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# THE HEMOLYMPH NODES OF THE RAT

BY

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In man, and probably in all mammals we find organs which resemble ordinary lymph nodes, but are red and contain blood in the sinus; such organs have been termed hemolymph nodes. Opinions are strongly divergent as to whether these nodes are organs *sui generis* or ordinary lymph nodes in the sinuses of which the red blood cells are present as a result of congestion, stasis, diapedesis, or resorption of extravasates. The structural resemblance of some hemolymph nodes to the spleen and the fact that this type of nodes is lacking afferent lymphatics are the principal reasons why many authors take them to be specific organs, closely related to the spleen. Other investigators regard the hemolymph nodes as modified lymph nodes because gradual transitional structures can be demonstrated between ordinary lymph nodes and hemolymph nodes. The great divergence in the views concerning the nature of the hemolymph nodes is undoubtedly due to the fact that most authors have been inclined to generalize their observations in a single or a few species. The term "hemolymph nodes" can hardly be said to denote an anatomical unity but more likely a common designation for two different forms of organs: (1) hemolymph nodes that are lacking afferent and efferent lymphatics and thus are situated in the blood stream exclusively; (2) hemolymph nodes which have afferent and efferent lymphatics, and are situated in the lymph stream as ordinary lymph nodes. Functionally the hemolymph nodes appear to constitute a unity, as their main function seems to be a phagocytic decomposition of red blood corpuscles (a review with a comprehensive list of literature on the hemolymph nodes has been given by WELLER, 1938).

Among the animals whose hemolymph nodes most frequently have been the subjects of investigation, the white rat holds a prominent place. The regular occurrence of the nodes is evident from works by VINCENT and HARRISON (1897), DRUMMOND (1900),

LEWIS (1902), WEIDENREICH (1902), KELLER (1922), MACMILLAN (1928) and many others. LEWIS failed to find communications between the sinuses of the nodes and neighbouring lymphatic channels, but later investigators have established the presence of lymphatics (HELLY, 1902; WEIDENREICH, 1905; MEYER, 1913; KELLER, 1922). On the other hand there is much difference of opinion in respect to the interpretation of the blood-vascular connections of the sinuses. The description given by various authors of the more detailed structures of the nodes is largely the same. SELYE and FOGLIA (1939), however, state that the hemolymph nodes of the rat do not contain blood, but only pigment-storing phagocytes in their sinuses and reticulum, and that these "iron pigment lymph nodes"—as the two authors propose calling them—are rarely if ever observed in immature animals.

In a fairly comprehensive work on the lymphatic system we have had good opportunities of observing the hemolymph nodes in rats at different ages. As thus we were able to ascertain some interesting features of the hemolymph nodes that have not been reported in the literature, we decided to carry out a systematic microscopic examination of these nodes. The result of this work supplemented with experimental studies on the mechanism by which blood seeps into the sinuses will be reported in what follows.

## Gross Anatomy.

The hemolymph nodes of the rat are situated retroperitoneally in the brown adipose tissue on the posterior abdominal wall between the inferior vena cava and the cranial pole of the kidney. On the left side the nodes are easily accessible, whereas on the right side it is necessary first to detach the liver from its attachment to the kidney and the adrenal. One or two nodes are found on each side. As a rule they are oval, sometimes spherical. The longest diameter varies between 1 and 4 mm. They are slightly flattened with a smooth surface, and do not differ from the ordinary prevertebral lymph nodes (the lumbar lymph nodes and the cisternal group) except by being mottled or red.

This description makes it evident that it is the nodes here mentioned, from one or both sides, that have been the subject of the studies on the hemolymph nodes of the rat cited in the literature. A few authors think that also certain other groups of lymph nodes ought to be classified with the hemolymph nodes. Thus Vincent and Harrison (1897) and Lewis (1902) reported that a group of small hemolymph nodes can be demonstrated in the fold of the peritoneum between the spleen and the stomach; these nodes are very diminutive and often it is impossible macroscopically to distinguish them from ordinary lymph nodes. In some cases we have looked for this group but we have never observed a red colour of the nodes. Keller (1922) states that in a few cases he found some hemolymph nodes along the thoracic vertebrae, and Macmillan (1928) and Selye and Foglia (1939) classify the lymph nodes of the thymus as hemolymph nodes. In our material we have submitted these groups of lymph nodes to a very thorough inspection, but never found them to be mottled or red. Nor have we found on microscopic examination that these nodes differ from the ordinary lymph nodes.

Our investigations include observations made on about 300 albino rats; for details as to the strain of rats, their nutrition and growth, see Andreasen, 1943. As a rule the nodes were dissected out in animals killed by bleeding under ether anesthesia. In many cases we further observed and dissected out the nodes in living animals (under light ether anesthesia) without being able to demonstrate any characteristic differences in the colour of the nodes in living and dead animals.

Our investigations showed that the hemolymph nodes do not appear till the latter half of the first month of life. Prior to this age, it is true, lymph nodes were found corresponding to the location, but microscopically these nodes did not differ from ordinary lymph nodes. Among 17 animals that were from 29 to 31 days old we found that these nodes were definitely red in 16 animals; they were perfectly white only in 1 animal (one of the smallest of the examined animals in the 1 month group).

In all the older animals examined the nodes were constantly mottled or red. In the animals of the 1 month group, one of the poles as a rule was mottled. With advancing age the red areas increase in size (cf. Fig. 1.) so that in the animals of the 1-year and 2-years groups the red colour often was predominant.

The weight of the nodes was recorded in 125 normal animals. The age and sex distribution is given in Table 1.


Fig. 1. Camera lucida drawings of sections of hemolymph nodes from rats of different ages, showing the characteristic development of the blood-filled sinuses with increasing age.

A graphical presentation of the individual weights of the hemolymph nodes is given in Fig. 2; and weight curves are plotted on the basis of the average weights for the age groups examined. From these diagrams it is evident that in the females the growth of the organs is conluded at the age of 2 months, in the male at the age of 4 months, after which the weight of the organs largely appears to remain stationary throughout life. In the males the weight appears to fall slightly towards the age of 2 years, but no reduction in the weight can be demonstrated statistically.

Table 1.

Age and Sex Distribution of Normal Animal Material.

| Age | Designations of groups | Females | Males |
|---|---|---|---|
| 1 month .............. | I | 7 | 9 |
| 2 months .............. | II | 11 | 11 |
| 3 — .............. | III | 12 | 10 |
| 4 — .............. | IV | 13 | 11 |
| 5 — .............. | VI | 9 | 10 |
| 1 year.................. | XXI | 2 | 4 |
| 2 years................. | XXIV | 9 | 7 |
| Total...... | .. | 63 | 62 |

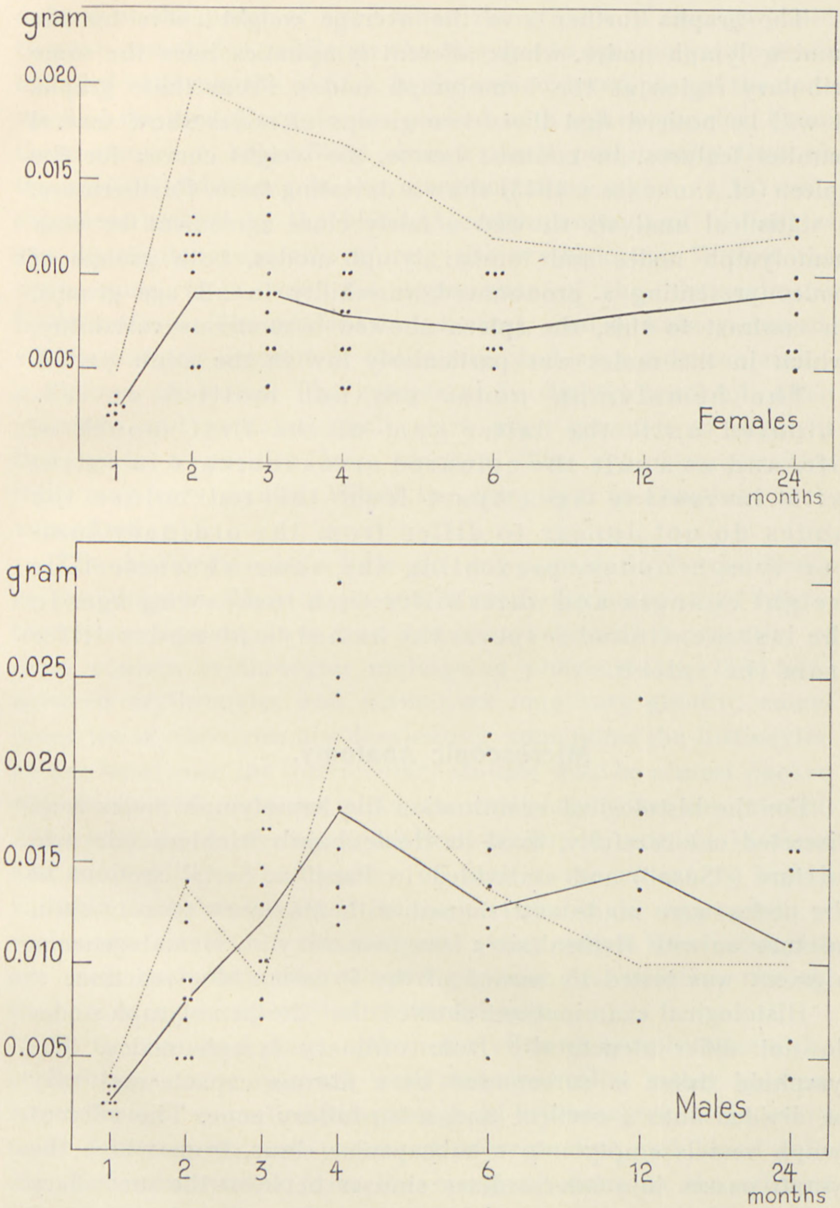


Fig. 2. Graphical presentation of the individual and the average weights of the hemolymph nodes in females and males. The graphs further give the average weight curves for the ordinary lumbar lymph nodes, represented by the dotted lines. The weights of the organs are plotted as ordinates while the age groups are plotted along the axis of abscissa.

The graphs further give the average weight curve for the lumbar lymph nodes, whose afferent lymphatics have the same tributary region as the hemolymph nodes. From these graphs it will be noticed that these two groups of nodes show several parallel features. In contrast hereto, the weight curves for the spleen (cf. ANDREASEN, 1943) show a deviating form. Furthermore, a statistical analysis showed a fairly close agreement between hemolymph nodes and lumbar lymph nodes, both groups of nodes presenting a pronounced variability in all age groups. In contrast to this, the spleen showed a moderate variability, which in the males was particularly low in the adult period.

The hemolymph nodes are not mottled or red-coloured until the latter part of the first month of life, and as a rule the coloured areas increase in extent with increasing age. Apart from the red colour the nodes do not appear to differ from the ordinary lumbar lymph nodes, presenting the same characteristic weight changes and variability with increasing age. In the last mentioned respects the hemolymph nodes differ from the spleen.

## Microscopic Anatomy.

For the histological examination the hemolymph nodes were dissected out carefully, fixed in Heidenhain's trichloracetic acid mixture ("Susa") and embedded in Paraffin. Serial sections of the nodes were made and stained with Maximow's azure-eosin mixture or with Heidenhain's iron hematoxylin. Hematogeneous pigment was tested by means of the Prussian-blue reaction.

Histological examinations showed that the hemolymph nodes do not differ structurally from ordinary lymph nodes. The lymphoid tissue is surrounded by a fibrous capsule and may be divided into a cortical and a medullary zone. The afferent lymph vessels empty into a subcapsular sinus, from which the lymph passes into intermediary sinuses between the medullary cords and from here into the terminal sinus at the hilus of the node and then into the efferent lymph vessels.

For the description of the features characteristic of the hemolymph nodes we shall pick out a typical node removed from an animal at the point of time when the growth of the lymphoid tissue is concluded, while no changes due to age can yet be made out (3rd—4th months of life). In the red-coloured part of the node we find the medullary sinuses packed with red corpuscles. From these sinuses a blood-filled sinus extends peripherally through the cortical substance to the marginal sinus which is to some extent permeated by the blood. A certain amount of histiocytes are distributed regularly among the erythrocytes. Within the same node, as a rule, the appearance of the histiocytes is fairly uniform. The nucleus is spherical, oval or kidney-shaped, with a varying amount of chromatin, and as a rule containing a distinct nucleolus. The cytoplasm is slightly basophil, often greenish, and contains small, dark-green granules of pigment that gives a positive iron reaction. The cytoplasma-nuclear ratio may vary greatly. When the cytoplasm is abundant it is vacuolized.

The histiocytes as a rule do not appear to be markedly erythrophagous. They may contain moderate amounts of hematogeneous pigment, it is true, but as a rule only a minority of them contain erythrocytes undergoing phagocytosis. The ratio between erythrocytes and histiocytes may vary greatly; sometimes the erythrocytes are dominating, sometimes the histiocytes. In the latter case the intermediary sinuses may be almost packed with histiocytes, between which the erythrocytes are compressed. As a rule the erythrocytes are well-preserved, normal in form and stainability. The density of their arrangement is highly variable from well-defined solitary elements to aggregates of eosinophil masses. In the marginal sinus only a few histiocytes are seen, and they may contain pigment, but seldom; nor do they show any evidence of erythrophagy.

In the terminal sinus and in the efferent lymph vessels the erythrocytes as a rule are well preserved. One rather gets an impression of a continuous passage of erythrocytes, as in every node sinuses with well preserved, close-packed, red blood cells can be demonstrated from the marginal sinus to the efferent lymph vessels. The efferent lymphatics often contain a few histiocytes that appear to be carried along by the lymph stream. In the bends of the blood-filled intermediary sinus one now and then has the impression of a marked retardation or complete cessation of the flow; here, as a rule, the erythrocytes are relatively scanty, often sticking to the histiocytes in a rosette pattern, often in close relation to mast cells that have migrated into the sinus from the adjacent lymphoid tissue. Further the pigmentation of the histiocytes is farther advanced here than in the other parts of the sinus.

The lymphoid tissue that is in direct contact with the blood-filled sinus shows a characteristic differentiation, as it is made up of plasma cells—in contrast to the rest of the lymphoid cortex in which plasma cells are not represented.

The microscopic picture of the hemolymph nodes presents such characteristic changes with increasing age that it is practicable from the histological examination of the nodes with a fair degree of accuracy to determine to which age group the animal belongs.

In infantile animals—in the first two weeks of life—red or mottled nodes, as mentioned, were never observed. Microscopy of the renal lymph nodes will now and then reveal a few erythrocytes in the marginal or intermediary sinuses, and they may stick to the histiocytes in a rosette-like pattern. Otherwise, no evidence of erythrophagy is seen; nor do the histiocytes contain any hematogeneous pigments. The presence of a few erythrocytes in the sinuses is not characteristic of the renal lymph node and is seen just as often in the other lymph nodes of the rat. In older infantile rats (about 1 month old) blood-filled sinuses in a small area of the node are practically always found. The blood admixture is not massive, and as a rule the histiocytes make up the dominant component in the blood-filled sinus. Erythrocytes and histiocytes are rather close-packed. The histiocytes are large, with light basophil protoplasm, in which a few granules of pigment are found, although exceptionally. Most of the specimens show no evidence of erythrophagy, but in a few of them some histiocytes are seen to contain red cells. Free erythrocytes are found in the terminal sinus and efferent lymphatics, so there can be no doubt that a flow of blood takes place.

In somewhat older animals (2 months old) the blood admixture to the sinuses becomes more abundant. The erythrophagy has become more lively, and the number of pigment-containing histiocytes is highly increased. The histological picture does not appear to deviate from that encountered in full-grown young animals (3—4 months), as described on page 9.

Increasing age is now associated with a shift in the histological picture as the number of blood-carrying sinuses increases progressively, and this also applies to the pigment contents of the histiocytes (cf. Fig. 3). In animals 6 months old, histiocytes contain red blood cells in various phases of disintegration; the pigment contents may now be further increased so that the cells in unstained sections appear as markedly yellowish-green or brownish, the protoplasm being filled with close-packed golden granules or drops of varying size. With the increasing accumulation of pigment (Plate 1, fig. 6), which is observed especially in animals of 1 and 2 years, the nuclei become pycnotic, eccen-

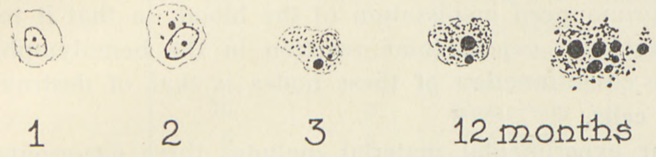


Fig. 3. Diagrammatic sketches of pigment cells showing the progressing accumulation of pigment with increasing age.

tric, and the protoplasm is increased markedly, giving the cell body an irregular outline. Now the cell is often surrounded by extracellular pigment, undoubtedly given off by the pigment-containing cells. In the pigment cells the nuclei seem finally to disappear, the cells being represented merely by a large aggregate of pigment. In sections stained by the method of Turnbull the pigment gives a positive reaction for iron; the blue colour may vary from light blue to bluish-black. Exceptionally a few phagocytes are seen to contain large granules of pigment that gives no positive reaction for iron. In contrast to Selye and Foglia (1939) we always found red blood cells in he pigment-storing phagocytes.

In general structure and arrangement of lymphoid tissue the hemolymph nodes resemble the ordinary lymph nodes and they are only characterized by the presence of erythrocytes and erythrophages in the sinuses. With increasing age, then, the number of sinuses containing blood increases, and this applies also to the amount of pigment taken up by the erythrophages, so that hematogeneous pigment may be found extracellularly after the 6th month of life.

## The Hemolymph Nodes during Inanition.

Apparently the changes of the hemolymph nodes during inanition have not been investigated previously. Still, a paper by Retterer (1902) is of some interest in this connection, as he mentions that the sinuses of the ordinary lymph nodes in the cat under normal conditions are filled with erythrocytes; after starvation, however, very few erythrocytes are seen in the sinuses, and then these erythrocytes are small and deformed. Further, we were led to take up this question as we have observed that the hemolymph nodes of animals died of starvation were often found to be quite pale. Besides, inanition is associated with pronounced inspissation of the blood, so that it might be reasonable to expect some reaction in the hemolymph nodes, as the chief function of these nodes is that of destroying red blood cells.

Our experimental material includes three age-groups (1, 3 and 12 months old) so that the hemolymph nodes were examined at 3 different stages: (1) at a time when the rate of growth of the nodes is very great, (2) when the lymphoid tissue has reached its maximum development, and (3) when the tissue has already long been undergoing involution due to age.

In all three age groups we found the hemolymph nodes atrophied in the same degrees as seen in ordinary lymph nodes; after food supply regeneration takes place apparently at the same rate as in the ordinary lymph nodes. In the infantile animals the hemolymph nodes have become completely white after extreme inanition (3 days), while in the older animals the red colour usually was visible even though the atropic nodes did not show the lively red colour seen in the control animals.

The microscopic examination includes serial sections of hemolymph nodes from a group of animals, 3 months old, which had been submitted to inanition of varying duration. A survey of this material, with notes on the blood contents of the sinuses is given in Table 2.

From this survey it is evident that the amount of erythrocytes in the sinuses decreases during starvation so that after extreme starvation the red blood cells have disappeared, only a remnant of them often persisting in the form of eosinophil

Table 2.
Survey of Blood Admixture in Sinuses during Inanition and Restitution Periods.

| Animal No. | Inanition period in days | Restitution period in days | Erythrocytes in sinuses |
|---|---|---|---|
| III, 31 | 5 | .. | Numerous |
| III, 32 | 7 | .. | Numerous |
| III, 33 | 7 | .. | Few |
| III, 34 | 9 | .. | None, but very scanty eosinophil aggregates |
| III, 35 | 10 | .. | None, but eosinophil aggregates |
| III, 36 | 7 | 2 | None, but eosinophil aggregates |
| III, 37 | 10 | 3 | Numerous |
| III, 38 | 8 | 6 | Numerous |
| III, 39 | 8 | 8 | Numerous |
| III, 40 | 10 | 10 | Numerous |

aggregates; during the restitution period the erythrocyte supply to the sinuses soon appears to be established again. In all phases of atrophy and regeneration the sinuses present numerous pigment-containing phagocytes (cf. Plate 2).

During starvation, then, the hemolymph nodes undergo atrophy—just like the ordinary lymph nodes. The erythrocyte supply to the sinuses decreases gradually and finally it ceases completely, and the hemolymph nodes come to look like ordinary lymph nodes—except for the presence of pigment-containing histiocytes in the formerly blood-filled sinuses.

## How does Blood Seep into the Sinus?

The question as to how the blood gets into the sinuses of the hemolymph nodes in the rat has been the subject of several investigations. These works were based on injection into blood vessels—which will always imply some risk that the injection pressure may cause ruptures of the vessels.

Lewis (1902) states that arterial injection fills the sinuses of the hemolymph nodes with injection fluid, whereas they are filled but partly or not at all by venous injection; and he claims that small arteries open directly into the sinuses. He further states that the injection mass may be found also in the sinuses of ordinary lymph nodes, but he is unable to decide whether there is any communication between arterial capillaries and the sinuses, or whether the delicate capillary walls may have ruptured.

In contrast, Helly (1902) stated that by injection into the nodes he had been able to ascertain a complete separation between the blood vessel system and the lymph vessel system, and that the former is not regularly in direct connection with the sinuses of the hemolymph nodes.

On arterial injection through the aorta, Keller (1922) observed that certain parts of the hemolymph nodes were constantly filled with the injection mass which entered the sinus from a thin-walled vessel resembling an afferent lymph vessel. According to Keller, this vessel was no vein that had been ruptured by the injection pressure and thus put in communication with the sinus; for also after vital injection India ink was sometimes found free in the sinuses. On the basis of his experiments Keller concludes that the blood is brought to the hemolymph nodes by way of lymph vessels that are in rather intimate connection with the blood vessel system; but he does not mention how this connection is established.

To us it seems more obvious to associate the admixture of blood in the sinuses with the lymphaticovenous communications which in many mammals and in man can be demonstrated retroperitoneally in the lumbar region. Such communications have been described also in the rat by Job (1915), who says (p. 452): "On the left side the number of lymph vessels leading from the lumbar node may vary from one to four, or form a network, depending somewhat on the mode of attachment with the right lymph vessel. However, all the vessels lead along the left side of the vena cava, in any case. If there is only one vessel, it will open into a single node, just anterior to the left renal vein, from which a branch is given to the renal vein, and one to the group of single nodes lying to the left of the cisterna chyli. If there be more than one lymph vessel leaving the lumbar node, some one of them will enter the renal node, the rest may join the cisterna group, the cisterna directly, the renal vein directly, or any combination thereof. The latter conditions are fewer than the single vessel method."

In a subsequent paper (1918) Job says (p. 469) that "barely 8 per cent of the material showed renal-vein communications that could be satisfactorily demonstrated. In a large number of cases a lymphatic vessel branched off the main system about 1 cm. posterior to left renal vein and outward toward the hilus of the kidney, but only occasionally was it possible to demonstrate its connection with the vein." Strange to say, Job does not mention that the lymph nodes he designates as the renal nodes show a red colouring and are hemolymph nodes, and hence he does not associate this red colouring with the special vascular communications applying to the lymphatics of these nodes.

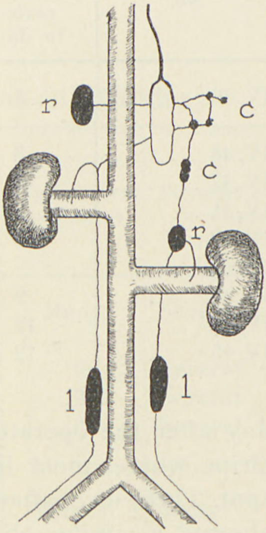

Fig. 4. The lymphatico-venous communications in the renal region (after Job, 1915). c: Cisternal lymph nodes, *l*: Lumbar lymph nodes, *r*: Renal hemolymph nodes.

From Job's reports it seemed obvious to assume that the blood supply to the sinuses of the hemolymph nodes might take place through reflux from the lymphatic that connects the lymph node with the renal vein (cf. Fig. 4).

This connection may be severed by excision of the kidney after binding its stalk. We therefore performed left-sided nephrectomy on several rats, about 4 months old, and examined the homolateral hemolymph node a varying number of days after the operation. After inspection *in situ*, the hemolymph node was dissected out, fixed and later examined microscopically in serial sections. A survey of the material and finding is given in Table 3.

From Table 3 it is evident that the hemolymph node on the operated side loses its characteristic red spots. Already one

Table 3.
Left Renal Hemolymph Nodes after Removal of Kidney.

| Animal No. | Duration of post-operative period in days | Macroscopic appearance of the hemolymph node | Erythrocytes in sinuses | Pigment cells in sinuses |
|---|---|---|---|---|
| IV, 46 ........ | 1 | Amber-coloured stripe | Few | Many |
| IV, 48 ........ | 3 | » » | 0 | Many |
| IV, 47 ........ | 3 | Quite pale | 0 | Many |
| IV, 41 ........ | 7 | » » | 0 | Very few |
| IV, 42 ........ | 9 | Narrow reddish-yellow ring | 0 | Several |
| IV, 43 ........ | 9 | Quite pale | 0 | Few |
| IV, 44 ........ | 12 | » » | 0 | Few |
| IV, 45 ........ | 12 | » » | 0 | Very few |

day after the operation it presented merely an amber-coloured stripe where, most likely, prior to the operation it had a red spot. Three days after the operation one of the examined animals showed a similar change in colour, while in the other animal (IV, 47) the node was now quite pale, looking exactly like the ordinary lumbar lymph nodes (Plate 3, fig. 9). In the animals examined a longer time after the operation (7, 9, 12 days) the red colour likewise had disappeared (Plate 3, fig. 10). The hemolymph nodes on the operated side seemed normal in size, form, and consistence, so that the operation appeared to have had no effect on the node except for the change in colour. The contralateral hemolymph node was mottled and the red areas did not appear to be more extensive than usual in animals of this age-class.

Examination of the serial sections of the left node showed the sinuses to be empty of erythrocytes; only in one animal, killed on the day after the operation, a few erythrocytes were still left. Otherwise the histological picture did not deviate from the normal. Mitotic figures were seen in the lymphoid tissue, and there was no suggestion whatever of any damage to the node from the operation. The contralateral hemolymph nodes showed blood-filled sinuses and neither qualitative nor quantitative evidence of changes could be demonstrated on microscopic examination.

This experimental series was later supplemented by another —this time with employment of younger animals (about 2 months old). The results are presented schematically in Table 4.

Table 4.
Left Renal Lymph Node after Removal of Kidney.

| Animal No. | Duration of post-operative period in days | Macroscopic appearance of hemolymph node | Erythrocytes in sinuses | Pigment cells in sinuses |
|---|---|---|---|---|
| II, 100 ........ | 2 | Pink stripe | Many | Several |
| II, 103 ........ | 3 | Quite pale | .. | .. |
| II, 105 ........ | 5 | » » | 0 | Several |
| II, 107 ........ | 6 | » » | 0 | Several |
| II, 101 ........ | 6 | Yellow marmorate spot | 0 | Several |
| II, 106 ........ | 7 | Quite pale | .. | .. |
| II, 102 ........ | 7 | Rust-coloured spots | Several | Many |
| II, 108 ........ | 8 | Quite pale | 0 | Few |
| II, 104 ........ | 9 | Rust-coloured spot | A few | Many |
| II, 109 ........ | 10 | Quite pale | 0 | Very few |

On the whole, the results obtained in this series agreed very well with those obtained in the first series though here they were hardly as clear-cut as in the first series. Two of the animals, killed respectively 7 and 9 days after the operation, still contained erythrocytes in the sinuses; the number of erythrocytes, however, had decreased considerably.

So the outcome of these experiments has been that the supply of blood to the sinuses can be interrupted by nephrectomy, probably because a reflux from the renal vein through an efferent lymph vessel is rendered impossible. The correctness of this view is further rendered probable by the fact that we have been able in infantile animals with perfectly pale hemolymph nodes by cautious compression of the renal vein in the living animal (under light ether anesthesia) to produce a red colour of some parts of the node. Subsequent microscopic examination of the node showed the sinus to be partly filled with erythrocytes, and the appearance of the node corresponded to that of the hemolymph node in somewhat older infantile animals. As the venous pressure rises on compression of the renal vein, the supply of blood to the sinuses may conceivably result from a rupture of one of the veins of the hemolymph node. So, naturally, we do not consider this compression as an *experimentum crucis*.

For that matter, certain objections may be raised to the interpretation of our experiments with nephrectomy. Thus one might conceive that the lymphatics of the hemolymph nodes drain the kidneys, and that red cells are present in the renal lymph; *a priori*, this possibility cannot be excluded, but it is still improbable that lymph from the kidneys should contain such a large amount of blood cells, for in the sinuses the erythrocytes are so closepacked that their mixing with lymph has to be regarded as out of the question. Previous authors who have investigated the renal lymph have not mentioned that it was mixed with blood cells (Schmidt and Hayman, 1929—30; Drinker and Field, 1931).

Further, one might think that ligation of the stalk of the kidney would involve also the small arteries which, according to Lewis, carry blood to the sinuses of the node; but this is rather a theoretical proposition. For Helly, as mentioned, has shown that such a connection between the sinuses and the circulatory system does not exist, and the studies reported by Keller show that blood seeps into the sinus through a thin-walled vessel that cannot be an artery.

A universal vascular effect produced by unilateral nephrectomy that would prevent a possible diapedesis to the sinuses is also improbable, as the contralateral hemolymph node shows normal red colour and a normal amount of erythrocytes in the sinuses.

The question whether the blood is conveyed to the sinuses jerkily or as a continuous stream is closely connected with that of the mechanism of the supply of blood to the sinuses. A cyclic function has been attributed to the hemolymph nodes by several observers (e. g., LEWIS, 1902; KELLER, 1922). This view has been based on the fact that different appearances have been noticed in nodes taken from the same positions suggesting different phases of activity. KELLER further states that the varying ratio between macrophages and free blood cells from the convexity of the node to the hilus might lend support to such a view. Thus, according to KELLER, the macrophages usually fill the intermediary sinus completely, whereas the free erythrocytes are predominant in the marginal sinus.

In contrast to previous assumptions, we think that our observations plainly show that the blood is conveyed to the node continuously. Macrophages are always rather scarce in the marginal sinus, it is true, but this is only natural as the phagocytic potency of the reticulo-endothelium under all conditions has proved to be lower in the marginal sinus than in the intermediary one. In the intermediary sinus the density of macrophages is the same peripherally and centrally, and on the whole the content of blood pigment in the cells is also the same peripherally and centrally in the same node. The plasma cell reaction of the lymphoid tissue is strongly suggestive of a continuous flow of blood through the same parts of the node; and only in exceptional instances have we found pigment cells in other parts of the node signifying that the sinus has previously contained erythrocytes.

Thus it seems safe to sum up the outcome of these experiments as follows:

By unilateral nephrectomy it is practicable to stop the supply of blood to the sinuses of the homolateral hemolymph node, thus making it impossible macroscopically and microscopically to distinguish this node from an ordinary lymph node. It seems most likely that the blood is conveyed to the renal hemolymph node by reflux through an efferent lymph vessel that opens into the renal vein. Microscopic findings seem to indicate that the blood is conveyed to the sinuses of the hemolymph node continuously.

## How Long do Erythrophages Stay in the Hemolymph Nodes?

Whether the erythrophages are cells with a short lifetime that are carried away by the lymph stream immediately after phagocytosis and destruction of the red blood cells, or whether they remain and keep functioning in the hemolymph node for a considerable length of time—perhaps throughout the lifetime of the individual—is a question about which it is difficult *a priori* to form any opinion. On the basis of the afore-mentioned (p. 11) age changes in the pigment content of the erythrophages, one might perhaps be inclined to think that here we meet with a progressive development of pigment within the same cell, commencing intracellularly and terminating extracellularly.

The question as to how long the erythrophages stay in the sinuses of the hemolymph nodes has been elucidated, however, through the above examination of the hemolymph node after nephrectomy. For here we found that when the supply of blood to the sinuses ceased a disappearance of the pigment cells might be observed at the same time (Plate 3). In Tables 3 and 4, in one of the columns a rough estimate is made of the number of pigment cells in the sinus. About one week after the nephrectomy, most of the pigment cells had disappeared, and 10—12 days after the operation the sinuses are wide, empty and almost completely rid of erythrophages. In a couple of the animals (II, 102 and II, 104) the sinuses still contained many pigment cells as late as 7 and 9 days after the nephrectomy. Thus individual variations may assert themselves—above all, probably with regard to the rate of flow of the lymph and thus the liberation of the pigment cells.

The increasing pigment content of the erythrophages with advancing age therefore indicates an increasing erythrophagy in the hemolymph nodes, even though it cannot be excluded that the slower lymph flow with advancing age may contribute to prolonging the stay of the erythrophages in the sinuses, which implies the possibility of a more prolonged erythrophagic function.

After unilateral nephrectomy, which stops the supply of blood to the sinuses in the homolateral hemolymph node, the number of pigment cells in the sinuses decreases rapidly, indicating that the function of the erythrophages in hemolymph nodes and other lymph nodes is of brief duration.

## Are the Hemolymph Nodes of the Rat Organs "*sui generis*"?

In conclusion, on the basis of our own studies and the reports in the literature we shall now try to place the hemolymph nodes of the rat systematically. The statements made below apply only to the hemolymph nodes of the rat and we take no stand on the systematics of the hemolymph nodes in other species beyond emphasizing that the term "hemolymph nodes" can hardly be said to denote an anatomical unity and that generalization from one species to another is not justifiable till thorough studies have been carried out on a fairly large number of species.

The following facts show how closely the hemolymph nodes are related to the ordinary lymph nodes. Morphologically the hemolymph node of the rat thus does not differ from the ordinary lymph nodes except for a characteristic red colour and the presence of erythrocytes and erythrophages in the sinuses. Like the other lymph nodes, the afferent lymphatics belonging to the hemolymph nodes drain a skin area, the lumbar region. This was stated already by Keller (1922); and by subcutaneous injection of India ink in this region we have been able to ascertain that the India ink was found distributed in the sinuses of the hemolymph nodes just as in the other lymph nodes. After subcutaneous injection of India ink in the hind legs we have likewise been able to demonstrate this substance in the hemolymph nodes (as well as in the popliteal and lumbar nodes)—which indeed was to be expected after Job's establishment of the lymphatic trunks. Consequently, also the hemolymph nodes are situated in the lymph stream passing from the hind leg to the cisterna chyli, and thus they do not differ in this respect either from the ordinary lymph nodes located on the posterior abdominal wall (lumbar lymph nodes).

The hemolymph nodes appear to be closely related to the lumbar lymph nodes also in other respects. Thus changes with advancing age, variability and changes in weight during inanition show a close relationship between the two groups of lymph nodes. On the other hand, on comparison of the hemolymph nodes with the spleen, to which an erythrocyte-destroying function is generally attributed, we find no such conspicuous relationships, and the variability of the two organs within various age-groups is even quite deviating in several respects.

The close relationship with the ordinary lymph nodes manifests itself also in other respects. Thus the hemolymph nodes develop from quite ordinary lymph nodes, only that blood is conveyed to their sinuses in the latter half of the first month of life; before that time the hemolymph nodes do not differ in any respect from ordinary lymph nodes. Later in life the red colour of the hemolymph nodes is constant, it is true, but it is practicable in young animals by starvation to transform hemolymph nodes into ordinary lymph nodes in so far as the admixture of blood to the sinuses may be checked completely by extreme inanition, so that only the presence of pigment cells in the sinuses indicates the previous function of erythrophagy. Further, it is possible also by unilateral nephrectomy to transform the homolateral hemolymph node to an ordinary lymph node as erythrocytes and pigment cells after the operation may disappear so completely that the node cannot be distinguished from the adjacent lumbar lymph nodes—neither macroscopically nor microscopically. Conversely, Selye and Foglia (1939) found that hemolymph nodes may be produced experimentally in the rat following exposure to damaging agents capable of eliciting an "alarm reaction" (excessive muscular exercise, exposure to cold, and toxic doses of formaldehyde).

Considering the question on the systematics of the hemolymph nodes, it will further be of importance to settle whether there exists a continuous series of intermediate forms between hemolymph nodes and ordinary lymph nodes. If such types really exist, it will only be reasonable to hold that the hemolymph nodes are not entitled to a special classification, as the possibility for transition from one form to another under normal conditions then would be present continually. For the elucidation of this question we are able to state that the ordinary lymph nodes in the normal animals never show any red colour like the hemolymph nodes. The presence of free erythrocytes and erythrophages in the sinuses of ordinary lymph nodes is a frequent finding—but never in such massive accumulation as in the hemolymph nodes. In our opinion, the constant location of the hemolymph nodes, their characteristic changes with advancing age and the pronounced cellular erythrocyte destruction render it justifiable to maintain the term "hemolymph node".

We shall not here enter into the capacity of the erythrophagic function of the hemolymph nodes as compared to that of the other blood-destroying organs, but merely state that undoubtedly large amounts of erythrocytes are decomposed daily in the hemolymph nodes of the rat. This is suggested strongly by the rapid disappearance of the numerous pigment cells from the sinuses of the hemolymph nodes after unilateral nephrectomy. Studies reported by Drinker, Field, and Ward (1934) lend support to this view. By perfusion of an ordinary popliteal lymph node from a dog with an autogeneous erythrocyte suspension in heparinized plasma and examination of the lymph collected from the efferent lymphatics, these investigators found that the filtration had been fairly complete. The morphological features observed in the course of the blood destruction in this experiment appear to correspond completely to the normal picture of the activity of the hemolymph nodes in the rat. It is evident also from papers by Müller (1879) and Keller (1922) that the phagocytic activity of the lymph nodes is very great during resorption of blood extravasates.

In our opinion the term hemolymph nodes should be used for the renal lymph nodes of the rat notwithstanding the fact that they are derived from ordinary lymph nodes and that it is possible to reverse them into the ordinary lymph node type. We think this view is justified by the constancy of the occurrence of the hemolymph nodes, the characteristic changes with advancing age and the special function of the nodes: a pronounced cellular destruction of erythrocytes.

## Summary.

The hemolymph nodes of the rat belong to the group of hemolymph nodes, which have afferent and efferent lymphatics, and are situated in the lymph stream as ordinary lymph nodes. They are not mottled or red until the latter part of the first month of life, and as a rule the coloured areas increase in extent with increasing age. Apart from the red colour the appearance of the nodes does not differ from that of ordinary lumbar lymph nodes, and the nodes present the same characteristic weight changes and variability with increasing age as the ordinary lymph nodes. In the last mentioned respects the hemolymph nodes differ from the spleen.

In general structure and arrangement of lymphoid tissue the hemolymph nodes resemble the ordinary lymph nodes and they are only characterized by the presence of erythrocytes and erythrophages in the sinuses. With increasing age the number of sinuses containing blood increases and this also applies to the amount of pigment ingested by the erythrophages, so that hematogeneous pigment may be found extracellularly after the 6th month of life.

During inanition the hemolymph nodes undergo atrophy— just like ordinary lymph nodes. The erythrocyte supply to the sinuses decreases gradually and finally it ceases completely, and the hemolymph nodes côme to look like ordinary lymph nodes— except for the presence of pigment-containing histiocytes in the formerly blood-filled sinuses. By unilateral nephrectomy it is also practicable to stop the supply of blood to the sinuses of the homolateral hemolymph node, thus making it impossible macroscopically and microscopically to distinguish this node from an ordinary lymph node.

It seems most likely that the blood is conveyed to the renal hemolymph node by reflux through an efferent lymph vessel opening into the renal vein. Microscopic findings seem to indicate that the blood is conveyed to the sinuses of the hemolymph node continuously.

After unilateral nephrectomy the number of pigment cells in the sinuses of the homolateral hemolymph nodes decreases rapidly, which indicates that the function of the erythrophages in these nodes and probably also in ordinary lymph nodes is of brief duration.

On the basis of these studies and reports in the literature we feel justified in maintaining the term hemolymph nodes for the renal lymph nodes of the rat notwithstanding the fact that they are derived from ordinary lymph nodes and that it is possible to reverse them into the ordinary lymph node type.

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Only after this paper went to press we have had an opportunity to go through the English and American literature, published during the war, on the subject dealt with here. SELYE and SCHENKER (J. Anat. **73**: 413—415, 1939) have succeeded in reverting hemolymph nodes to the ordinary lymph node type by homolateral nephrectomy, especially when combined with adrenalectomy. If these operations were performed in the immature rat before the transformation of the renal lymph node into an "iron pigment lymph node", they prevented the occurrence of such a transformation. It appears, therefore, that in the absence of these glands, the node discontinues its blood-destroying activity but remains different from other lymph nodes in that it contains no germinal centres. LASNITZKI and WOODHOUSE (J. Anat. 78: 121—129, 1944) after subcutaneously administrations of 1:2:5:6: dibenzanthracene found that many of the lymph nodes of the animals (rats) were transformed into more or less pronounced hemolymph nodes.

Indleveret til Selskabet den 2. Juli 1945.
Færdig fra Trykkeriet den 27. April 1946.

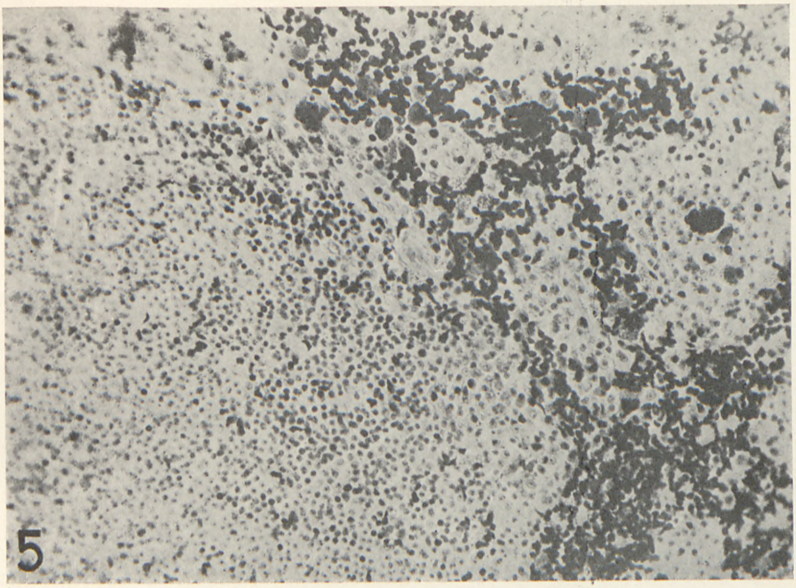

Fig. 5. Microphotograph. Cortical substance and blood-filled intermediary sinuses of a hemolymph node. The histiocytes are regularly distributed among the erythrocytes. From a normal animal, 1 year old. Heidenhain's iron hematoxylin. × 250.

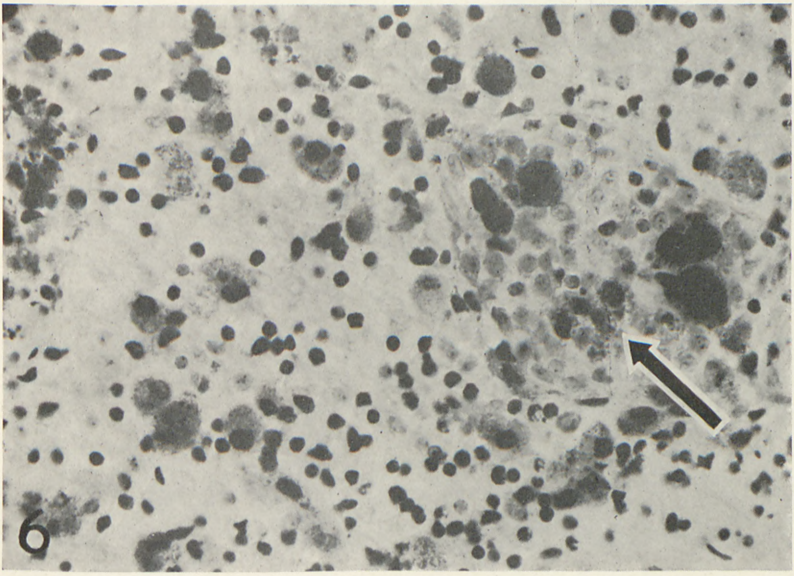

Fig. 6. Microphotograph. Intermediary sinus from the same node as in Fig. 5. The histiocytes are filled with hematogenous pigment; extracellular pigment is seen in the medullary cord on the right Heidenhain's iron hematoxylin. Hom. imm. 1/7. Oc. 5. Zeiss. × 450.

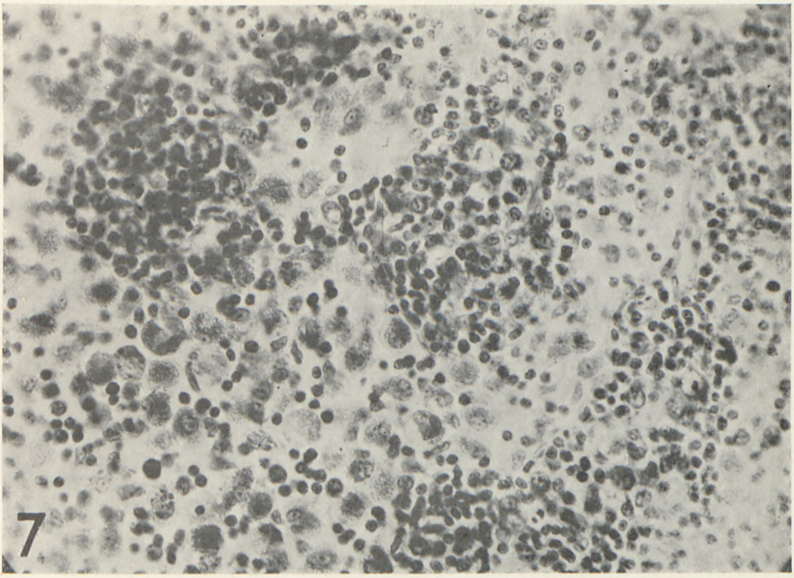

Fig. 7. Microphotograph. Intermediary sinus from an animal which had been submitted to inanition for nine days (III, 34). The red blood cells have disappeared, the pigment-containing histiocytes persist. Maximow's azure-eosin. Hom. imm. $^{1}/_{7}$. Oc. 5. Zeiss. $\times$ 450.

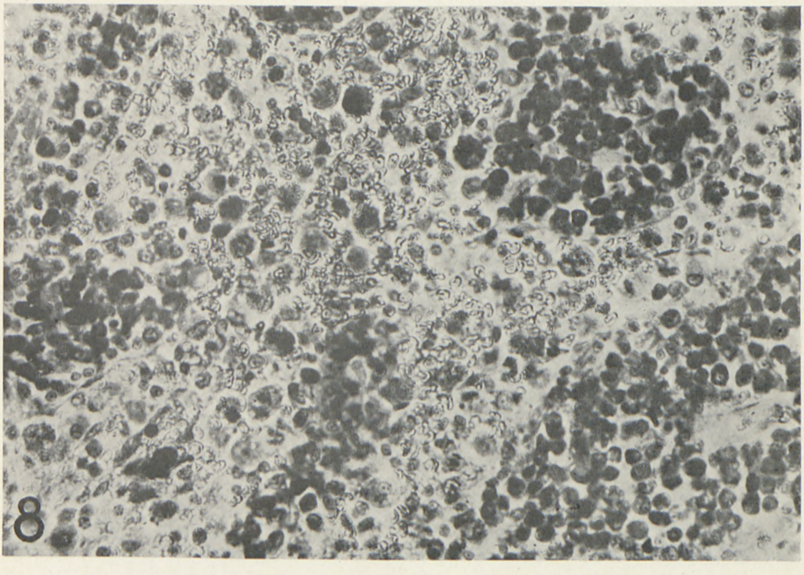

Fig. 8. Microphotograph. Intermediary sinus from the control animal. The sinus packed with erythrocytes and pigment cells. Maximow's azure-eosin. Hom. imm. $^{1}/_{7}$. Oc. 5. Zeiss. $\times$ 450.

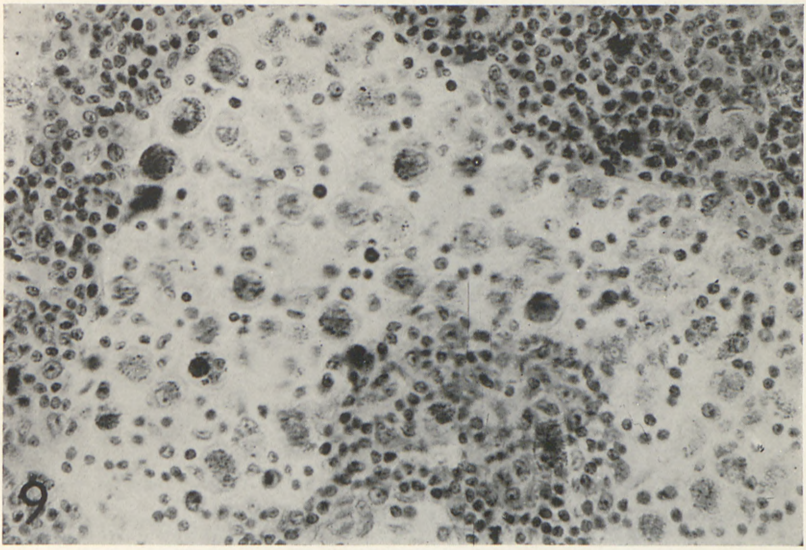

Fig. 9. Microphotograph. Intermediary sinus of a hemolymph node from an animal three days after unilateral nephrectomy (IV, 47). The sinuses are empty of erythrocytes, but they still contain many pigment cells. Maximow's azure-eosin. Hom. imm. $^1/_7$. Oc. 5. Zeiss. × 450.

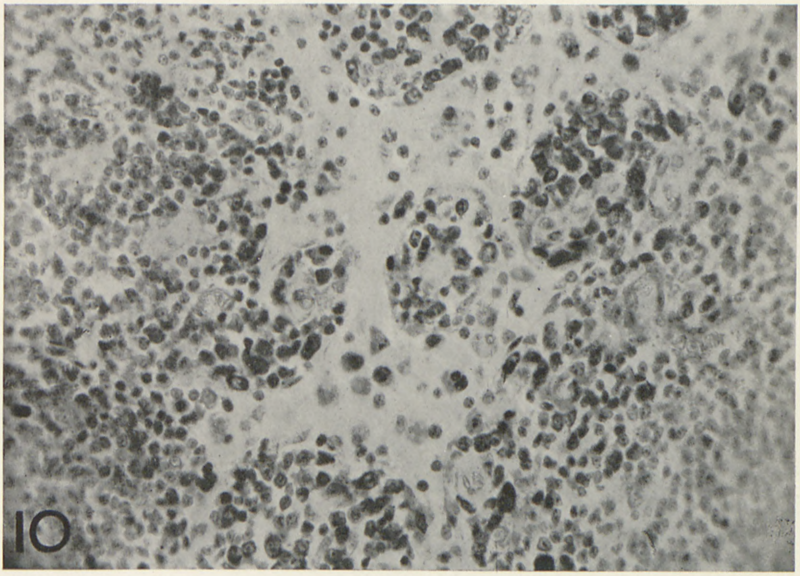

Fig. 10. Microphotograph. Intermediary sinuses of a hemolymph node from an animal twelve days after the operation (IV, 45). The sinuses are completely rid of erythrocytes and pigment cells. Maximow's azure-oesin. Hom. imm. $^1/_7$, Oc. 5. Zeiss. × 450.