

## The Demonstration of the Lymph Pathways in the Haemolymph Nodes of Cattle, and their Relationship to the Lymphatic System

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### Summary

Improvements in the morphological demonstration of the lymph vessels in bovine haemolymph nodes were achieved with the help of vital dyes (patent blue violet and Japanese ink) and by the lymphokinetic effect of a drug. In this way evidence was produced of the relationships between the haemolymph nodes and the lymphatic system via afferent and efferent lymph vessels.

In recent decades the latest findings regarding the various aspects of the structure and function of lymph nodes, including the well-known blood-containing lymph nodes, have aroused progressively more interest in scientific discussion. Thus, one finds statements about their macroscopic, microscopic and ultramicroscopic structure, in the course of which a special significance is accorded to the haemolymph nodes from the immunological point of view. In the literature there are contradictory statements regarding the relationships between the haemolymph nodes and the lymphatic system. Having regard to already proven injection techniques and the present-day newer combined methods of investigation, it is hoped to prove to what extent there are direct connexions between the haemolymph nodes and the lymphatic system.

### Literature

In the most recent literature there are relatively numerous articles to be found about lymph nodes (*nodi lymphatici*, *lymphonodi*) in the traditional sense (1, 5, 7, 8, 11, 29, 30, 34, 35, 45, 47, 52, 53, 54, 55, 56, 57, 58, 59, 60, 64, 65, 75, 79, 80, 81, 83, 85,

87, 96) which are also designated as true lymph nodes (15) or as 'typical' lymph nodes (73) in contrast to the haemolymph nodes - 'lymphonodi haemales' (19, 61, 65, 66, 68, 88, 89). These blood lymph nodes are also called haemolymph nodes - haemolymphonodi (2, 10, 65, 73, 77, 78, 86, 91, 95), haemal nodes - *nodi haemalis* (31, 62, 65), blood lymph glands - haemolymphoglandulae (6, 37, 67, 69, 76, 92), and as their structure occupies an intermediate position between lymph nodes and the spleen, they are also designated as lymphoid or splenic nodes (36, 40, 41, 62).

The above mentioned true or typical lymph nodes occur in man and other mammals. The colour of lymph nodes is normally grey but it can be changed to yellow as a result of fat deposits, or to black by coal dust (anthracosis) (33). Moreover a red colouration of lymph nodes can appear in man and animals. *Starck* (82) has written that in the embryo the formation of red blood corpuscles takes place to some extent in the lymph nodes. This erythropoiesis however, ceases very early. He also notes that e.g. in the horse, the lymph nodes acquire connexions with the vascular system and have blood running through them. He calls these lymph nodes "blood lymph nodes". *Watzka* (91) declares that in man - in the new-born and in infants - as well as in many animals, the blood capillaries often run into the lymph sinuses in the retroperitoneal and intestinal lymph nodes, so that these lymph nodes take part in the destruction of erythrocytes. He speaks here

of haemolymph nodes. According to *Ferner* (26) blood-containing lymph nodes are found in man at the hilum of the spleen and liver, and after destruction of the blood behave again as ordinary lymph nodes. On the other hand he noted that in animals there are "true blood lymph nodes" which do not have any afferent lymphatic vessels. This observation has also been made by *Benninghoff* and *Goertler* (4). Although in man some individual authors point out the occurrence of blood in the abdominal lymph nodes and that the function of the spleen was taken over from the haemolymph nodes, it is predominantly the view that this is concerned with the lymphatic transport of blood (38, 39, 42, 71). *Bargmann* (2) concludes that normally man has no haemolymph nodes, a view also expressed by *Lennert* (43). In man the presence of blood in lymph nodes is usually the result of some disease process, whereby the sinuses are used as blood pathways, e. g. in congestion of the portal circulation, as in cirrhosis of the liver (2, 10, 97), where blood cells are also conveyed by the lymph pathways with the lymph from the spleen (63, 74). The true haemolymph nodes which have already been mentioned here, according to *Grau* (33), only been observed in ruminants, cattle, sheep and the goat (3, 12, 13, 14, 16, 17, 18, 28, 32, 37, 40, 67, 70, 72, 76, 90, 93, 94) and in the rat (14, 44, 65, 66, 72, 78, 88, 90, 97).

The characteristic feature of these haemolymph nodes is the complete absence of lymphatic vessels (37, 46, 76, 93, 94), which, according to *v. Schumacher* (76), only occurs secondarily during the embryonic period as a result of the obliteration of the afferent and efferent lymph vessels. In one site viz. the hilum, a small artery enters and a wide vein comes out (95). As in the spleen there are no lymph vessels opening into the parenchyma but there are blood vessels and accordingly there are no afferent or efferent lymphatic vessels. The mesh-work of the organ therefore contains no lymph, but blood and hence it is not connected to the lymphatic but to the vascular system (33). Efferent lymphatic capillaries are described (66, 88,

90, 97) and these arise in the connective tissue framework of the nodes (33). According to *v. Schumacher* (76) the haemolymph nodes cannot be sharply distinguished from the other lymph nodes and they must be regarded as rudimentary forms of ordinary lymph nodes.

#### *Material and methods*

For this investigation haemolymph nodes were obtained from nine freshly removed bovine fetuses of varying size — crown — rump measurements between 29.8 and 51.1 cm. Similar nodes were taken from twelve adult Friesian cattle of both sexes. They were taken from the thorax and abdomen in the vicinity of the abdominal aorta and the caudal vena cava. The previous, well-tried injection technique was used (20, 21, 22) together with the methods already employed in the investigations of the Pacinian corpuscles (24, 25). They were used here in a modified form and with more solutions (25). They were injected with a 2.5% patent blue violet solution<sup>1)</sup>, with a solution of Japanese ink and also with "Venalot"<sup>2)</sup>. Recently such injections of patent blue violet and indian ink have also been used successfully for the demonstration of the lymphatic system of birds (9). In order to display them the haemolymph nodes were injected with a very fine cannula under the stereo-microscope. In the bovine fetuses the injection of patent blue violet was made into the interdigital space of the hind leg and then immediately after opening the abdominal cavity, the thoracic duct and the lymph pathways which were thus demonstrated were further injected both orthograde and retrograde with Japanese ink. In the adult animals the injection was made directly into the haemolymph nodes or into the adjacent lymph vessels which had been made visible by the patent blue violet injections. The injection with Venalot, a coumarin (5,6-benzopyron)-rutin derivative, was given intravenously or else directly into the haemolymph nodes, in view of the lympho-kinetic effect of this drug. In animal experiments a 1.7–2.6-fold increase in lymph transport has been demonstrated (27).

1) Manufactured by Byk-Gulden, Constance

2) Manufactured by Schaper and Brummer, Salzgitte-Ringelheim



### Results

Although the so-called haemolymph nodes are the main subject of this investigation, attention must also be given to the lymph capillaries and vessels in their vicinity as well as to the removal of the injected materials – the patent blue violet and the Japanese ink.

The haemolymph nodes investigated in the bovine fetuses vary in size from 3 mm to 6.5 mm. In the adult animals they also measure 3 mm, but have a greater range, up to 17.5 mm. On the medial cut surface of the haemolymph nodes the following features can be recognized.

In a 29.8 cm fetus one finds under the thin capsule an extremely wide marginal sinus, which is packed with red blood corpuscles and, even macroscopically, is quite striking on account of its red colour. From here, via intermediate sinuses it advances centripetally

right into the medulla. The parenchyma forms for the most part a uniform lymphoreticular tissue in which scattered germinal centres are found. No clear distinction can be recognized between the marginal and medullary tissue.

In a 45.6 cm fetus there is a strikingly smaller marginal sinus, also filled with red blood corpuscles. The marginal sinus is very small in a 51.1 cm fetus, and there is a relatively very thin capsule.

There are also afferent and efferent lymph vessels to be seen in the aforementioned haemolymph nodes, and these are visible as very fine vessels going into the hilum and through the capsule. They are particularly striking after an injection of Venalot because this drug has a three-fold myotropic effect on the lymph vessels, so that there is a striking increase in the frequency of pulsation and in the size as well as the tone of the lymph vessels (50). There



Fig. 1 Bovine haemolymph nodes with their attached lymph vessels which have been injected first with patent blue violet and later with Japanese ink. The vessels are joined by bridging anastomoses (arrow) and there are also afferent and efferent lymph vessels together with a smaller haemolymph node which has a lymphatic attachment.

Negative enlargement 1 x, positive enlargement 3,33 x

are also solitary smaller lymph vessels from the capsule which connect up with efferent vessels from other haemolymph nodes, as occurs also in the bovine adult. These are shown in Fig. 1 and larger lymph vessels run into the lumbar lymph trunks through the cisterna chyli into the thoracic duct.

The injection of Japanese ink into a haemolymph node through an afferent lymph vessels after preliminary irrigation gives a picture completely similar to the findings described above not only in the fetus, but also as might be expected, in the adult animals. There are afferent and also efferent vessels from the haemolymph node, where the latter finally connect up with the thoracic duct.

Even the direct injection of dye centrally into the haemolymph nodes shows a pathway through the marginal sinus via the capillaries of the capsule. With a direct injection of the dye into the marginal sinus, its removal takes place through the lymphatic capillaries of the

capsule into the vicinity of the haemolymph nodes.

The haemolymph nodes of adult cattle also resemble the previous descriptions, in that afferent and efferent lymph vessels are also present (28, 76) which are demonstrated particularly clearly with the lymphokinetic effect after an injection of Venalot (Fig. 2), because one can achieve an opening up of the finest lymph capillaries, which previously were regarded as collapsed or even as obliterated. This is also shown by a more rapid removal of the dye, so that it was often very difficult to obtain a pictorial record of the events. An increased and very fine delineation of the lymph vessels in the vicinity of the haemolymph nodes could be observed but these were in the marginal sinus, the trabeculae and the parenchyma (Fig. 3) but there was also a display of the lymph capillaries in the capsule, where fine lymph vessels had already been confirmed by *Helly* (37).

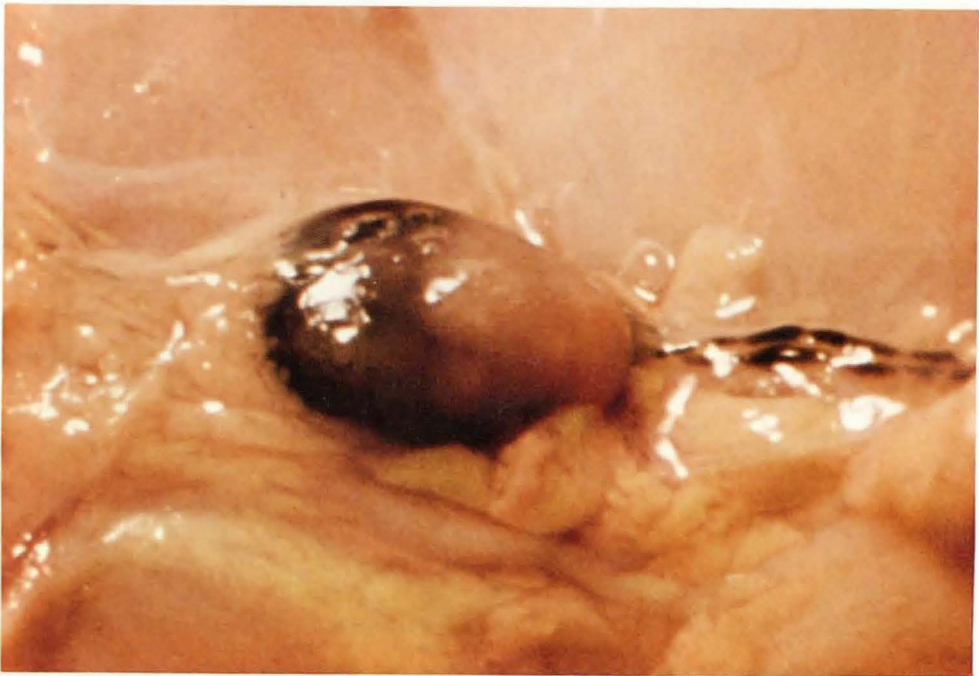


Fig. 2 Bovine haemolymph nodes with two afferent lymph vessels with a beaded appearance (right half of picture), also several efferent vessels (left half of picture), after administration of the pharmacological agent together with injection of patent blue violet and Japanese ink. Negative enlargement 1 x, positive enlargement 12,5 x



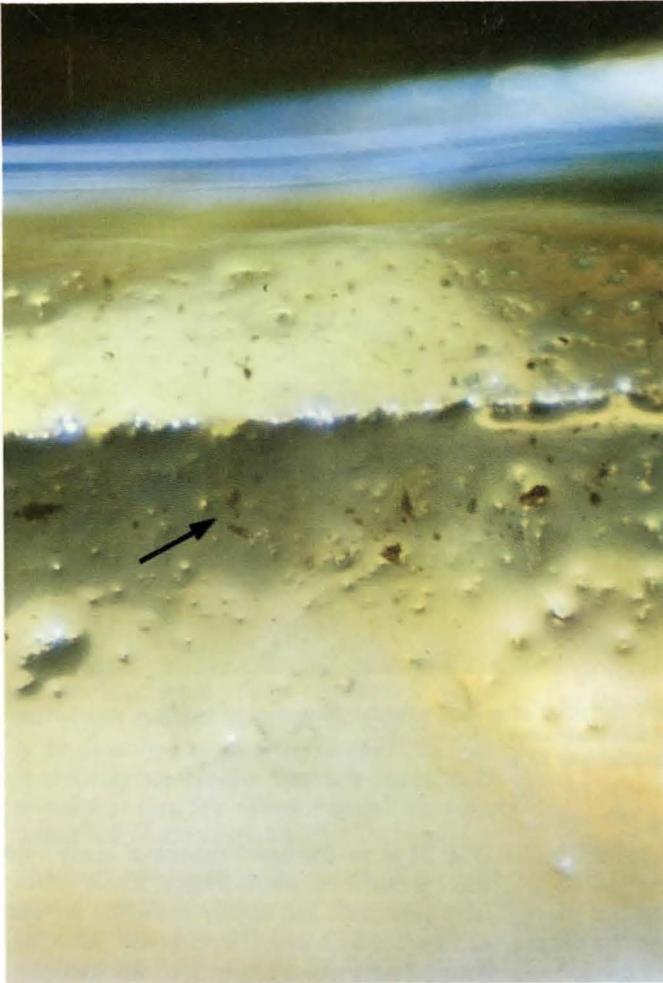


Fig. 3 Section through a bovine haemolymph node after injection with patent blue violet and the start of an injection with Japanese ink. Lymph capillaries of the capsule draining into the underlying marginal sinus (arrow) and into the trabecule (right side of picture). Negative enlargement 32 x, positive enlargement 107 x

Haemolymph nodes were found whose afferent lymph vessels entered the hilum, as already described in the haemolymph nodes of the fetus. These haemolymph nodes were particularly prominent here, besides those described in the literature where afferent vessels enter the convexity of the node and then leave again as efferent vessels from the hilum. In these haemolymph nodes the injection of the dye can be carried out through larger afferent vessels on their convexity, which here is comparable

to a retrograde injection. It goes through the capsule through very fine capillaries to the inside of the gland where it splits up into a network. The demonstration of the capillaries was very dense in the marginal sinus and particularly so at its base. From here an increased number of capillaries ran along the trabeculae into the interior of the haemolymph node.

Both patterns, however, do not change in their functional significance. It is only the observation which changes, that the injection which



Fig. 4 Section through a bovine haemolymph node after a peripheral injection of patent blue violet. Afferent lymph vessel at the hilum with two semilunar valves (arrow). Negative enlargement 6,25 x, positive enlargement 21 x

normally runs orthograde was injected retrograde, which is similar to the course and topography in the lymph nodes of the pig, in which there is also a departure from the usual state of affairs. This is also the case in the dolphin and also apparently in the rhinoceros (33).

The haemolymph nodes which are the basis of these investigations display a more or less reddish tinge and were injected as described above. One can give quite a definite answer to the question that in bovine haemolymph nodes afferent and efferent lymph vessels can be demonstrated. These lymphatics stand out very clearly after injection with Venalot, as shown in Fig. 2. The flow of the dye from the hilar region of the node into its centre proceeds very rapidly after the injection, so that there is often great trouble in recording the different stages of the process. The two beaded and parallel running lymph vessels

(Fig. 2) show the development of many valves throughout their whole length. These valves have two cusps and clearly show the direction of flow in the haemolymph nodes. Also the lymph vessel which drains into the haemolymph node is firmly fixed at the hilum and shortly after its entry into the node shows semilunar valves on its medial cut surface (Fig. 4).

The lymph vessel which goes to the hilum of the haemolymph node as an afferent vessel is accompanied in its course by a supplying artery and a draining vein.

#### Discussion

As *v. Schumacher* (76) has already reported confirmation of the finer lymph vessels is only possible when they are not collapsed. It is hence apparent that without an injection of dye, the lymph vessels or capillaries connected with the haemolymph nodes, com-



pletely escape detection. It is only after injection with patent blue violet and subsequent orthograde or retrograde injection of Japanese ink that they can be easily recognized (Fig. 1). A characteristic property of the lymph vessels is their great ability to dilate, so that after the injection the vessels take on a beaded appearance (Fig. 2) and, indeed, appear as afferent vessels before their entry into the haemolymph nodes. This was predominantly the case in almost all the haemolymph nodes investigated and recalled very much the anatomy of lymph nodes in the pig, where, in contrast to other domestic animals the lymph goes into the hilar notch and not to the periphery of the node. *V. Schumacher* (76) states that he was not able to find any haemolymph nodes in the pig, although there were lymph glands containing blood, which were connected to afferent and efferent lymph vessels. In his diagram of the pig there is an afferent lymph vessel in the marginal region of a retroperitoneal lymph gland which opens into the marginal sinus. If one considers the typical lymph node of the pig this should actually be described as an efferent vessel, as here it certainly runs in the opposite direction. He noted that in a few afferent lymph vessels he found red blood corpuscles.

The demonstration of the course of the lymphatic system, including the thoracic duct, resulting from the injection of patent blue violet in the hind limb of the bovine fetuses of varying length, and with the later injection of Japanese ink, can be significantly improved by an intravenous injection of Venalot. This was shown in earlier investigations on the Pacinian corpuscles in the meso-jejunum of the cat (25). The observations made in these investigations after the injection of Japanese ink, furnish evidence that apart from the lymph vessels around the haemolymph nodes, there are also afferent and efferent lymph vessels.

In order to enable some further information to be obtained about the relationship of the haemolymph nodes to the lymphatic system, an attempt was made to remove the blood from the haemolymph nodes by irrigating

with physiological saline (84) via the upper part of the abdominal aorta. This largely succeeded. However, it must be stated that the function of the haemolymph nodes here, as also in the adult animals, has not so far been clarified (65). According to *Turner* (88) a few blood capillaries in the medullary cords open into the sinuses. *Oláh, Röhlich* and *Törö* (65) on the other hand hold a different view, as according to their observations no capillaries drain into the sinuses. They conclude from this that there is a closed circulation and if, per chance, any direct connexion exists between the blood and lymph sinuses, this can only be quite fleeting. In spite of the vague relationship between the haemolymph nodes and the blood circulation and also their marked phagocytosis of erythrocytes, the haemolymph nodes certainly show more similarity to normal lymph nodes than to the spleen. These writers therefore assume that it is a specialized form of lymph node.

The relationship of the haemolymph nodes to the vascular system will not be discussed in detail here. What is clear is that afferent and also efferent blood vessels do exist. At the present time there is still a difference of opinion about the afferent and efferent lymph vessels of the haemolymph nodes. However, one can in general advocate the view that there are no afferent and indeed no efferent vessels (33). What are described are merely efferent lymph capillaries which may arise in the connective tissue reticulum of the node (66, 88, 90, 97).

According to *v. Schumacher* (76) the presence of many valves is exclusively the characteristic of lymph vessels. The valves, well-known as endothelial folds occur at regular intervals, so that because of this segmentation the vessel has a beaded appearance (Fig. 2). Each intervalvular section (valve segment) is called by *Mislin* (48, 49, 50) a 'lymphangioma'. From this he deduces that there are two types of lymph vessel. Firstly those vessels where extramural forces play the principal part in lymph transport, which therefore are purely transport vessels. The other show rhythmic contractions and represent special-

ized transport vessels. Contrary to other views these assumptions have been confirmed by more recent investigations (51).

These investigations have shown that here also, there are lymph vessels which take over only a pure transport function and are attached to the haemolymph nodes and connected to each other by bridging anastomoses (Fig. 1). Those with a smaller diameter go to the hilum of the lymph node as afferent vessels (Fig. 2, right half) and leave it again on its outer surface as efferent vessels (Fig. 2 left half). The latter represent specialized transport vessels.

The microscopic appearance of the interior of the haemolymph nodes does not show any actual distinction between the medullary and cortical zones. The individual follicles are more centrally situated, as in the pigs lymph nodes. In general there is a very clearly outlined lymphatic vascular network injected with ink, ending up with a curved pattern of very fine lymph capillaries in the vicinity of the hilum, which further divide into a sort of honeycomb and apparently represent a labyrinth of tortuous sinuses. Individual larger lymph capillaries make their way peripherally in the trabeculae and divide at the base of the marginal sinus. These lymph capillaries line the marginal sinus in a circular manner, so that there appears to be a completely closed lymphatic circulation. From there more very fine capillaries extend into the capsule. Some of these seem to end there, while other penetrate it and leave the haemolymph node as efferent vessels (Fig. 3), joining the larger lymph vessels which finally empty into the thoracic duct.

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