

## EXPERIMENTAL BIOLOGY

### THE REGENERATION OF LYMPH NODES AFTER THEIR PARTIAL REMOVAL OR INJURY

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The regeneration of lymph nodes and the possibility of their re-formation is of real theoretical and practical interest. This is a question which has been much discussed but which is still far from being solved, and the opinions of different researchers are extremely contradictory. A. P. Avtsyn [1], J. Auché [2], E. Clark and E. Clark [6], S. Groot [8], J. Horii and T. Sakai [10], M. Ohkubo [14] and others believe that lymph nodes are capable of regeneration. H. Baum [4], P. Heuter [9], A. W. Meyer [11], L. Moriconi [12], G. Ottaviani and M. Cavalli [15], A. G. Sanders and H. W. Flory [20], A. Vecchi [22] and others disagree with this.

Data describing the conditions favorable and unfavorable to lymph node regeneration are extremely limited [7, 17, 19].

The opinion that lymph nodes completely lack the ability to regenerate is contradictory to all the accumulated clinical and experimental data, which indicate that these organs are highly reactive. It is difficult to believe that lymph nodes, which change sharply in pathologic conditions and are made up of plastic lymphoid tissue, are unable to either regenerate or form anew.

#### EXPERIMENTAL METHODS

In this article, we present data on the regeneration of injured iliac lymph nodes in white rats weighing 100-130 g, depending on the degree to which lymph circulation to these nodes was disturbed (experimental series I, II, III and V) and on the degree to which the nodal tissue was altered (experimental series IV). In experimental series I, the lymph nodes were seared in one or several places (2-5); in series II, from 1/5 to 1/2 of the lymph node was resected. In all of the experiments of this series, the part of the lymph node with the afferent lymphatics was left on the right side, and on the left side, the part with the efferent lymphatic was left; the afferent lymphatics, for a distance of 2-3 cm of their lengths and the corresponding portion of the node were removed. In experimental series III, the middle portion of the nodes was left, and their poles with the afferent and efferent lymphatics removed. The lymphatics were dissected out and cut at a distance of 2-3 cm. In experimental series IV, after half of the node had been removed, the remaining part was seared along the line of the cut. In series V, 1/3-1/4 of the lymph node was left, and, at the same time, the iliac or the femoral vein was carefully separated to avoid damage to the near-by lymph vessels and ligated below the node. The amount of lymph circulating from the limb into the node was greatly increased in these experiments due to the stoppage of circulation through the vein. Fifty experiments were conducted in all (9 experiments in series I, 17 experiments in series II, 9 experiments in series III, 10 experiments in series IV and 6 experiments in series V). Most of the rats were killed after intervals of up to 30 days after the operation, a few, after 2-3 months. Histological processing was combined with Trypan blue or India ink injections of the lymph system. The first injection was done during the operation in order to render the lymph bed more completely visible; a second was sometimes done with the second laparotomy to show the changes occurring after the operation, and third injection was done when the animal was killed. The lymph nodes were measured before and after the operation in all the experiments.

## EXPERIMENTAL RESULTS

All the experiments in series I had similar results; the lymph nodes regenerated in the seared places as early as 10-12 days after the operation and could not be distinguished from the undamaged nodes. No deformation of the nodes due to the searing was observed. When the nodes were injected, the color spread evenly; there were no portions to which the color did not penetrate. Along with regenerated lymph tissue in the formerly damaged nodes, microscopic examination frequently showed connective tissue rods growing up from below into the body of the node. These rods, however, never became very thick; subsequently, they became somewhat thinner, looking like thin bands along the greater part of their length, sometimes somewhat thickened near the capsule. In other cases, the connective tissue rods were only preserved on the periphery of the nodes and did not penetrate very deeply. Without going into a detailed description of the course of regeneration in the damaged part of the lymph nodes, we shall only mention that the reticular syncytium proliferated first of all, growing further and further beyond the undamaged places as the wound healed. This syncytium, distinguished by the extreme polymorphism of its component elements, contained a varying amount of lymphocytes, distributed at random, most of which probably infiltrated from the remains of the node. At the same time, foci of lymphopoiesis, containing lymphocytes in all stages of formation and differentiation, appeared in the syncytium. At first, these foci were distributed apart from each other, later, however, as they grew and changed form, they grew together. Sinuses were gradually differentiated, and their connection with the sinuses of the undamaged part of the lymph node was established. The color injections confirmed the latter. In spite of known variations in the different experiments, the general course of regeneration was homologous in all of the experiments.

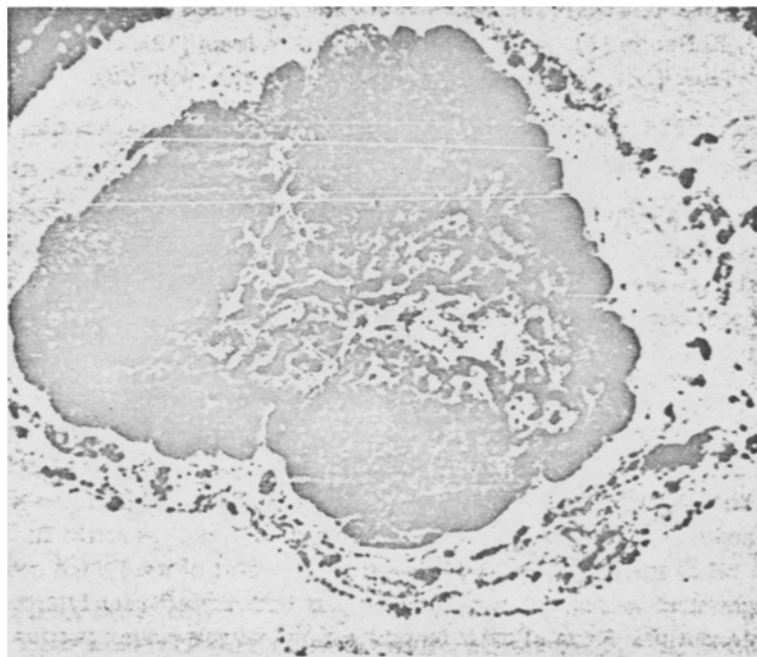


Fig. 1. General view of iliac regional lymph node of a white rat which was completely regenerated by the 23rd day of the experiment after 3/4 of it and the efferent vessel had been resected. Photomicrograph. Magnification - 15 times.

In experimental series II, the remaining portion of the lymph node with the afferent lymphatics (on the right) had, after 15-20 days, become a macroscopically well-formulated node, easily filled with color by the injection (Fig. 1). The color extended from the node along the efferent lymphatic, which was usually located at the pole opposite to the afferent lymphatics. This vessel was evidently formed from the regeneration of the remaining part of the efferent lymphatic, which grew up to meet the remnant of the node. Sometimes, one of the afferent lymphatics became the efferent.

The course of regeneration was generally analogous to that described earlier: first the reticular syncytium

began to grow, and individual foci of lymphoplasia appeared. The largest of these appeared along the periphery, near the differentiated capsule. Then they increased in size. The individual foci in the center of the node also became larger and grew together. At the same time, the number of blood vessels which had appeared at first among the elements of the reticular syncytium decreased somewhat. The remnant of the lymph node also underwent a known reconstruction. In it, during the first few days after the operation, the normal relationships between the sinuses, myelinated septa and follicles were disturbed; the contours of the latter became more defined. A considerable amount of lymphocytes passed into the intermediate sinuses, thus concealing their contours. The preserved part of the lymph node became more and more like an unformed accumulation of lymphoid tissue. The marginal sinuses, on the other hand, became much larger. However, this process gradually ceased, and by the 20-25th day after the operation, at the latest, (often before the 15th day), the normal structural picture was regained. Regeneration always occurred, although, in some cases, it was very slow. The size of the regenerated node varied in different experiments; in some cases, it was only  $2/3$  as large as the original node, and in others, it was either about the same size as before or only slightly smaller. Only in one experiment did the regenerated node remain very small. Histological examination of this node on the 20th day after the operation showed a structure typical of this stage, but the regenerated part was still not clearly delimited from the surrounding connective tissue, and there was no capsule at this place.

Where the portion of the lymph node including the afferent vessels and the afferent lymphatics themselves had been resected for a length of 2-3 cm (on the left), results analogous to those already described were obtained in 2 cases out of 11. The injection showed that, in these two experiments, the lymphatics from the opposite side, where the part of the node with afferent lymphatics remained, grew onto the remaining portion of the node. There was no regeneration in the other 9 experiments. Connective tissue grew along the line of the cut; the lymph node remnant became disorganized, causing it to look at first like a non-differentiated accumulation of lymphoid tissue, lacking sinuses and without the follicles with their centers of division (Fig. 2). There were different numbers of macrophages in different portions. Lymph circulation through the node was not restored, since the regenerated lymphatics were located some distance from it; more and more connective tissue grew from the remaining portion of the node; the number of lymphocytes decreased sharply, and the lymphocytes themselves were distributed in the form of separate islands, differing greatly in shape and without definite borders, due to the gradual migration of lymphocytes beyond the limits of the lymph node remnant.

The 9 experiments in series III gave the following results. The several injections of Trypan blue showed that all of the lymphatics approaching the node could not be distinguished in 3 experiments. The lymph circulation seemed rather disturbed, and the lymph nodes regenerated, but quite slowly. Two of the nodes remained small, and four other approximated those observed in experimental series II on the right in size. Regeneration did not occur in the other 6 experiments, and the results in these experiments were the same as those observed in the 9 experiments of series II in which the operation was done on the left.

The results obtained in experimental series IV were generally the same as those obtained in the experimental series II with the operation on the right, i.e., without the searing. In these experiments, regeneration sometimes occurred somewhat later, and the sizes of the regenerated parts were smaller. In experimental series V, on the other hand, regeneration was accelerated, and signs of a compensatory reaction in response to the intensified lymph circulation were often found in the regenerated lymph nodes, which signs we have already described (sinus expansion, proliferation of reticular syncytium, etc. ([A. L. Braude, 1956])).

After considering all the data presented, one can conclude that the remaining part of a lymph node can only regenerate when lymph circulation through the node is continued (experimental series I) or when it is comparatively quickly restored (experimental series II with operation to the right). On the other hand, regeneration does not occur when the lymph circulation is cut off, and the remaining part becomes gradually reduced and is replaced by connective tissue. This occurred in experimental series III and the experiments in series II with the operation on the left, when the collateral lymph circulation which developed by-passed the remaining part of the lymph node. On the other hand, when the node's connection with the lymphatics was restored (by the growth of the lymphatics from the opposite side, or due to their incomplete resection), in two experiments of series II with the operation to the left and in three experiments of series III, the node regenerated. Regeneration occurred especially rapidly when lymph circulation through the node was intensified (experimental series V).

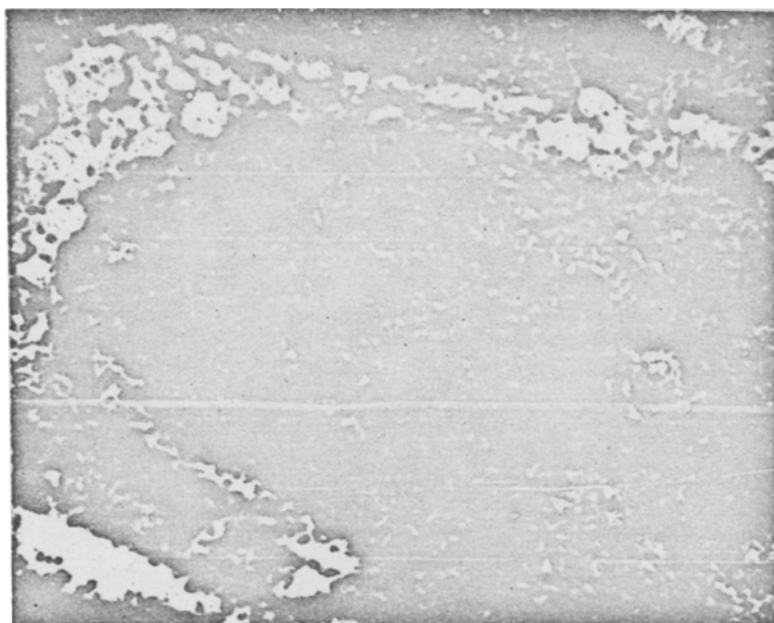


Fig. 2. Disorganization of iliac lymph node remnant in white rat, under conditions of lymph circulation stoppage, on the 20th day of the experiment. Photomicrograph. Magnification - 120 times.

The degree to which the nodal tissue was altered affected the experimental results. The data received in experimental series IV and II with the operation to the right were generally analogous, except for the fact that the sizes of the regenerated nodes varied somewhat. However, this circumstance alone is not sufficient basis for any sort of conclusion. In the course of the experiments in series II, III, IV and V, additional lymph nodes usually appeared at a greater or lesser distance from the regenerated nodes. Due to their appearance, the lymph flowing out from the hind leg entered several (as many as 3-4) iliac nodes on each side instead of just one, as happens normally. Therefore, there was somewhat less lymph circulating through the regenerating node than before the operation. It seems likely that this phenomenon, which was expressed to varying degrees in the different experiments, was the reason that the regenerating node was not always as large as the original node. Turning back to the results of experimental series IV, it should be emphasized that the degree of injury to the lymph nodes evidently has less influence on their regeneration than the preservation or disturbance of lymph circulation. The latter has the deciding role in the process studied. With this in mind, we consider it impossible to consider the question of whether or not lymph nodes are capable of regeneration after being injured or partially removed without considering the concrete conditions in which regeneration occurs. Our study of regeneration clearly demonstrated the constructive role of lymph circulation through the node, in the absence of which, the node not only does not regenerate, but changes from a structurally complicated organ into an accumulation of lymphoid tissue, lacking a clear contour and containing a greater or lesser amount of macrophages. As opposed to the comparatively brief disorganization stage occurring in the lymph node remnant during lymph circulation disturbance, these phenomena appear to be irreversible.

#### SUMMARY

A series of experiments were conducted on albino rats, weighing 100-130 g. It was established that iliac lymphatic nodes are able to regenerate after injury or removal of their greater part. However, regeneration takes place only in conditions of continued circulation of lymph in the node or in relatively rapid recovery of the circulation. In stable obstruction of lymph drainage, on the contrary, there is no regeneration in the lymph node and gradual fibrosis takes place in its residual part.

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