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Changes in the cellular architecture of a lymph node after blocking its lymphatic circulation

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INTRODUCTION

The lymph node has a dual circulation and the maintenance of its cellular architecture depends upon both its blood and lymph circulation. According to Holman & Self (1938) and Tilak & Howard (1964), the popliteal lymph node of dogs may survive *in situ* even after complete blockage of blood supply, as long as lymph flow through the node is maintained. However, our earlier experiments on the popliteal lymph node in the rabbit (Osogoe & Courtice, 1968) have demonstrated that lymphatic circulation alone is not sufficient to sustain viability of the node, and that it merely accelerates regeneration of lymphatic tissue. Subsequent experiments revealed that complete blockage of the lymphatic circulation, without interrupting the blood supply, does not interefere with viability of the node, but that it does produce profound alterations of its cellular architecture. The results of these experiments are reported here.

MATERIALS AND METHODS

A total of forty-nine rabbits (New Zealand White) of both sexes and weighing more than 1·5 kg were used. All operations were performed on the popliteal lymph node using sterile technique under general anaesthesia induced with Nembutal and maintained with ether. For visualization of the lymphatics, Evans blue dye was injected subcutaneously into the hind footpads.

The anatomy of blood and lymph vessels of the popliteal lymph node in the rabbit was described in an earlier paper (Osogoe & Courtice, 1968). The main artery and vein of the node furnish large branches to the adjacent muscles (the semimembranosus and biceps femoris) and several small ones to the perinodal fat and overlying fascia before they enter or leave the hilum. The afferent lymphatics (three or four) course along the superficial vein under the skin before entering the node, while the efferent lymphatic (usually one, occasionally two or three) runs closely with the nodal artery and vein after leaving the hilum. For this reason, it is difficult to ligate the efferent lymphatic separately near the node. In a number of earlier experiments, therefore, the efferent lymphatic was blocked at the groin, where it courses along the femoral artery and vein and can be tied separately with ease. However, it was further revealed that blockage of the main artery and vein, together with the efferent lym-

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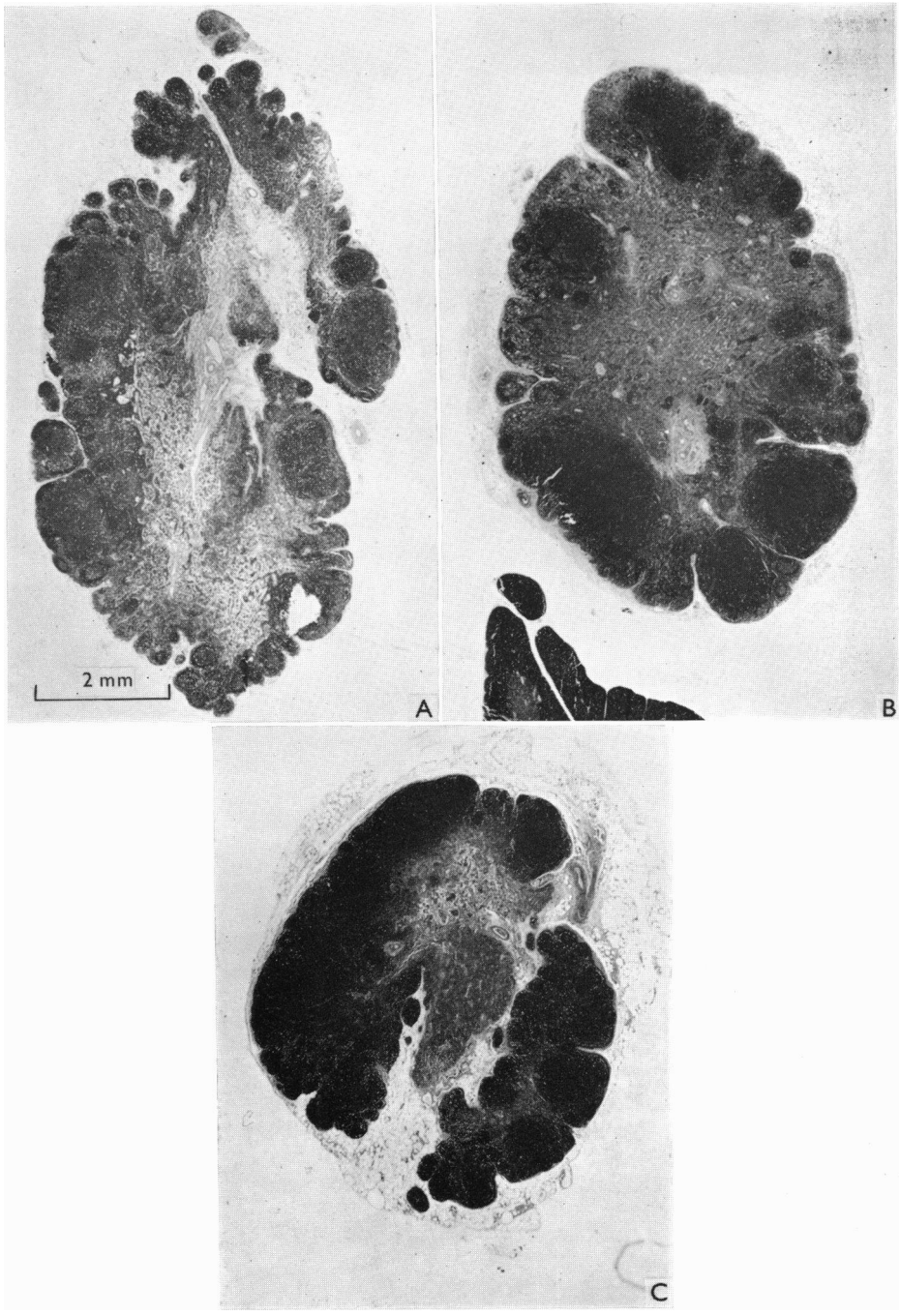
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phatic, does not interefere with viability and reactivity of the node, as long as their branches remain intact.

In a series of twenty-one rabbits, all the efferent and afferent lymphatics to the node of one hind limb were blocked. Obstruction of the efferent lymphatic was performed either at the groin (five rabbits) or in the popliteal region (sixteen rabbits). At the groin, a longitudinal incision was made along the femoral artery and vein and the efferent lymphatic running along these vessels tied by two ligatures and divided. In the popliteal region, an incision was also made longitudinally and the node exposed with its efferent and afferent lymphatics. The efferent lymphatic was doubly ligated and divided about 2 cm from the hilum of the node, together with the main artery and vein which run closely with the lymphatic.

After the division of the efferent lymphatic, all the afferent lymphatics (three or four) running along the superficial veins under the skin were double ligated and divided just before they enter the node.

In another series of twenty rabbits, either efferent or afferent lymphatics of the popliteal lymph node of one hind limb were blocked. The efferent lymphatic was obstructed in the popliteal region together with the main artery and vein without interrupting any of the afferent lymphatics (ten rabbits). Obstruction of the afferent lymphatics was performed as mentioned above without blocking the efferent lymphatic (ten rabbits).

In each animal the popliteal lymph node of the opposite hind limb served as a control. In most instances, the control node was also exposed and manipulated, but without blocking any of the lymphatics (sham-operated control).

After completion of the operation, the wound was closed, and 5–10 mg of Terramycin per kg body weight was given in a single intramuscular injection.

At successive intervals of 3–62 d after the operation, the animals were killed and the popliteal lymph nodes of both sides were excised for examination. In most instances, carbon suspension C11/1431*a* (Gunther Wagner, Hannover) diluted 1:3 with 1·0 ml physiological saline was injected subcutaneously into the hind footpads on both sides 1 h before sacrifice in order to determine whether the lymph flow through the node was actually blocked or not. Provided that the carbon suspension was injected by 1 h before sacrifice, this procedure itself did not produce either oedema or any change in the basic pattern of cellular architecture of the draining nodes, even in the cases where the afferent lymphatics were not blocked.

In still another, supplementary, series of eight rabbits, all the lymphatics of the popliteal node of one hind limb were blocked without interrupting any of the nodal blood vessels, and the node examined 3–7 d after the operation. Since such nodes

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Fig. 1. Longitudinal sections through the middle of the popliteal lymph node, illustrating increase in the cellular density and mass of cortex and shrinkage of medulla, after blockage of both the efferent and afferent lymphatics.

A: control node, 3 d after sham-operation without lymphatic obstruction (left popliteal node of rabbit no. 72, 2·6 kg, ♀). B: operated node, 3 d after obstruction of its lymphatic circulation (right popliteal node of rabbit no. 72, the same animal as shown in Fig. 1 A). Notice that the cellular density and mass of its cortex is greatly increased whereas its medulla is shrunken. Lower left to the node, a portion of the thymic cortex is shown for comparison. C: another operated node, 5 d after obstruction of its lymphatic circulation (left popliteal node of rabbit no. 31, 2·75 kg, ♂). The cellular density of cortex is much greater than in the node shown in Fig. 1 B.

served as a control in our earlier experiments in which the blood supply to the popliteal node was obstructed either partially or completely (Osogoe & Courtice, 1968), the data are not listed in Table 1. In such instances the carbon suspension was not injected into the hind footpads.

The excised nodes were cleared from the surrounding fat and weighed. They were then fixed either in formol saline or in Carnoy's fluid embedded in paraffin and cut serially at 5–7 μm. Haematoxylin and eosin was the standard stain used, supplemented with pyronin–methyl green.

In order to compare the activity of secondary nodules (germinal centres) in the popliteal lymph nodes under various experimental conditions, the number of these nodules which appeared in a longitudinal section through the middle of the node was counted. So-called 'solid' secondary nodules without germinal centres were not included.

RESULTS

Effect of blockage of both efferent and afferent lymphatics

Complete obstruction of the lymph flow through the popliteal lymph node was produced in sixteen of twenty-one operated rabbits and maintained for a period of 3–28 d, as shown in Table 1. In the remaining five rabbits obstruction of the lymph flow into the node was incomplete and these cases are not listed in the table. After lymphatic obstruction, each animal developed marked oedema of the distal part of the limb, reaching its peak on the third or fourth day and subsiding thereafter. By the 14th day oedema disappeared nearly completely.

Table 1. *Effect of blockage of both efferent and afferent lymphatics on popliteal lymph node*

Days after operation	Rabbit no. and sex	Body weight (kg)	Blockage of main artery and vein	Weight of popliteal lymph node (g)		Number of germinal centres per section		Reconstitution of lymphatics	
				Operated	Sham-operated	Operated	Sham-operated	Efferent	Afferent
3	68 F	2·6	Yes	0·288	0·452	32	55	No	No
	72 M	2·0	No	0·217	0·318	7	25	No	No
5	52 M	2·2	Yes	—*	—	14	52	No	No
	53 F	2·4	No	—*	—	46	85	No	No
7	66 M	2·1	Yes	0·264	0·198	14	57	No	No
	69 F	2·6	No	0·437	0·236	36	46	No	No
10	64 M	2·0	Yes	0·161	0·192	41	72	No	No
	56 M	2·2	No	0·238	0·150	38	37	No	No
14	58 M	2·4	No	0·294	0·372	26	63	No	No
	59 F	2·2	Yes	0·289	0·319	30	59	No	No
28	96 M	2·56	Yes	0·112	0·284	5	22	No	No
	115 M	2·95	Yes	0·153	0·196	25	62	No	No
	131 F	2·0	Yes	0·330	0·200	18	39	No	No
62	105 M	2·75	Yes	0·327	0·193	23	29	Yes	Yes
	106 M	2·5	Yes	0·093	0·108	44	38	Yes	Yes
	110 M	2·9	Yes	0·320	0·225	68	61	Yes	Yes

* The node was considerably smaller than the control node.

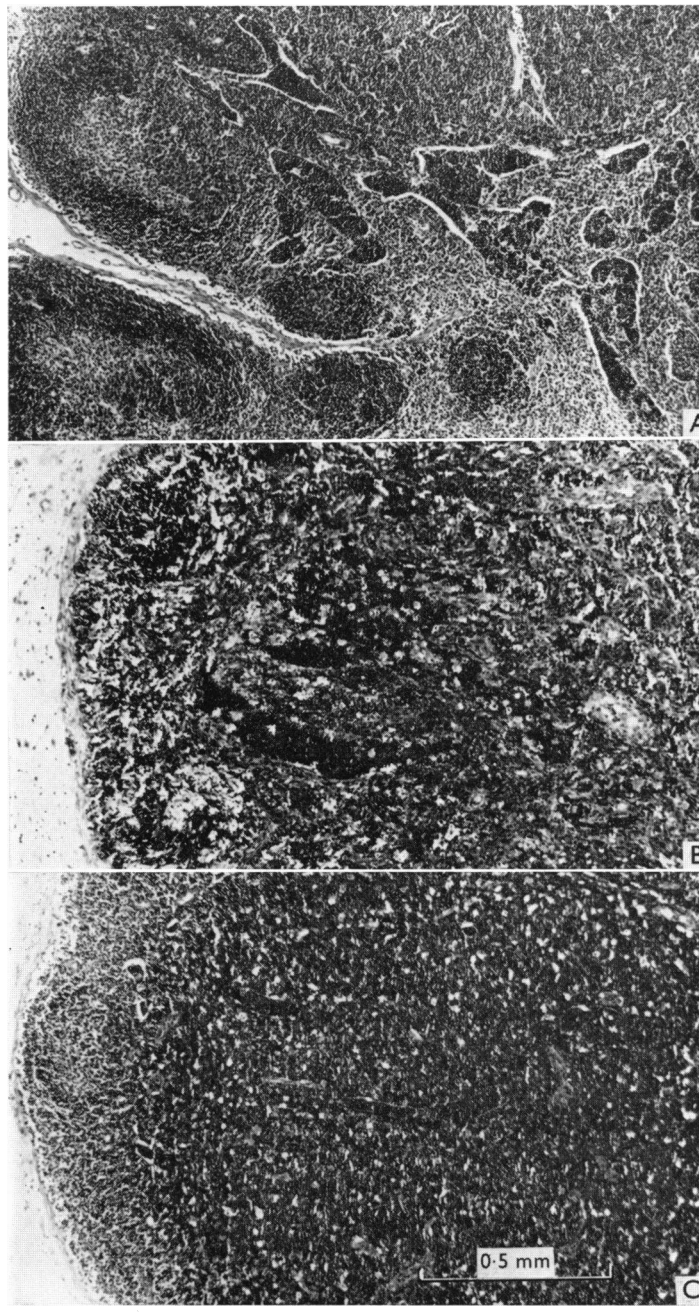


Fig. 2. Section through cortex of the popliteal lymph node, illustrating accumulation of lymphocytes in the lumen of lymphatic sinuses, 3 d after obstruction of both efferent and afferent lymphatics.

A: cortical area with normal cellular density (right popliteal node of rabbit no. 43, 2·5 kg, ♀). B: cortical area with moderately increased cellular density (right popliteal node of rabbit no. 72, 2·0 kg, ♀). C: cortical area with greatly increased cellular density (right popliteal node of rabbit no. 72, 2·0 kg, ♀).

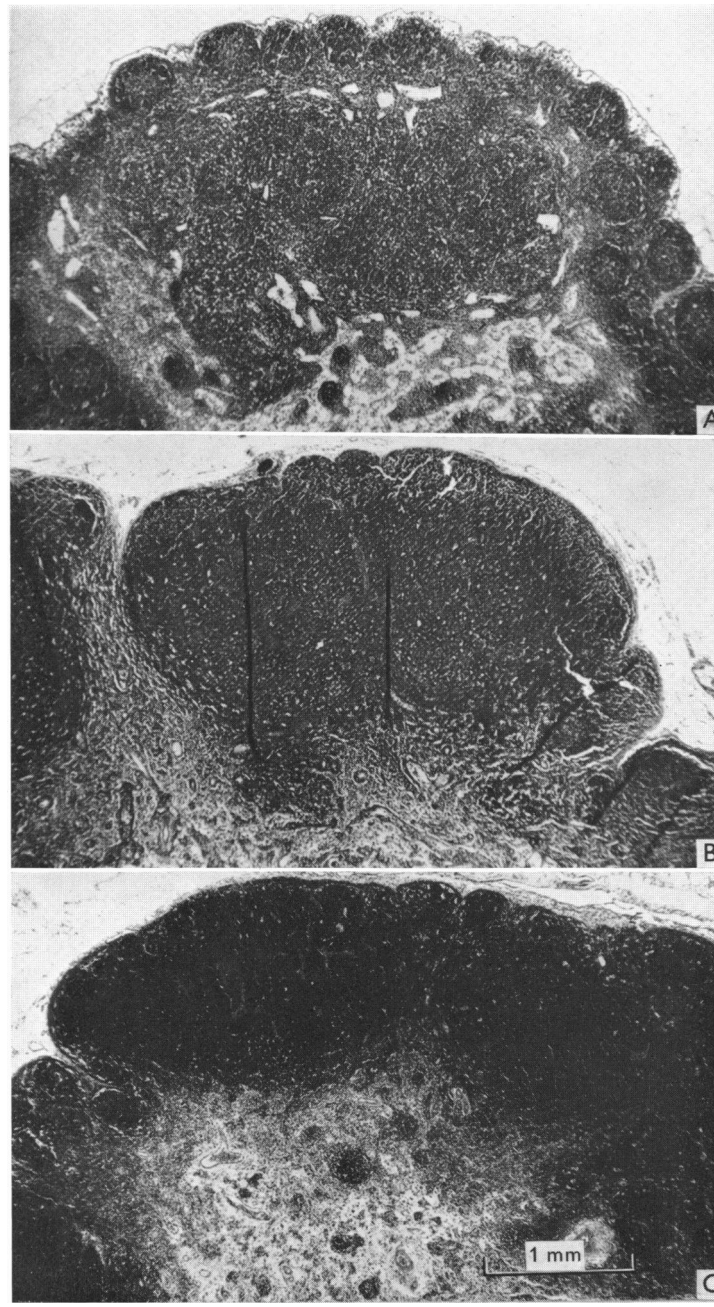


Fig. 3. Sections through the popliteal lymph nodes, illustrating the process of increase in the cellular density of cortex, after obstruction of both the efferent and afferent lymphatics.

A: cortical area of a normal popliteal node (left node of rabbit no. 67*b*, 2·3 kg, ♀). B: cortical area of an operated node having increased cellular density, 3 d after lymphatic blockage (left popliteal node of rabbit no. 25, 2·85 kg, ♀). C: cortical area of another operated node showing greatly increased cellular density, comparable to that of thymic cortex, 5 d after the lymphatic blockage (left popliteal node of rabbit no. 31, 2·72 kg, ♀).

One of the immediate effects of this condition was a marked shrinkage of medulla (Fig. 1), chiefly due to reduction in the amount of lymph in the lymphatic sinuses resulting from the interruption of the lymph flow into the node. No massive degeneration of cells occurred anywhere within the node. However, this does not mean that there was no increase in the number of pyknotic nuclei, although the pyknotic index of the node was not determined in the present study.

Another immediate effect was a striking accumulation of lymphocytes, particularly those of smaller size, within the node. As a consequence, the lumina of numerous lymphatic sinuses in the cortex were filled with these cells (Fig. 2). Such

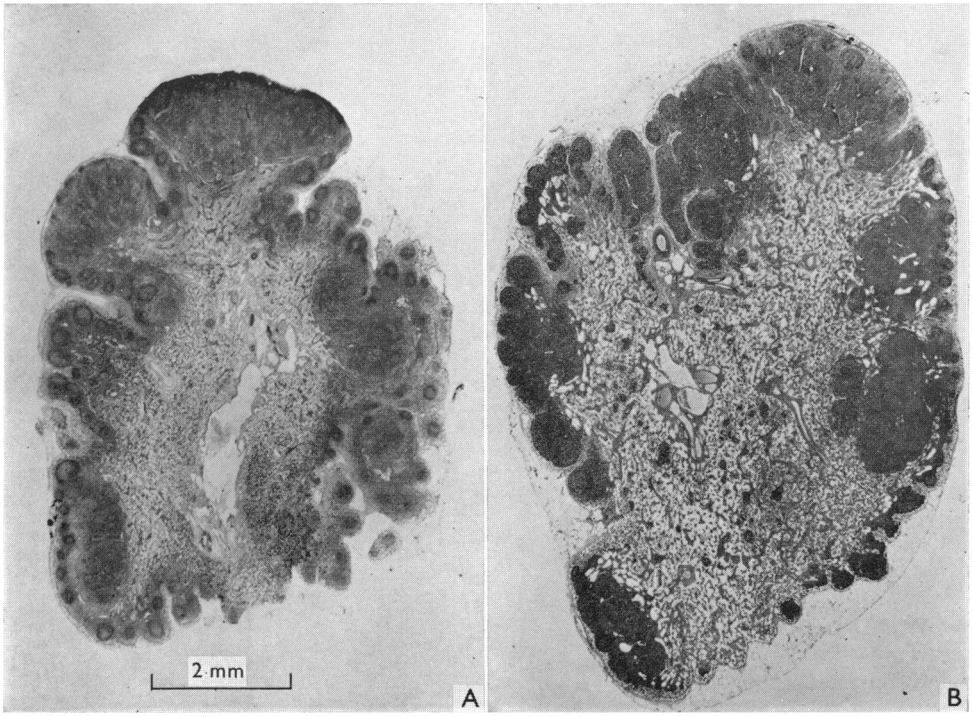
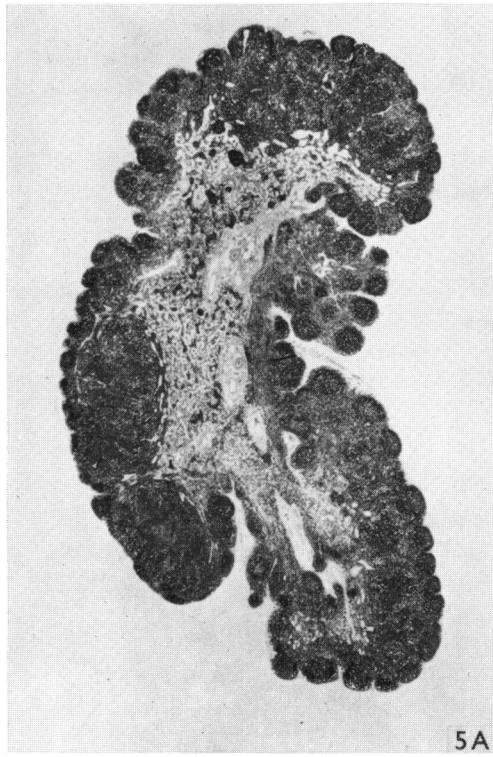


Fig. 4. Longitudinal sections through the middle of the popliteal lymph node 28 d after blockage of both the efferent and afferent lymphatics (rabbit no. 131, 2·0 kg, ♀).

A: control node that received sham-operation; B: operated node. Notice that the number of secondary nodules (germinal centres) is markedly reduced in the operated node as compared with the control.

accumulation of lymphocytes in the cortex resulted in a marked increase in its cellular density, particularly in cortical masses of diffuse lymphatic tissue. Thus within several days the cellular density of the nodal cortex approached that of thymic cortex. Secondary nodules (germinal centres), on the other hand, were considerably reduced in number and often disappeared nearly completely in some areas, so that the cellular architecture of nodal cortex resembled that of thymic cortex (Figs. 1, 3).

The above observations were based on the experiments in which all the lymphatics of the popliteal node were blocked without interrupting any of the nodal blood



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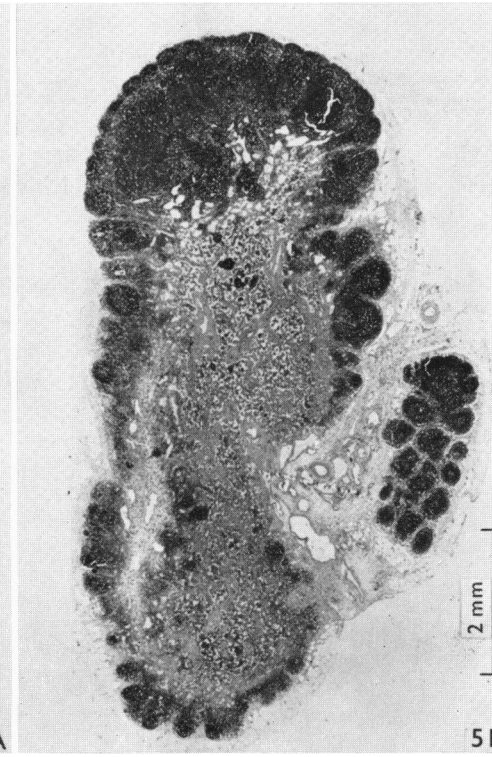

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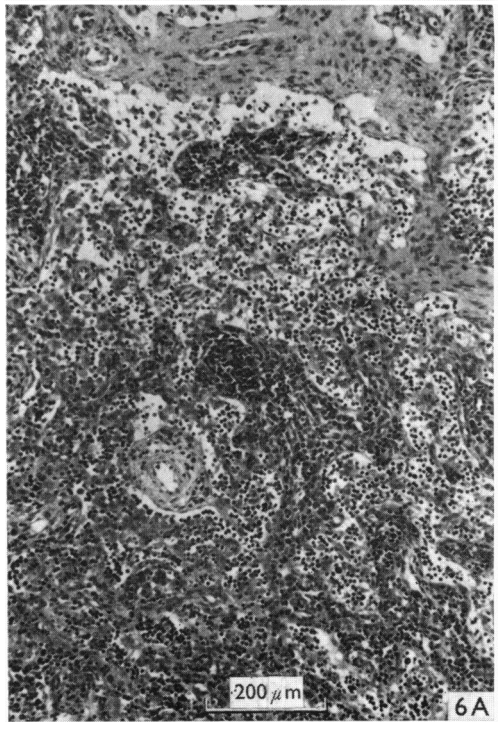

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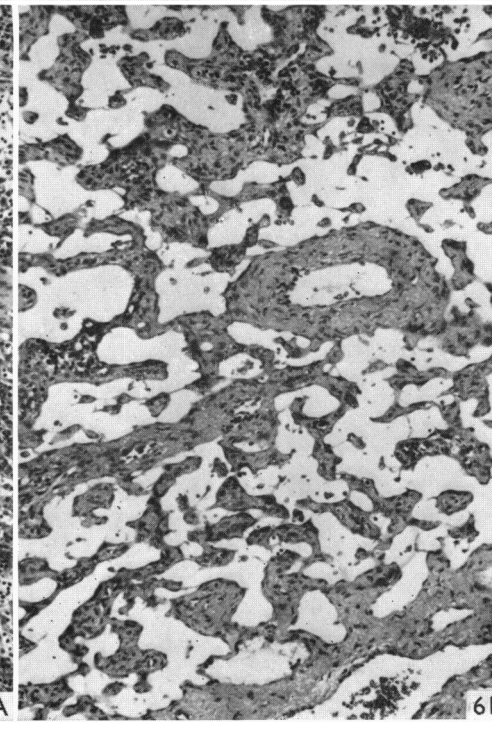

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vessels. Where the main artery and vein of the node were interrupted simultaneously, again no massive degeneration of cells was observed anywhere within the node, as in the former experiments. In addition, nodal changes characteristic of the lymphatic obstruction occurred in essentially the same manner as in the former experiments, regardless of whether the main artery and vein of the node were interrupted or not; both series are therefore listed together in Table 1.

After reaching its maximum, the cellular density of the cortex gradually diminished and returned almost to normal by the 14th day, whereas secondary nodules of the cortex remained significantly reduced in number until the 28th day (Table 1). However, such nodules did not disappear completely from the node in any instance. The medulla, which had earlier shrunk considerably, gradually enlarged to a size greater than in the control nodes at 28 d (Fig. 4).

Reconstitution of either efferent or afferent lymphatics occurred during the period between 28 and 68 d after the obstruction of both vessels. In several instances, however, lymph began to enter the node again before the 14th day, due either to the existence of unblocked channels or to the development of new lymphatics. The efferent lymphatic, on the other hand, without exception showed no sign of regeneration until the 28th day.

Table 2. *Effect of blockage of the efferent lymphatic alone on popliteal lymph node*

Days after operation	Rabbit no. and sex	Body weight (kg)	Weight of popliteal lymph node (gm.)		Number of germinal centres per section		Reconstitution of efferent lymphatic
			Operated*	Sham-operated	Operated	Sham-operated	
3	123 M	2·4	0·374	0·189	—	—	No
	155 M	2·2	0·670	0·330	45	92	No
7	76 M	2·95	0·435	0·300	14	36	No
	146 M	2·4	0·495	0·364	64	96	No
14	130 M	1·65	0·242	0·192	44	33	No
	142 M	2·45	0·327	0·188	32	30	No
28	122 M	2·5	0·143	0·160	8	13	No
	125 F	1·6	0·299	0·234	30	48	No
48	120 M	1·6	0·168	0·090	25	15	Yes
62	109 M	2·5	0·149	0·095	6	7	Yes

* The efferent lymphatic was blocked in the popliteal region together with the main artery and vein, without interrupting any of the afferent lymphatics.

Fig. 5. Longitudinal sections through the middle of the popliteal lymph node, 7 d after obstruction of the efferent lymphatic without interrupting any of the afferent lymphatics (rabbit no. 76, 2·95, ♂).

A: control node that received sham-operation; B: operated node. Notice marked enlargement of medulla and hypoplasia of cortical lymphatic tissue in the operated node.

Fig. 6. Sections through medulla of the popliteal lymph nodes, illustrating dilatation of lymphatic sinuses and fibrosis of medullary cords, 14 d after obstruction of the efferent lymphatic without interrupting any of the afferent lymphatics (rabbit no. 130, 1·65 kg, ♂).

A: control node that received sham-operation; B: operated node. Notice that free cells such as lymphocytes and macrophages in the lumen of lymphatic sinuses are greatly reduced in numbers in the operated node.

Effect of blockage of either efferent or afferent lymphatics

Obstruction of the efferent lymphatic alone produced stagnation of lymph within the node, leading to marked nodal swelling. The lymphatic sinuses were greatly dilated and free cells within the lumen such as lymphocytes and macrophages became scanty. The medullary cords were thickened through fibrosis. The diffuse lymphatic tissue of the cortex was reduced both in cellularity and amount (Figs. 5, 6). Secondary nodules also diminished in number but to a lesser extent than after the obstruction of both efferent and afferent lymphatics (Table 2).

Such structural alterations of the node remained almost unchanged until the 28th day, up to which time the efferent lymph flow from the node was blocked completely. Reconstitution of the efferent lymphatic occurred during the period between 28 and 42 d after its blockage. When the afferent lymphatics alone were blocked reconstitution of afferent channels rapidly occurred, restoring the lymph flow into the node within several days.

Effect of sham-operation

Following sham-operation without blocking any of the lymphatic channels of the popliteal lymph node, nodal swelling and cortical hyperplasia occurred to an unexpectedly great extent. As reported in an earlier paper (Osogoe & Courtice, 1968) the lymphocyte content in the efferent lymph was considerably increased after this operation. However, structural alterations of the node characteristic of the condition of lymphatic obstruction were never produced by this operation.

DISCUSSION

In the present work, the main artery and vein of the popliteal lymph node were interrupted simultaneously with the obstruction of its lymphatic circulation in many instances. Therefore, the effect of blockage of these blood vessels must first be considered. Our earlier study (Osogoe & Courtice, 1968) has demonstrated that the main artery and vein of the popliteal node furnish large branches to the adjacent muscles (the semimembranosus and biceps femoris) and several small ones to the perinodal fat and overlying fascia, before they enter or leave the hilum. Complete obstruction of all these blood vessels results in an almost total necrosis of the node, whereas blockage of the main artery and vein without interrupting their branches does not. In the present experiments, there was no massive degeneration of cells anywhere within the node, even when the main artery and vein were blocked simultaneously with the lymphatic obstruction. In addition the nodal changes which are characteristic of the condition of lymphatic obstruction were essentially the same whether the main artery and vein were blocked simultaneously or not. Furthermore, an immune cellular proliferation may be produced in the popliteal lymph node after blocking all its lymphatics, and simultaneous division of the main artery and vein does not seriously affect the nodal response (B. Osogoe, unpublished observations). From these observations it is evident that the division of the main artery and vein of the popliteal lymph node does not interfere with viability and reactivity of the node because of the establishment of a collateral circulation through their branches.

Since the lymphocyte output through the efferent lymph from the popliteal lymph node of the adult rabbits is about $2{\cdot}6 \times 10^6$ per hour (T. Ido, Y. Kanai & B. Osogoe, unpublished observations), it was expected that interruption of the efferent lymph flow through the node would be followed by intranodal accumulation of lymphocytes which normally would be transported from the node by the lymph; and in fact a striking accumulation of lymphocytes within the lymphatic sinuses after complete obstruction of the lymph flow through the node did occur. Moreover, as a consequence of accumulation of lymphocytes the cellular density of the cortex was greatly increased and secondary nodules were considerably reduced in number, so that the cellular architecture of nodal cortex resembled that of thymic cortex. Such thymic transformation of the node, however, was transient and incomplete.

The present findings must also be discussed in relation to the recirculation of lymphocytes from blood to lymph through lymph nodes, because this phenomenon has attracted much attention since Gowans (1959) first pointed out its importance. It is evident that lymphatic obstruction interrupts such recirculation of lymphocytes completely. However, from the present study it cannot be determined whether the intranodal accumulation of lymphocytes occurring after lymphatic blockage is due to the multiplication of lymphocytes within the node or to the migration of these cells from blood to lymph node. Further experiments are needed to settle this question.

The effect of blockage of both efferent and afferent lymphatics differs from that of obstruction of the efferent lymphatic alone. The former effect is characterized by intranodal accumulation of lymphocytes, reduction in the number of secondary nodules and shrinkage of the medulla, whereas the latter is characterized by nodal swelling, dilatation of lymphatic sinuses and fibrosis of medullary cords. Free cells such as lymphocytes and macrophages within the dilated lymphatic sinuses became scanty when the efferent lymphatic alone was blocked. All the latter changes may be ascribed to the high lymph pressure produced by obstruction of the efferent lymph flow from the node. This is supported by the observation of Drinker & Field (1933) that the lymph pressure may rise as high as 99 cm of water when the vessels are completely obstructed.

As regards regeneration of lymphatic vessels after their division, it is worthy of notice that after obstruction of afferent lymphatics alone re-establishment of the afferent lymph flow into the node occurred rapidly, within a few days, whereas it was much delayed when the efferent lymphatic was also interrupted. The mechanism by which regeneration of afferent lymphatics is inhibited by the simultaneous obstruction of the efferent lymph flow is not known.

SUMMARY

Changes in the cellular architecture of the popliteal lymph nodes in the rabbit after blocking their lymphatic circulation were studied.

When all the lymphatics, both efferent and afferent, to the node were blocked a striking accumulation of small lymphocytes occurred within the node and the cellular density of cortex was greatly increased. Secondary nodules (germinal centres), diminished considerably in number and often almost disappeared in some areas, so

that the cellular architecture of nodal cortex resembled that of thymic cortex. Such thymic transformation of lymph nodes, however, was transient and incomplete. Secondary nodules did not disappear completely even though complete obstruction of the lymph flow through the node was maintained for a period of up to 28 d.

Obstruction of the efferent lymphatic alone produced no accumulation of lymphocytes anywhere within the node. After blockage of afferent lymphatics alone, reconstitution of afferent channels occurred rapidly, restoring the lymph flow into the node within several days.

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