THE ABILITY OF LYMPH TO MAINTAIN VIABILITY IN "DEVASCULARIZED" LYMPH NODES *

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In the course of experimental work concerning the physiology of the lymphatic system it occurred to the authors that some light might be shed on the function of lymph if it could be shown that lymph *per se* could keep tissues alive. In the following experiments all vascular connections to the popliteal lymph nodes of dogs were severed, but one or more afferent and one or more efferent lymphatic channels were left intact and it was found that lymph sufficed to maintain viability. These results imply a nutritive function on the part of lymph which, so far as we are aware, has not been demonstrated *in vivo* in mammals.

METHODS

Normal, healthy, adult mongrel dogs maintained on kennel diet and allowed water *ad lib.*, were used as experimental animals. Under ether or nembutal anesthesia the popliteal node was exposed through a linear 4–6 cm. incision. Sterile technique was observed in all operative procedures.

In the *control* group of animals the node was completely excised with a minimum amount of hemorrhage and trauma and replaced in the popliteal space where it was held by suturing the subcutaneous fascia and skin over it. Attempts were made to ligate all severed afferent and efferent lymphatic channels as well as blood vessels.

In the *experimental* group all tissue which could not definitely be identified as lymphatic trunks was severed and these remaining trunks were cleaned as thoroughly as possible of any connective tissue that might carry blood capillaries. With experience one learns to identify lymphatic trunks with certainty by their translucency and the characteristic fashion in which they "bead" below

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a temporary obstruction. Especially is the identification of lymphatic trunks certain when a poorly diffusible dye (approximately 1 per cent Chicago blue or pontamine blue dissolved in saline, or preferably dog serum) is injected into the subcutaneous tissues between the toes, and the foot gently massaged. This procedure, which can be carried out before the operation is begun, or by an assistant after the operative field is exposed, almost immediately outlines the popliteal node and its afferent and efferent channels in sharp contrast. These dye injections were made in most instances in the experimental group, but in a number of experiments they were purposely omitted to exclude the possible (antiseptic?) effect of the dye.

At varying intervals after the operation the nodes were removed by biopsy or at autopsy, fixed in Zenker's solution, embedded in paraffin, sectioned, and stained with hematoxylin and eosin, the elastic tissue method followed by van Gieson's counterstain, and Foot's modification of Bielschowsky's silver stain. In several instances Gram and methylene blue stains were also made.

In obtaining samples of afferent and efferent lymph for chemical analysis the technique described by Drinker and Field [1] was used. Reducing substance was determined by the Hagedorn-Jensen method [2]; bound carbon dioxide and carbon dioxide combining power by a volumetric method.*

Experimental Observations

The results are summarized in Table I. In the control group, *i.e.* dogs with all blood vessels and lymphatics severed, all 15 nodes rapidly underwent massive necrosis, as illustrated in Figure 1. These necrotic changes, which affected primarily the lymphocytic elements but also involved the reticulum and blood vessels, were maximal by 36 hours, following which the nodes underwent liquefaction, reabsorption and fibrous tissue replacement. It was difficult to follow these changes longer than 3 days as the nodes usually became infected and tended to slough out; in fact, 7 experiments were discarded because the node could not be identified in the depths of the open wound and no remnants were recognizable in histological sections of regional tissue. The almost constant presence of infection in this group at first made us suspect our oper-

* The authors are indebted to Dr. Joseph Victor for carrying out these analyses.

ative technique, but from histological studies of these nodes and especially from bacteriological studies of nodes obtained from normal dogs sacrificed for other purposes, we are convinced that in apparently normal healthy dogs, organisms (*Staphylococcus albus* and *aureus*, hemolytic and non-hemolytic streptococcus) are continually being filtered out of subcutaneous lymph by peripheral lymph nodes. The presence of necrotic tissue, which serves as a favorable medium for the growth of bacteria already present in the nodes, plus the absence of the normal defensive mechanism offered by the lymphatic system, affords an ample explanation for the high incidence of infection in this control group.

In the *experimental* group, *i.e.* dogs with all vascular connections severed but with one or more afferent and one or more effer-

TABLE I

Maintenance of Viability in "Devascularized" Lymph Nodes

Group	Interval between operation and biopsy	Number of nodes	Number of nodes completely necrotic	Number of nodes partially viable	Number of normal nodes	Number of infected nodes
	days					
Control *	1–3	15	15	0	0	15
Experimental †	1–9	20	2	14	4	3

* All vascular and lymphatic connections severed.
† All vascular connections severed, but one or more afferent and one or more efferent lymphatic channels left intact.

ent lymphatic channels remaining intact, the results, though not wholly consistent, are suggestive. Some degree of viability was maintained in 18 of 20 experiments (90 per cent); 4 of the nodes remained grossly and histologically normal (Fig. 2), and only 3 became infected. The explanation for the varying degrees of viability illustrated in Figures 3 and 4 is not clear; possibly mechanical and anatomical factors are responsible, such as kinking, thrombosis and leakage from lymphatic trunks, occlusion of trunks by pressure from without as by edema or fibrin, variation in the number and anatomical arrangement of trunks remaining patent, and variation in the amount of movement of individual dogs and the consequent variation in lymph flow through the nodes. The only evidence we have to support the suspicion that the number of patent channels is important is that when deliberate efforts were made to leave a single afferent and a single efferent channel intact so quantitative chemical studies could be done, all of the nodes

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growing through the capsule save along the afferent and efferent lymphatic trunks at the poles of the node which, of course, penetrated the rubber sheath. The experimental node appeared normal when it was excised 7 days later. The opposite popliteal node, which was completely removed and replaced in the popliteal space enclosed in a rubber sheath, showed massive necrosis and infection when it sloughed out 3 days later.

The deprivation of vascular supply was also confirmed by intra-arterial injection of India ink. One to 3 days after isolating the popliteal node on one side, save for its lymphatic connections, the dog was anesthetized and a cannula placed in each femoral artery and tourniquets placed about 5 cm. above and below each "knee." Saline (about 150 cc.) was then perfused through the arteries until the fluid that returned through the cut end of the femoral veins was clear. Full strength Higgins' India ink (about 30 cc.) was then run through the cannulas until the returning fluid was black. Whereas practically every blood vessel in the node on the unoperated side was distended with ink, of the 6 devascularized nodes on which this procedure was tried ink was found in only one small arteriole in 1 node. The necrotic changes in this node were as great as in the other 5 nodes which contained no ink.

Evidence from all three types of experiments indicates that the blood supply to the nodes had been excluded. Any nourishment to the nodes, therefore, must have come by way of the lymphatic trunks or by diffusion through the capsule from the surrounding tissues. That the latter mechanism is totally inadequate to maintain the vitality of the nodes is evident from the uniform necrosis in the control group. Nutritive material must have reached the nodes through the lymphatic channels.

In a limited number of experiments, sugar, bound carbon dioxide, and carbon dioxide combining power determinations were made on afferent and efferent lymph obtained by cannulating lymphatic trunks leading to and from these "devascularized" nodes. The results of 3 experiments are given in Table II.

The data in Table II indicate certain relations between glucose disappearance and decrease in both carbon dioxide and carbon dioxide combining power of lymph during its passage through the lymph nodes. The mean decrease in lymph carbon dioxide and carbon dioxide combining power was 12–13 volumes per cent re-

TABLE II

Chemical Analyses of Lymph Flowing to and from "Devascularized" Lymph Nodes

Number of dog and node	Number of hours after severance of blood vessels	Lymph flow through node †	Sugar		Carbon dioxide				Histology of node
					Bound		Combining power		
			A	E	A	E	A	E	
		cc./hr.	*mg./100 cc.*	*mg./100 cc.*	*vol./%*	*vol./%*	*vol./%*	*vol./%*	
37–144 (RPN)	2½	0.45	116	59	38	27	61	48	Normal
37–145 (RPN)	3½	1.05	109	66	41	31	61	48	Normal
37–145 (LPN)	24	1.40	146	110	45	30	58	45	Slight necrosis of medulla, follicles viable

A = afferent lymph; E = efferent lymph; RPN = right popliteal node; LPN = left popliteal node.
These figures represent amount of efferent lymph collected from a single trunk.

spectively, which is equivalent to 48–52 mg. of lactic acid. This indicates that under the conditions of this experiment all of the glucose that disappeared can be accounted for by its conversion to lactic acid. With intact blood supply there is no evidence of decrease of glucose from the lymph.[3] Either glucose is supplied to the node directly by the blood or else the loss from the lymph is masked by diffusion replacement from the blood. Under aerobic conditions lymph nodes from mice placed in Ringer's solution produce very little lactic acid from glucose but considerable quantities may be produced anaerobically.[4] Normal lymph nodes also oxidize glucose. Therefore, since under the conditions described above the changes in lymph glucose and lymph carbon dioxide and carbon dioxide combining power are equivalent, they indicate that during the period in which the *lymph gland remains viable its metabolism is mainly anaerobic*. This is not surprising when it is recalled that the rate of oxygen consumption of lymphoid tissue of the mouse is about 1.02 cc. per gm. per hour.[4] If the oxygen content of afferent lymph is equal to that of plasma, then 1 cc. would contain about 5 cmm. of oxygen. Since the rate of lymph flow through a node is about 3 cc. per gm. per hour,* the lymph could supply only about 1 per cent of the oxygen required for respiration.

The obvious step from these *in vivo* experiments, which amount to nothing more than perfusion with lymph, to experiments *in vitro* in which the nodes are perfused with "artificial lymph" and in which there is no question of persistent blood supply, has been taken. The results of early experiments confirm the *in vivo* studies listed in Table II in all respects. These results will be reported later. Suffice it to state here that the chemical studies to date have yielded confirmatory evidence that the blood supply to the nodes has been severed and have indicated that anaerobic glycolysis is one of the metabolic processes effective while they are maintained in a viable state.

Discussion

When it is taken into consideration that lymph was one of the first substances used as a tissue culture medium and that the com-

* This figure is based on an average weight of 1.5 gm. for popliteal lymph nodes from dogs, an average flow of 1.5 cc. per hour per lymphatic trunk,[5] and an average of three afferent trunks.

position of the plasma-Tyrode mixture commonly employed today for tissue culture work closely approximates that of lymph in glucose, electrolyte and protein content, and further that bland infarction of lymph nodes is a rarity, if it ever occurs, the observation that lymph *per se* will maintain the viability of lymph nodes is not surprising. But does this observation give an indication as to the normal function of lymph?

The great bulk of evidence today indicates that lymph, at least in the peripheral portions of the body, is derived from the blood plasma. The rate of lymph formation and the constant presence in the lymph of appreciable quantities of proteins indistinguishable from those in the blood plasma render any other interpretation untenable. Without entering into the controversy as to the identity of tissue fluid and lymph, it is fair to state that all workers are agreed that the fluid which flows through the lymphatic channels represents that portion of the blood plasma filtrate not reabsorbed by the blood capillaries and not utilized by the body cells which are bathed by it before it enters the endothelial lined channels of the lymphatic system to become lymph. That the body cells may add to as well as subtract from the tissue fluid and that all these changes may alter the composition of lymph is also generally recognized. The experimental observations in this paper indicate that this fluid, which has been "rejected" by certain tissues of the body, is still capable of maintaining viability in other tissues. The simplest explanation for this observation is that the excess nutritive material is merely passed on. The magnitude of this "factor of safety," however, is not great * and it is possible that qualitative as well as quantitative factors are involved. How long such nodes can be maintained free from blood supply, whether any morphological changes in the node will occur with time, and

* If the amount of tissue whose lymph drains into the popliteal node of a dog weighing 10 kilos be estimated at 200 gm., and if a pulse rate of 100 and a cardiac output of 30 cc. per beat be assumed, and further if the assumption be made that each gram of tissue in the dog receives the same amount of blood, it can be calculated that the blood flow to the 200 gm. of tissue is 3600 cc. per hour. The total amount of lymph flowing through the node, even under conditions of activity, probably would not exceed 10 cc. per hour; from statistical analysis * the actual lymph flow would be nearer 5 cc. per hour, a factor of safety of about 0.2 per cent. On the same admittedly crude basis of computation, the blood flow through the node (assuming a weight of 1.5 gm. for the node) would be 27 cc., or about six times the lymph flow. The actual blood flow through the lymph node is probably much greater.

whether such nodes are capable of hyperplasia or antibody production in response to appropriate stimuli are all questions for future investigation.

Summary and Conclusions

Popliteal lymph nodes of dogs when replaced in the popliteal space after complete severance of all vascular and lymphatic connections rapidly undergo massive necrosis. These nodes usually become infected and may slough out.

When, however, all vascular connections are severed but one or more afferent and one or more efferent lymphatic channels remain intact, infection does not ensue and the nodes remain viable. Chemical analyses on lymph flowing to and from these "devascularized" nodes show a sharp drop in reducing substance, bound carbon dioxide and carbon dioxide combining power in the lymph during its passage through the node, and indicate that anaerobic glycolysis is one of the metabolic processes taking place in the viable node.

These observations imply a nutritive function on the part of lymph which, so far as we can determine, has not been demonstrated *in vivo* in mammals.

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DESCRIPTION OF PLATE

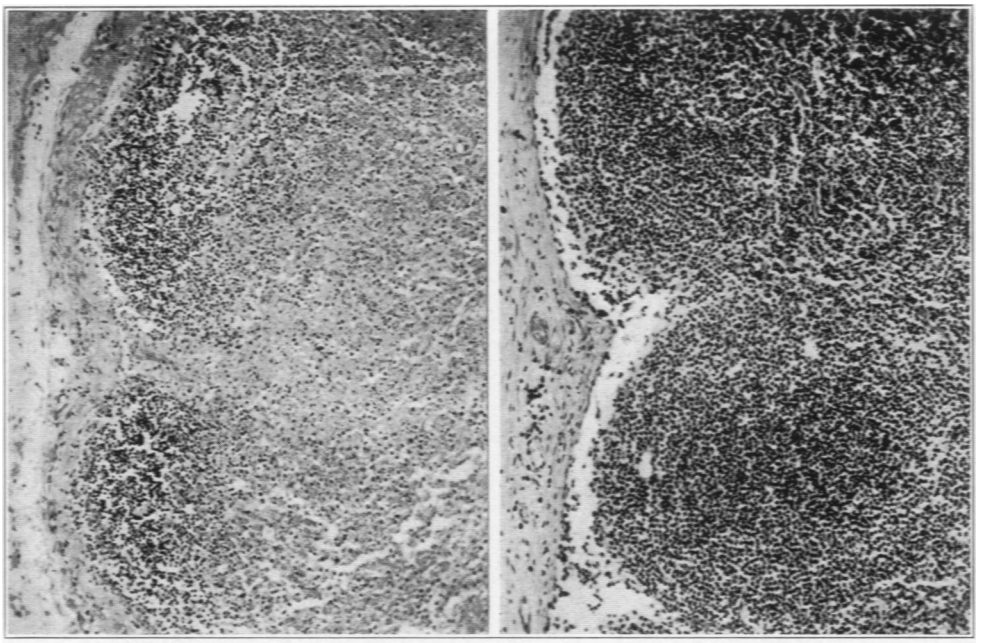
PLATE 126

FIG. 1. Complete necrosis in lymph node 29 hours after severance of all lymphatics and blood vessels.

FIG. 2. Normal structure of node preserved 7 days after severance of blood vessels; lymphatics left intact.

FIG. 3. Partial viability in node 1 day after severance of blood vessels; lymphatics left intact.

FIG. 4. Partial viability in node 2 days after severance of blood vessels; lymphatics left intact.



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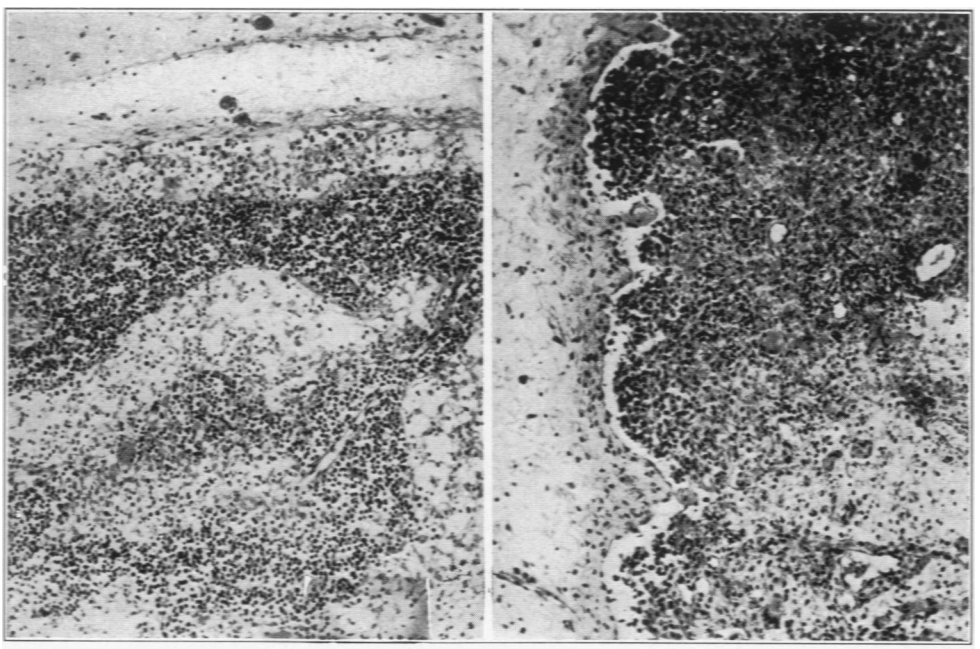

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