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Received November 21, 1963. P.S.E.B.M., 1964, v116.

Comparative Immunology. Lymph Nodes in the Amphibian, *Bufo marinus** (29278)

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There are several statements that lymph nodes are not found in animals which are phylogenetically more primitive than birds (1-4). During comparative studies of antibody formation in poikilothermic vertebrates (5-7), structures resembling the lymph nodes of mammals were observed in the marine toad, *Bufo marinus*. Because of the potential importance of this finding to studies on the phylogeny of the immune response, experimental observations on the lymph nodes of *B. marinus* are presented here.

Methods and materials. Six adult marine toads were injected in the dorsal lymph sac with 0.25 to 1 ml of India ink 24 hours before sacrifice. The body cavities, neck, axilla, and inguinal areas were dissected. Samples of tissue that contained India ink on gross examination were fixed in neutral buffered formalin and subsequently embedded in paraffin.

Forty-nine additional adult animals being used in the study of antibody formation to bovine serum albumin (BSA) were also dissected. Twenty-six of these animals received a single injection of 10 mg of BSA in the dorsal lymph sac 4 to 24 days before sacrifice. Seventeen animals had received three 10 mg injections of BSA at intervals of one month or longer. They were sacrificed 2 to 18 days after the final injection. Six animals were non-injected controls. Samples of the liver, spleen, kidney and lymph nodes were fixed in cold 95% alcohol after the method of Sainte Marie(8). Using the fluorescent antibody technique, the tissues were examined for cells forming antibody to BSA(9). Fluorescein labelled BSA (0.5 mg/ml) was used for the direct demonstration of antibody forming cells. As controls, alternate sections were exposed to fluorescein labelled *B. marinus* serum protein (0.5 mg/ml). Tissue from non-injected toads as well as tissue from toads receiving BSA for the first time shortly before sacrifice were used as additional controls. Sections of all of the tissues were stained with hematoxylin and eosin and with methyl green pyronin(10). Sections were also stained with Masson's Trichrome stain, with Giemsa stain and by Gridley's reticulum stain(10,11).

Results. On gross examination 24 hours after the injection of India ink the liver, spleen, kidneys and lungs were black. In addition circumscribed black nodes of lymphoid tissues were noted along the great vessels at the base of the heart and their branches in the neck and axilla (Fig. 1). These masses of lymphoid tissue were associated with the arteries and veins but could be dissected readily from these large vessels. Four to ten nodes measuring from 0.5 mm to 8.0 mm in greatest diameter were noted on either side of the mid line in the upper thorax, axilla, and neck. Most of the nodules were from 1 to 4 mm in diameter. They were rounded, usually slightly flattened and sometimes were "bean" shaped. Similar nodules were found in all 55 animals studied. In

* Supported in part by NIH grants.

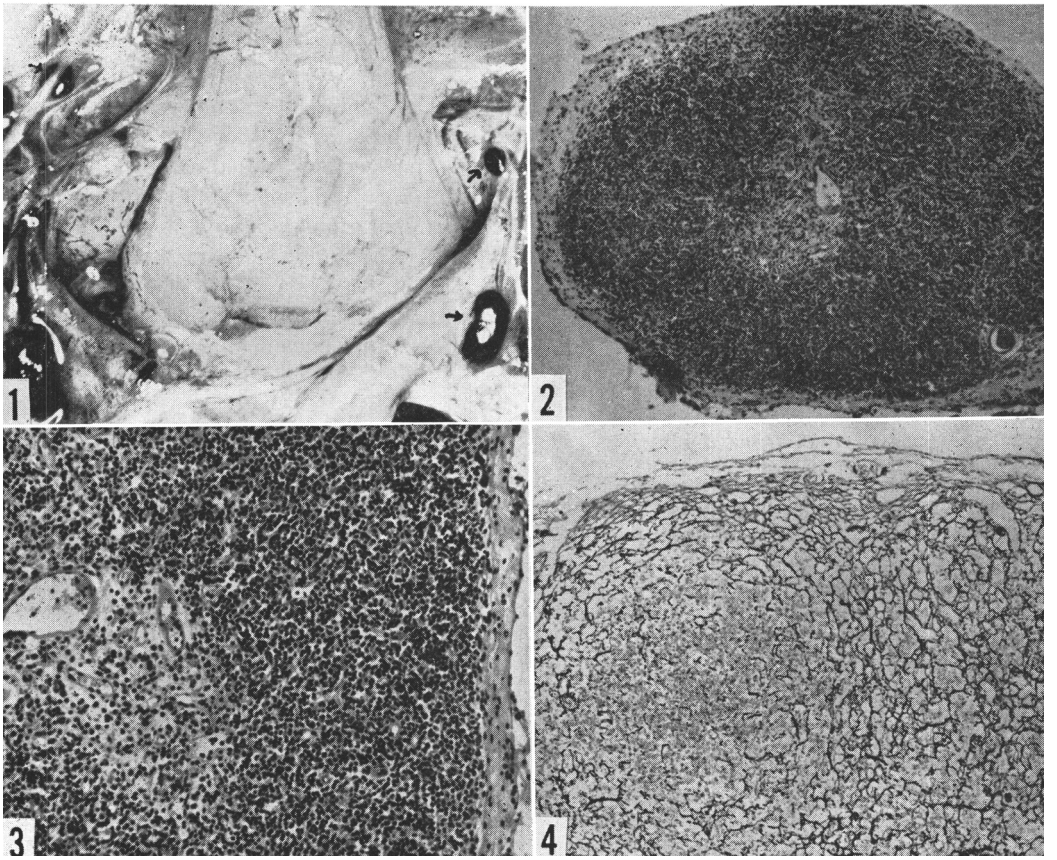

FIG. 1. Gross photograph of neck of a toad sacrificed 24 hr after injection of India ink. Note dark lymph nodes (arrow) on either side of esophagus which is in the mid line. Right and left truncus arteriosus are seen at bottom of picture. × 3.6.

FIG. 2. Photomicrograph of a lymph node showing capsule, a dense layer of lymphocytes beneath capsule and sparsely cellular central portion of node. Hematoxylin and Eosin. × 120.

FIG. 3. High power view of lymph node seen in Fig. 2. Note capsule (right) with underlying compact layer of lymphocytes. Center of the node (left) contains fewer lymphocytes. Hematoxylin and Eosin. × 300.

FIG. 4. Reticulum stain of a lymph node. Capsule (top) contains a few reticular fibers. Note paucity of reticular fibers in left center, an area densely populated with lymphocytes. The usual reticular framework of node can be seen on right. × 240.

animals that did not receive India ink the nodes were a light tan. Comparable masses of lymphoid tissue were not seen in the lower thorax, abdomen or inguinal areas.

Microscopically the nodules of lymphoid tissue were demarcated from the surrounding connective tissue by a thin capsule made up largely of collagen and reticular fibers (Fig. 2-4). Extensions of the capsule as trabeculae into the substance of the nodes were rarely seen. The internal architecture of the nodes varied but usually consisted of relatively homogeneous masses of lymphocytes set in a reticular framework (Fig. 2, 3, 4). The lymphocytes were at times more dense in the subcapsular areas and relatively sparse in other areas suggesting cortical and medullary zones. (Fig. 2, 3). However, these zones were not clearly demarcated and were not present in all nodes. Occasionally the masses of lymphocytes in subcortical areas had a nodular appearance suggesting a primary nodule. The reticular fibers in the center of a few such nodules were relatively sparse as is characteristic of the secondary centers seen in mammalian lymph nodes (Fig. 4). How-

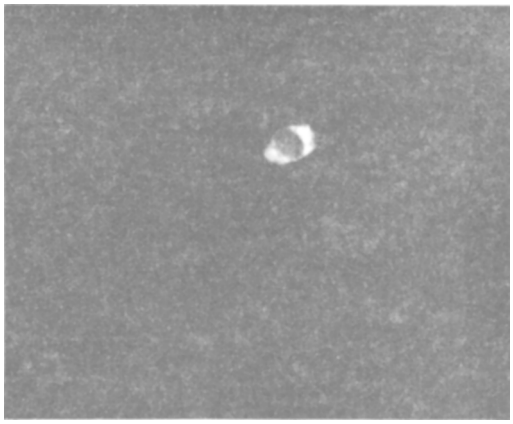

FIG. 5. An antibody forming cell as seen with ultraviolet microscope following exposure of tissue to fluorescein labelled BSA. Note non-fluorescent nucleus surrounded by brightly fluorescent cytoplasm. × 2160.

ever, secondary centers were not identified in the hematoxylin and eosin preparation of the lymph nodes.

Each lymph node contained a small artery with its many branches which usually penetrated well into the node. Associated venules were present. The intervening capillary network was evident. In addition irregular channels were noted in the intervening tissue which did not have a definitive basement membrane but which were partially lined by large irregular cells that phagocytized an abundance of India ink. These spaces usually did not contain erythrocytes. Phagocytic cells which accumulated India ink also were interspersed among the lymphocytes away from any definite vascular space.

All of the nodes contained cells with pyroniphilic cytoplasm. The nuclei of these cells were relatively large and surrounded by a thin rim of pyroniphilic cytoplasm. Occasionally cells with a nuclear-cytoplasmic ratio comparable to the mature plasma cells seen in mammals were noted. These cells were more abundant in animals known to be forming antibody than in non-injected controls.

The toads that were forming antibody to bovine serum albumin showed antibody forming cells in the lymph nodes in addition to the spleen, kidney and liver (Fig. 5). The number of antibody forming cells in the lymph nodes was comparable to the spleen, kidney and liver. Details will be reported elsewhere (12).

Discussion. Circumscribed encapsulated nodules of lymphoid tissue that form chains along the great vessels of the upper thorax, axilla and neck as described here in *Bufo marinus* are consistent with the gross pattern and configuration of lymph nodes in higher animals. The lymph nodes described in birds are also located in the region of the neck(2,4). On the other hand the lymph nodes in mammals are found not only in the neck but also in the thorax, abdomen and extremities. The basic microscopic structure of the lymph nodes, masses of lymphocytes set in a reticular framework and surrounded by a definitive capsule, is similar to mammalian lymph nodes. The absence of secondary centers even in animals stimulated by foreign antigens and known to be forming antibodies to the foreign antigens, the paucity of primary nodules, the poorly developed sinusoidal system, and the failure as yet to demonstrate connections between lymph nodes and lymph vessels are all points of difference between lymph nodes in toads and lymph nodes as they occur in mammals. However, the ability of these nodes to phagocytize foreign material such as India ink and to form antibody to bovine serum albumin indicates that the nodes function like the lymph nodes in mammals. We believe that the anatomical and functional characteristics of the lymph nodes as described in *Bufo marinus* are such that phylogenetically they may be considered comparable to mammalian lymph nodes.

The work presented here has made it evident that lymph nodes are not restricted to mammals and certain birds(1-4). Additional work is needed to assess the extent of their distribution in amphibian species and in other lower vertebrates; meanwhile projected studies of antibody forming tissues in amphibia and other poikilotherms should include a search for lymph nodes because of the potential significance of these organs in the immune response.

Summary. Encapsulated nodes of lymphoid tissue have been demonstrated in the upper thorax, neck and axilla of the amphibian, *Bufo marinus*. The nodes phagocytize India ink and form antibody to bovine serum

albumin. The anatomical and functional characteristics of these lymph nodes indicate that they are comparable to lymph nodes of mammals.

The authors express their grateful acknowledgement to Dr. Thomas E. Hunt and Dr. Clarence E. Klapper for helpful suggestions during preparation of the manuscript.

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Received March 10, 1964. P.S.E.B.M., 1964, v116.

Phospholipid Patterns in Livers Accumulating Fat. (29279)

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Although the rare earths so far have not been found in mammalian cells, these elements have been studied as anticoagulants and as possible internal emitters for therapeutic radiation; these earlier investigations have led to many reports of their biological effects(1-4). Our laboratory has been interested in the mechanism of triglyceride accumulation in fatty livers caused by intravenous administration of certain metals of the rare-earth series(5-9), since the fatty livers induced by these elements return to normal after one week and since the cycle of fatty infiltration can be repeated. The dual role of phosphatidic acid in triglyceride and lecithin synthesis in isolated enzyme systems (10) suggests that whereas we did not find a change in the total phospholipid phosphorus of the rare-earth fatty liver in an earlier study (5), alterations in the liver phospholipids probably occur under these conditions of rapid triglyceride accumulation in the liver. This paper reports our observations regarding the phospholipid components of fatty livers produced by cerium injections.

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Methods. Exerimental treatment of rats. The rats used in these experiments were CFN female rats weighing between 150 and 250 g. Fatty livers were produced by injecting cerous chloride (2 mg/kg) intravenously(5). The animals were decapitated 24 and 48 hours after the injection; all rats were fasted 24 hours before death with the exception of one group of control animals that were fed.

Extraction of liver lipids. The whole liver was removed from each rat promptly after killing and placed into an Osterizer blender bowl containing 100 ml of methanol. The blender was operated for approximately 5 sec to reduce the livers to small particles; longer periods produced emulsions or very fine suspensions that were difficult to separate in later stages. The contents of the bowl were transferred to a beaker and placed in a boiling water bath until the methanol had boiled for approximately 3 minutes. Then 100 ml of chloroform was added and the mixture was stirred. After the solids had settled, the clear supernatant was removed quantitatively by decantation into a course sintered glass funnel. The extraction was repeated 2 more times. The combined filtrates were trans-