tissue and a more proximal cannulation of the afferent lymphatics was necessary. In all cases a second lymphogram was achieved.

The rabbits did not develop clinical edema as a result of the lymphatic ligation.

Results of Afferent Lymphatic Ligation

Lymphangiography

The effects of ligating the afferent lymphatics to the popliteal node were assessed lymphographically in thirteen rabbits. Alternative pathways opened up or developed so that the Lipiodol completely bypassed the lymph node, and the lymphatic drainage of the limb was reconstituted. The typical findings are shown in Fig. 2.

Lipiodol filled the lymph node through a regenerated lymphatic in four cases; this was not a technical failure since a postligation lymphangiogram showed adequate ligation. In nine cases the Lipiodol bypassed the popliteal lymph node and also entered directly into the deep lymphatics of the lower leg. In seven of these rabbits the popliteal node eventually filled through small tributaries 'back filling' into the lymph node. The mainstream of Lipiodol completely bypassed the lymph node.

Since there was an adequate collateral lymphatic circulation, mainly via the deep lymphatics, the rate of flow between the site of cannulation and the lumbar nodes was more rapid as a result of bypassing the popliteal lymph node.

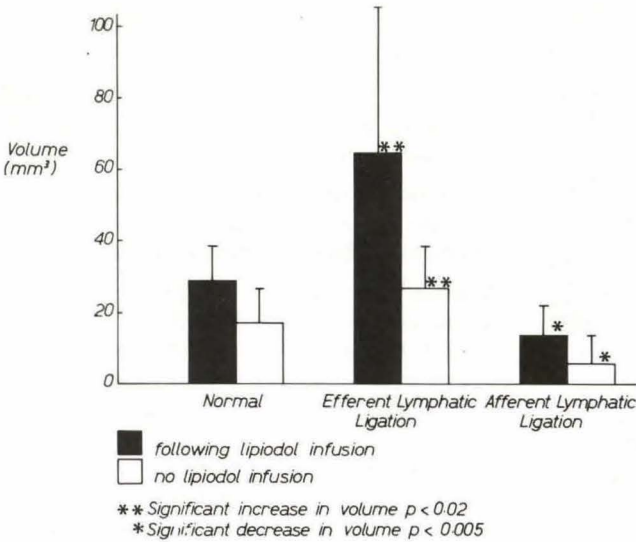


Fig. 3 Lymph node volumes following efferent and afferent lymphatic ligation

Lymph Node Size

There was a rapid initial decrease in the area of the lymph nodes and this did not recover during the course of the experiment. This reduction in lymph node area was borne out by the significant reduction in lymph node volume. This was true whether or not Lipiodol had been infused ($p < 0.005$ using Student's test separately for each group.) (Fig. 3).

The hilar artery bled in all cases when the lymph node was removed, so that there was no gross clinical evidence to suggest lymph node ischemia.

Lymph Node Histology

Perinodal fibrosis was a striking feature in seven of the thirteen Lipiodol-filled nodes and four of the eight Lipiodol-free nodes. This fibrosis was not related to the length of time that the lymphatic supply had been ligated, and was present in one lymph node that was removed at two weeks. An extensive dissection of the node was required to ligate all the afferent lymphatics and the fibrosis may have been the response to the lymphatic ligation.

The diminution in size of the lymph node was apparent in most of the nodes examined.

This was largely due to a marked shrinkage of the medulla. The cortex of the nodes was usually congested with large numbers of lymphocytes in the sinuses (Fig. 4) but the germinal centers appeared similar to the normal contralateral node. The four nodes that had a reduced number of germinal centers in the section examined were all removed within forty days of obstructing the afferent lymphatics, suggesting that the germinal centers return to normal after a short period of time. There was no evidence of an increase in the amount of central fibrosis in any node from this group.

In summary, despite the dramatic reduction in lymph node size following afferent lymphatic ligation there was no increase in the amount of central fibrosis. Although there was an increase in perinodal fibrosis this may not be the effect of lymphatic ligation.

Results of Efferent Lymphatic Ligation

Lymphangiography

Fourteen rabbits had successful lymphography following efferent lymphatic ligation. Seven had lymphatic ligation in the thigh only, and seven also had efferent lymphatics ligated in the popliteal fossa six weeks after the thigh

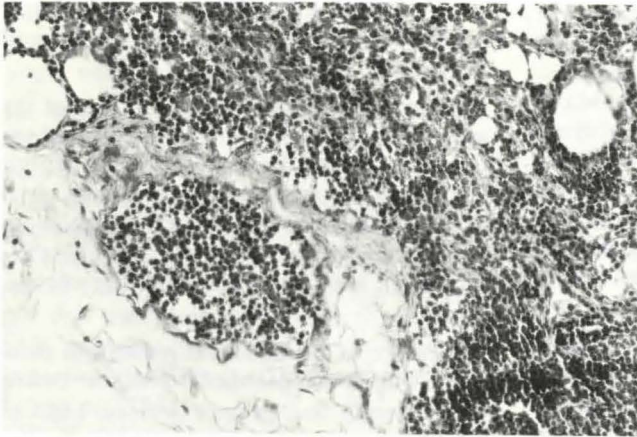


Fig. 4 Cortical sinus and adjacent lymphatic showing lymphocytic congestion following afferent lymphatic ligation (magnification x 250)

lymphatics were ligated. The second ligation was performed in an attempt to produce a more efficacious obstruction to flow. Unfortunately, there was rapid reconstitution of the lymphatic channels even following the second ligation.

In each of these rabbits adequate initial ligation of the lymphatics was shown lymphographically, but alternative lymphatic channels developed in all except one (a rabbit sacrificed at two weeks).

In only two rabbits did the Lipiodol enter

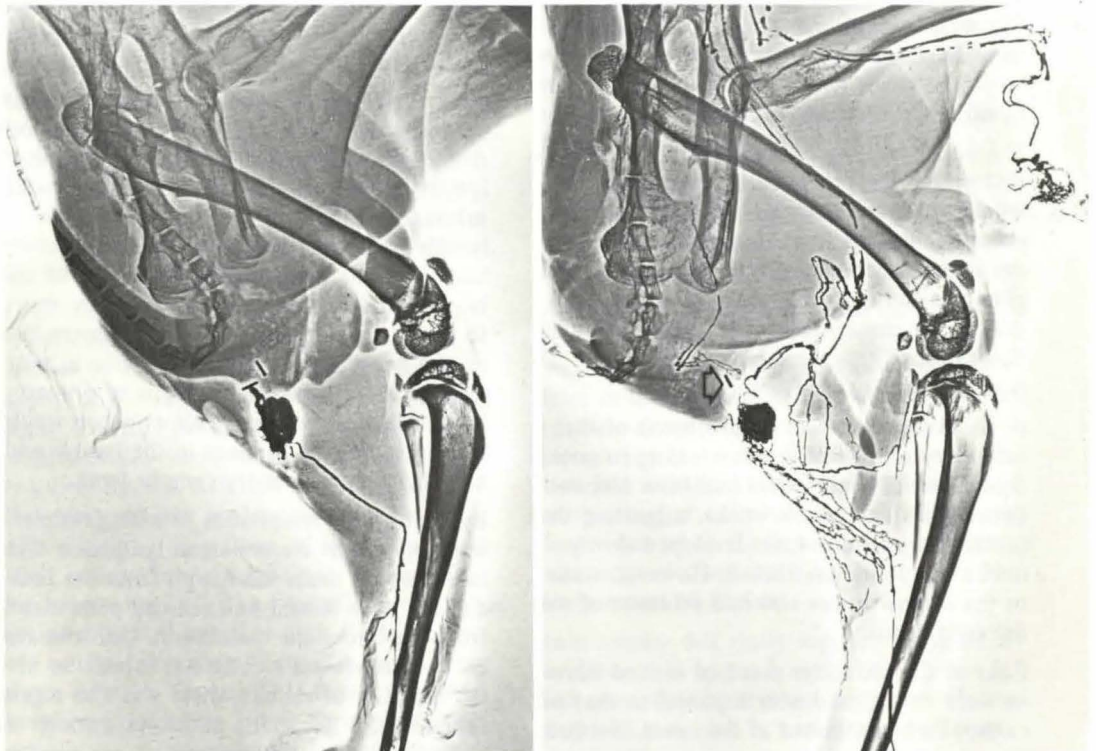


Fig. 5 Lymphangiogram immediately following efferent lymphatic ligation and 14 weeks later

the deep lymphatics of the lower leg and completely bypass the popliteal node. In the rest the Lipiodol passed through the lymph node, but there was considerable resistance to flow despite regeneration of lymphatics and collateral formation. The efferent lymphatics at the hilum of the node regenerated following ligation in all the rabbits except one. The thigh lymphatics either regenerated, or collaterals developed to bypass the obstruction (Fig. 5).

The mean transit time of Lipiodol from the needle site to the lumbar lymph node was twice as long as in the normal control studies.

Lymph Node Size

There was an initial increase in the lymph node area which started to resolve as the weeks passed. However, the lymph nodes remained larger than normal throughout the experiment. There was an increase in volume of nodes following efferent ligation whether or not they had Lipiodol infused ($p < 0.02$ using Student's *t* test separately for each group) (Fig. 3).

Lymph Node Histology

The nodal swelling following efferent lymphatic ligation was produced by stagnation of lymph. The lymphatic sinuses were dilated giving the node a less dense appearance, and the sinuses contained scanty numbers of lymphocytes and macrophages in contra-distinction to the sinuses following afferent lymphatic ligation. This gave the medulla of the nodes an 'empty' appearance.

In six nodes there was some fibrosis of the medullary sinuses. It was interesting to note that all six of these nodes had been biopsied between six and twelve weeks, suggesting that central fibrosis may have developed slowly unlike the perinodal fibrosis. However, some of the normal nodes also had evidence of medullary fibrosis.

Four of the six nodes that had central fibrosis were not infused with Lipiodol so that oil cannot be incriminated as the cause. Neither

can the fibrosis be attributed to surgical trauma since the popliteal fossa was not dissected in four of the affected nodes.

Perinodal fibrosis was present in three of the seven rabbits that had a popliteal fossa dissection and none of those that had efferent lymphatic ligation performed in the thigh only. This adds further evidence to the suggestion that perinodal fibrosis was the result of the dissection, and not of the lymphatic obstruction.

The cortex of the node was reduced in cellularity with fewer germinal centers in twelve of the twenty four nodes examined. Eight of the twelve nodes with a reduction in the number of germinal centers were removed six weeks or less after lymphatic ablation. This suggests that, as after afferent lymphatic ligation, the effect on the cortex of the node was transient.

In summary lymph stasis due to obstruction proximal to the lymph node may produce some medullary fibrosis but obstruction distal to the node does not have the same effect.

Discussion

Most experimental work on lymph stasis has concentrated on changes in the subcutaneous tissue and distal collecting vessels, and the lymph nodes have been destroyed by noxious substances in an attempt to produce this lymph stasis. As a result, any histological evaluation of the nodes was difficult and of dubious value. The purpose of this study was to assess the effects of lymphatic obstruction on the lymph node, and particularly to see whether the severe node fibrosis of primary lymphedema developed. This evidence would help interpret the findings in the lymph nodes of patients with primary lymphedema.

By ligating the lymphatics to a single node it was hoped that the collateral lymphatic circulation would drain the lymph from the limb and the node would be excluded permanently from the lymphatic circulation. Unfortunately, despite adequate collateral formation in the majority of rabbits, there was also regeneration of the lymphatic pathways through the

lymph node. Thus it was only possible to look at the relatively short term effects of lymph node obstruction.

The lymphograms showed early regeneration of collateral lymphatic pathways, and also showed a considerable decrease in the flow rate once the Lipiodol had transgressed a normal node. This flow is unphysiological in both the properties in the fluid and the rate of flow, but nevertheless it does suggest that the lymph nodes resist flow. Lymph node resistance has been shown more elegantly by *Browse, Doig et al. (12)* and *Papp, Makara et al. (13)*.

The rate of flow out of the limb was considerably impaired by efferent lymphatic ligation but not by afferent lymphatic ligation. This was because an adequate and rapid collateral circulation developed following afferent lymphatic ligation.

It has been shown that proximal obstruction results in enlargement of the lymph node even if Lipiodol has not been infused into the obstructed lymphatic system. This has relevance to the lymphadenograms of patients with primary lymphedema. Patients with proximal obstructive hypoplasia frequently have enlarged inguinal lymph nodes and this is probably due to the proximal obstruction. The lymph node atrophy that results from obliteration of the afferent lymphatics is well recognized, and is again of relevance to clinical lymphadenograms.

The only thorough study of total lymph vessel ablation to the lymph node was performed by *Osogoe (14)*. He studied the viability of the rabbit popliteal node following lymphatic ligation. In the majority of rabbits all the lymphatics and the hilar vessels were ligated. Afferent lymphatic ligation was probably inadequate since he reported ligating only three or four vessels and other authors have found seven to ten afferent lymphatics (11, 15). The efferent lymphatics were ligated together with the hilar vessels confounding the effects of a purely lymphatic ligation. *Osogoe (14)* found that total lymph vessel ligation led to an intranodal accumulation of lymphocytes, and shrinkage

of the medulla, whereas efferent lymphatic and hilar vessel ablation led to nodal swelling, dilatation of the lymphatic sinuses and fibrosis of the medullary cords; very similar findings to the present study. *Engeset and Neisheim (16)* also produced gross sinus lymphocytosis by ligating all the afferent lymphatics to the lymph nodes. They attributed this finding to the failure of lymph to wash the recycling lymphocytes out of the lymph node.

The histology of the present study has to be interpreted with care. Many of the lymph nodes had perinodal fibrosis which could be attributed to the dissection. The leg wound was also likely to affect lymph node histology so that a comparison was made with control nodes following a similar incision on the contralateral leg. Both Lipiodol and Patent Blue Violet effect lymph node histology, but the effects of Patent Blue Violet are probably small.

The surprising finding in both the enlarged and atrophic nodes was the lack of gross histological changes. In the enlarged lymph nodes the lymphatic spaces were dilated, especially in the lymph nodes that had been infused with Lipiodol. Some fibrosis of the medullary cords was seen in the nodes following efferent lymphatic obstruction.

This fibrosis in six of the twenty four nodes following efferent lymphatic ligation was the only convincing increase in fibrosis that could be attributed to the lymph vessel obstruction.

A reduction in active germinal centers was a feature of early lymphatic obstruction and was not present in lymph nodes removed later in the experiment suggesting a reversal of the process with time. Lymphocytic depletion is a feature of lymph node degeneration but in many nodes the lymphoid tissue was normal in both volume and cellular content. Even when the lymphocytic content was reduced there was no gross increase in the amount of fibrosis.

In summary this study supports the clinical evidence that severe lymph node fibrosis is not secondary to afferent lymphatic obstruction and may also be unrelated to efferent

lymphatic obstruction. A fibrotic process within the nodes leading to further resistance of lymph flow may be the initial event in severe primary lymphedema.

References

- 1 *Kinmonth, J.B., J.H.N. Wolfe*: Lymph Node Fibrosis in Primary Lymphoedema. *Ann. Roy. Coll. Surg.* 62 (1980) 344–354
- 2 *Tilak, S.P., J.M. Howard*: The influence of dual circulation on the viability of lymph nodes following interruption of their blood or lymphatic supply. *Surg. Gynec. and Obst.* 119 (1964) 349–352
- 3 *Osogoe, B., F.C. Courtice*: The effects of occlusion of the blood supply to the popliteal node of the rabbit on the cell and protein content of the lymph and the histology of the node. *Aust. J. Exp. Biol. Med. Sci.* 46 (1968) 515–524
- 4 *Callum, K.G., J.B. Kinmonth*: The effect of ischaemia on lymph nodes and the lymphatic circulation. *Lymphology* 7 (1974) 53–61
- 5 *Herman, P.G., Lyonnet, D., R. Fingerhut and R.N. Tuttle*: Regional blood flow to lymph nodes during the immune response. *Lymphology* 9 (1976) 101–104
- 6 *Kazem, I., A. Neowich, R. Mortel, T. Honda*: Comparative histological changes in normal lymph nodes following ethiodol lymphography and colloidal gold¹⁹⁸ lymph scanning. *Clin. Radiol.* 22 (1971) 382–388
- 7 *Carvalho, A. de*: Spontaneous alteration in the size of lymph nodes after lymphography. *Lymphology* 9 (1976) 138–141
- 8 *Symmers, W.St.C.*: The lymphoreticular system in: *Systemic Pathology*. 2nd Edition (1978). Ed.: W.St.C. Symmers, p. 504–591, Churchill Livingstone, Edinburgh, London, New York
- 9 *Bourquin, J.*: Histologische Grundlagen des Lymphadenogramms. *Radiologe* 8 (1968) 150–153
- 10 *Bujar, H.*: Functional Interpretation of lymphography according to contrast medium persistence in the lymph nodes. *Rev. Roum Med. Int.* 10 (1973) 388–395
- 11 *Bach, C., G.P. Lewis*: Lymph flow and lymph protein concentration in the skin and muscle of the rabbit hind limb. *J. Physiol.* 235 (1973) 477–492
- 12 *Browse, N.L., R.L. Doig, D. Sizeland*: The resistance of a lymph node to lymph flow. *J. Physiol. (Lond.)* 208 (1970) 77–78
- 13 *Papp, M.E., G.B. Makara, B. Hajtman*: The resistance of in situ perfused lymph trunks and lymph nodes to flow. *Experientia* 27 (1971) 391–392
- 14 *Osogoe, B.*: Changes in the cellular architecture of a lymph node after blocking its lymphatic circulation. *J. Anat.* 104 (1969) 495–506
- 15 *Kett, K., L. Illenyi, L. Lukacs, J. Nyarady*: Passage through the lymph node. *Lymphology* 11 (1978) 75–80
- 16 *Engeset, A., A. Neisheim*: Sinus lymphocytosis and lymph flow. *Acta Path. Microbiol. Scand.* 68 (1966) 181–188

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