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Distribution of Lympho-epithelial Tissues in the Larval South African Clawed Toad, *Xenopus laevis* Daudin

By

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(With 1 Text-figure and 2 Plates)

In recent years, the amphibians have received much attention in terms of both phylogenetic and ontogenetic origins of immunity in lower vertebrates (for recent reviews, see Du Pasquier, 1973; Cooper, 1973). Among well-studied anuran amphibians, the correlation between the ontogeny of the immune response and the lymphoid system development has been best documented in *Xenopus laevis* (Horton, 1969; Kidder *et al.*, 1973). Thus, transplanted as well as certain humoral immunities may be dependent on the lymphoid differentiation in the thymus (Horton and Manning, 1972, 1974; Turner and Manning, 1974). In morphological aspects, however, it seems rather peculiar that the larval *Xenopus* reportedly possesses a negligible amount of lymphoid tissues associated with the post-pharyngeal region of the gastro-intestinal tract, as contrasted with other species of anuran larvae where an abundant lymphocytic infiltration has been found along the entire length of the digestive tract (Du Pasquier, 1968; Horton, 1971a, b). The importance of the lymphoid distribution along the intestinal canal has been suggested in an extensive study by Fichtelius *et al.* (1968) of the morphological bursal equivalency in bursaless animals. In this respect, a careful examination of the distribution of lymphoid tissues in *Xenopus*, particularly in its larval stages, is yet rewarding for a fuller understanding of the cellular basis of immunity.

The present observation of larval lympho-epithelial tissues was undertaken as a preliminary study to understanding the mechanism of the immune response in an anuran *Xenopus*. Although a wide spectrum of lymphoid organ histogenesis has been available on the same material (Sterba, 1950; Manning and Horton, 1969), the results presented below will show that several additional numbers of lympho-epithelial tissue bodies do occur in both branchial and gastro-intestinal regions, which have not been described by previous researchers.

Material and Methods

The material used was the South African clawed toad, *Xenopus laevis* Daudin, which were maintained in our laboratory for several years. Spawning was induced by injections

of chorionic gonadotropin (Gonotropin: Teikoku Zoki Co.) into mature male and female toads. Developmental stages were determined according to the normal table of Nieuwkoop and Faber (1956). Fertilized eggs were allowed to develop in aerated aquaria containing dechlorinated tap water at 22-24°C. After stage 45, larvae were fed boiled alfalfa leaf powder; water and food were changed every other day.

For the observations described below, only the larvae were used, which had reached the appropriate developmental stages at the age given in the normal table. Several larvae at stages 42-58 and toadlets of 2-3 months of age after metamorphosis were fixed in Bouin's solution and preserved in 70% ethanol. Out of them, fifty stage 54-58 larvae, either unstained or stained with borax-carmin, were dissected and inspected under the stereoscopic binocular microscope for the grossly visible lymphoid microorgans occurring in the branchial region. Other specimens were used for histological observations.

For the histological study, larval head and trunk regions were embedded in paraffin using the methyl benzoate-paraffin method, serially sectioned at 6-8 μ in thickness, and were stained with Delafield's hematoxylin and eosin. The alimentary tracts were isolated from the toadlets, and submitted for histological observations as described above.

Observations

A survey of several developmental stage larvae proved that those at stage 56 can be taken as representative samples for describing the lymphoid organs. Therefore, unless otherwise stated, the following description will be concerned with the stage 56 larvae.

Ventral cavity bodies (VCBs) and dorsal cavity bodies (DCBs) in the branchial region

On gross examination of the larval branchial region, lymphoid microorgans were detected as white bodies (Fig. 2). Fig. 1 illustrates the location of whole lympho-epithelial tissue (LET) bodies found in the branchial region. These LET bodies were designated as "ventral cavity bodies (VCBs)" and "dorsal cavity bodies (DCBs)" according to their location.

VCBs are pairs of 4 groups of lymphoid microorgans which are situated in the ventral region of the branchial chamber near the opening to the opercular chamber (Fig. 5). Of these, the first to third bodies lie at the sites described by Manning and Horton (1969). Each first to third VCB either consists of 2-3 smaller sub-bodies or forms a fused larger body. Occasionally, the third pair extends caudally to the subarcual muscle, which is situated at the opening of the third branchial chamber to the opercular chamber. Besides these 3 pairs, the fourth VCBs were consistently found at the base of the second branchial arch. These bodies differ from the above-mentioned ones in that they appear at separate positions and never fuse into one (Fig. 1), although sometimes each separate body has 2-3 sub-bodies.

Other lymphoid microorgans found in the branchial region are 2 pairs of DCBs, which occur on the ceiling of the branchial chamber (Fig. 1); the anteriorly located ones (anterior DCBs) lie anterior to the level of the thymus on each side, whereas the posterior DCBs lie in the depth of the hollows formed by the third

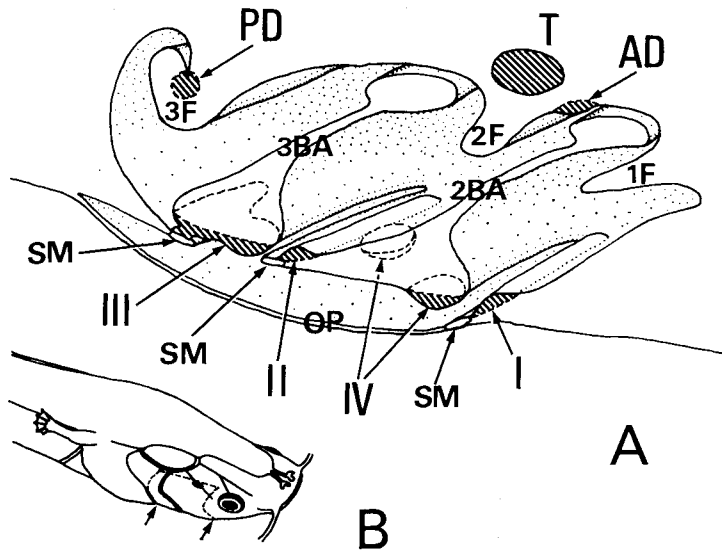


Fig. 1. Schematic illustration of the branchial region of stage 56 larva (A) cut at the level shown in B (dotted line). The location of 4 groups of VCBs (I, II, III and IV), anterior and posterior DCBs (AD and PD) are represented. Gill rakers are not shown in A. Anterior to the right. 2, 3BA, second and third branchial arch; 1, 2, 3F, first, second and third dorso-pharyngeal fold; OP, operculum; SM, subarcual muscle; T, thymus.

dorsal pharyngeal folds. Anterior DCBs, not consistently found in all larvae, are likely to correspond to the "extra-thymic lymphocytes" of Sterba (1950) and Manning and Horton (1969). The posterior DCBs were observed in all the larvae examined.

Histologically, VCBs and DCBs are characterized by a densely packed lymphoid accumulation immediately beneath the epithelium (Figs. 5 and 7). Although histological features of the epithelia in VCBs and the anterior and posterior DCBs are different from each other (*cf.* Figs. 3, 7 and 8), the epithelium covering the lymphoid accumulation is always heavily infiltrated by lymphoid cells, forming a "dome epithelium" by Fichtelius *et al.* (1968). Thus, at the base of such "dome epithelium" the basement membrane is not visible. Basal to the lymphoid mass there are no particular capsules or sacs. Blood and lymph capillaries are associated with the connective tissue surrounding these bodies (Fig. 4). The lymphoid accumulations are predominantly composed of small-to medium-sized lymphocytes. Besides these, other leukocyte series cells and macrophages are occasionally seen. It should be mentioned, however, that the latter types of cells are not especially abundant here as they are in the connective tissue. Mitotic figures are often encountered (Fig. 6). Another histological observation in the LET bodies is the occurrence of a peculiar "giant inclusion"

which possesses large and small particles deeply stainable with hematoxylin (Figs. 6 and 7). This inclusion is spherical in shape and measures more than $30\ \mu$ in diameter. Although this inclusion assumes a macrophage, it is apparently different from the latter in its lack of nucleus and unusually large size of body. Thus, this inclusion seems to be non-cellular in nature, possibly some fused body of disintegrated cells.

Lympho-epithelial tissues along the alimentary tract

Gross examination of the isolated larval alimentary tract revealed no lymphoid accumulations. Histological examinations revealed, however, that a vast number of lymphoid accumulations are present in several separate locations along the whole length of the alimentary tract, from the pharyngo-esophagus to the rectum. These lymphoid accumulations showed, without exception, an intimate relationship with the gut epithelium. The number of these scattered LET bodies was fairly variable, from 30 to 50 according to the individual larvae. In an extreme case, as many as 66 bodies were counted.

The long and coiled alimentary tract of a larva made it difficult to specify the exact locations of the LET bodies in the histological sections. It seems that most gut-associated LET bodies do not have a definite location, except for a pair of LET bodies which occur consistently on the ventral side at the boundary between the pharynx and esophagus (Fig. 9). In addition to the lymphoid aggregation just beneath the gastric epithelium, lymphoid accumulations were occasionally seen in the stomach among the gastric glands (Fig. 10). Since gastric gland cells are epithelial in nature, these accumulations may also be included in the LET bodies.

Among several gut-associated LET bodies, the degree of lymphoid invasion into the epithelium varies from case to case even in the same specimen. Thus, in a pair of bodies in the pharyngo-esophageal region and some of those in the ileum, the invasion is so extensive that they form "dome epithelia", like the LET bodies in the branchial region (Figs. 9 and 13). In other gut-associated bodies, the epithelium is invaded to a lesser extent without changes in the cell shape (Figs. 11, 12 and 14).

The cellular composition of the gut-associated bodies is essentially similar to that of the LET bodies found in the branchial region. Usually the basement membrane is not discernible and blood and lymph capillaries are always associated with the assemblage of lymphocytes (Figs. 9-14).

Lympho-epithelial tissues in developmental stages

The mode of differentiation of the thymus and the VCBs and anterior DCBs (extra-thymic lymphocytes) was similar to that described by Manning and Horton (1969); the thymic anlage appears at stage 42, detaches from the pharyngeal epithelium during stage 45, and the first thymic lymphoid differentiation occurs at stages 48-49. At stage 50, the first indication of both VCB and anterior DCB

differentiation occurs as a lymphocytic accumulation. This is also a case of both posterior DCBs and LET bodies in pharyngo-esophagus.

It should be mentioned that at stage 48 the small lymphoid aggregations closely associated with the gut epithelium appear for the first time. By stage 49 these lymphoid accumulations have grown to definite LET bodies (Fig. 15). During succeeding stages, the number of gut-associated LET bodies increases as elongation and differentiation of the alimentary tract progress and they reach maximum size at stages 55-57, as do the LET bodies in the branchial region.

The metamorphosed animals were found to possess numbers of separate LET bodies of relatively large size along the whole length of the alimentary tract. The lymphoid accumulations were particularly large in those located in the esophagus, stomach, and ileum. As contrasted with those in the larval stages, a "dome epithelium" with a heavy lymphoid infiltration was confined only to a pair of esophageal bodies. These observations on the metamorphosed *Xenopus* coincide well with those of Horton (1971) on a froglet of *Rana pipiens*.

Discussion

In their study of the histogenesis of larval *Xenopus* lymphoid organs, Manning and Horton (1969) found lymphoid tissues in the thymus, spleen, kidney (mesonephros), and liver. For the lympho-epithelial tissue, *i.e.* the assemblage of lymphoid cells closely associated with the epithelium, these researchers described 3 pairs of ventral cavity bodies, with the notion that there is a negligible amount of lymphoid accumulation in the post-pharyngeal alimentary tract. The present observation demonstrates additional numbers of LET bodies which constantly occur in the branchial region; the fourth VCBs and the posterior DCBs have not been described before. In addition, it should be stressed that, as contrasted with the previous observations (Manning and Horton, 1969), a vast number of scattered LET bodies were found along the whole length of the gastro-intestinal tract. A pair of bodies designated as anterior DCBs correspond to those previously referred to as "extra-thymic lymphocytes" (Sterba, 1950; Manning and Horton, 1969).

Because of the coincidence of the stage of their first appearance and of their apparent lympho-epithelial relationship, all the lymphoid bodies formed in the branchial region may well be referred to using the same terminology. However, the anterior DCBs might possibly differ from the others by their occasional absence as well as their greater dependency on the thymus, as discussed below.

The location and number of VCBs in larval amphibians differ considerably among several species (*cf.* for *Rana pipiens*, Horton, 1971; for *R. catesbeiana*, Cooper, 1967a, b). This difference is not surprising in view of the gross anatomical difference of the branchial region among species, particularly the uniqueness in *Xenopus*. The variability might also reflect the function of these temporary, gill-associated microorgans, although their exact function is still the subject of mere speculations (*cf.* Manning and Horton, 1969; Horton, 1971a, b).

The lympho-epithelial tissues associated with the digestive canal deserve attention because of their possible equivalency to the bursal function in birds. Thus, Fichtelius *et al.* (1968) described a number of examples in the poikilotherms in which the gut-associated LET bodies were found. However, these workers failed to find the LET bodies in question in the adult *Rana catesbeiana*, *Bufo marinus*, and *Necturus maculosus*. On the other hand, these tissues have been described in larval *Alytes obstetricans* (Du Pasquier, 1968) and in both larval and adult *Rana pipiens* (Horton, 1971a, b). The present observations clearly show that the gut-associated LET bodies do occur in both larval and adult *Xenopus*, alike in *Rana pipiens*. Because of the profound reorganization of the intestinal tract during the metamorphic stage, it is difficult to ascertain the identity of each gut-associated LET body found before and after metamorphosis.

Cooper *et al.* (1967) postulated several morphological criteria for the bursa-equivalency of lymphoid tissues. Among the criteria, relevant to the present observations are that they are (1) gut-associated lympho-epithelial tissues and (2) essentially early-life-tissues showing some involution later in life. The gut-associated LET bodies observed in the present study may fulfill these postulations. Further experimental analyses are required to clarify whether or not these lymphoid bodies in amphibians play the supposed role of lympho-epithelial tissues such as the Peyer's patches, sacculus rotundus, and the appendix in rabbits (Fichtelius *et al.*, 1968; Good, 1971).

Manning (1971) reported that the thymectomy from stage 49 *Xenopus* larvae caused a moderate or severe depletion of the lymphocyte population in the VCBs (and extra-follicular spleen). The thymectomy at stage 48 impaired the animal's subsequent alloimmune response capacity and its response following the administration of human gamma globulin and sheep red blood cells (Horton and Manning, 1972, 1974; Turner and Manning, 1974). Using the same material, a thymectomy at an extremely early stage (stage 45) was successful in the present laboratory (Tochinai, 1975). Histological examination of the thymectomized animals revealed that the lymphoid accumulation in all the LET bodies was considerably depleted and lacked a "dome epithelium". Of particular note, the effect was so severe in the anterior DCBs that no thymectomized animals possessed this organ. The effect was considerable also in the VCBs. However, it should be emphasized that there are certain degrees of lymphoid accumulations which are still discernible in the posterior DCBs and the LET bodies associated with the alimentary tract. It is quite interesting to ask where these lymphocytes originated in the thymusless animals, and whether or not these cells represent the thymus-independent system in the amphibians. The immunological reactivities in both the cellular and humoral aspects of these thymectomized animals will be presented elsewhere.

Summary

The locations and histological features of lympho-epithelial tissue (LET) bodies were described in the larvae of *Xenopus laevis*. There are pairs of 4 ventral cavity bodies (VCBs) and 2 dorsal cavity bodies (DCBs) in the branchial region. In addition, a number of scattered LET bodies were found along the alimentary tract from the pharyngo-esophageal region to the rectum, although their number and location were variable according to the specimens. All the LET bodies in the branchial region and some of those in the alimentary tract possess the epithelia which are extensively infiltrated by lymphoid cells, forming "dome epithelia". Examinations on the earlier stage larvae showed that lymphoid accumulation is first detectable at stage 50 in LET bodies of the branchial region and at stage 48 in gut-associated bodies. The larval LET bodies attain their maximum size at stages 55-57. Additional observations on the toadlets revealed the presence of LET bodies associated with the gut epithelia covering area from the esophagus to the rectum. Some discussion of the distribution and histological features of the LET bodies in comparison with those in other amphibian species is given, as well as of their possible function in the immune response.

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Explanation of Plates

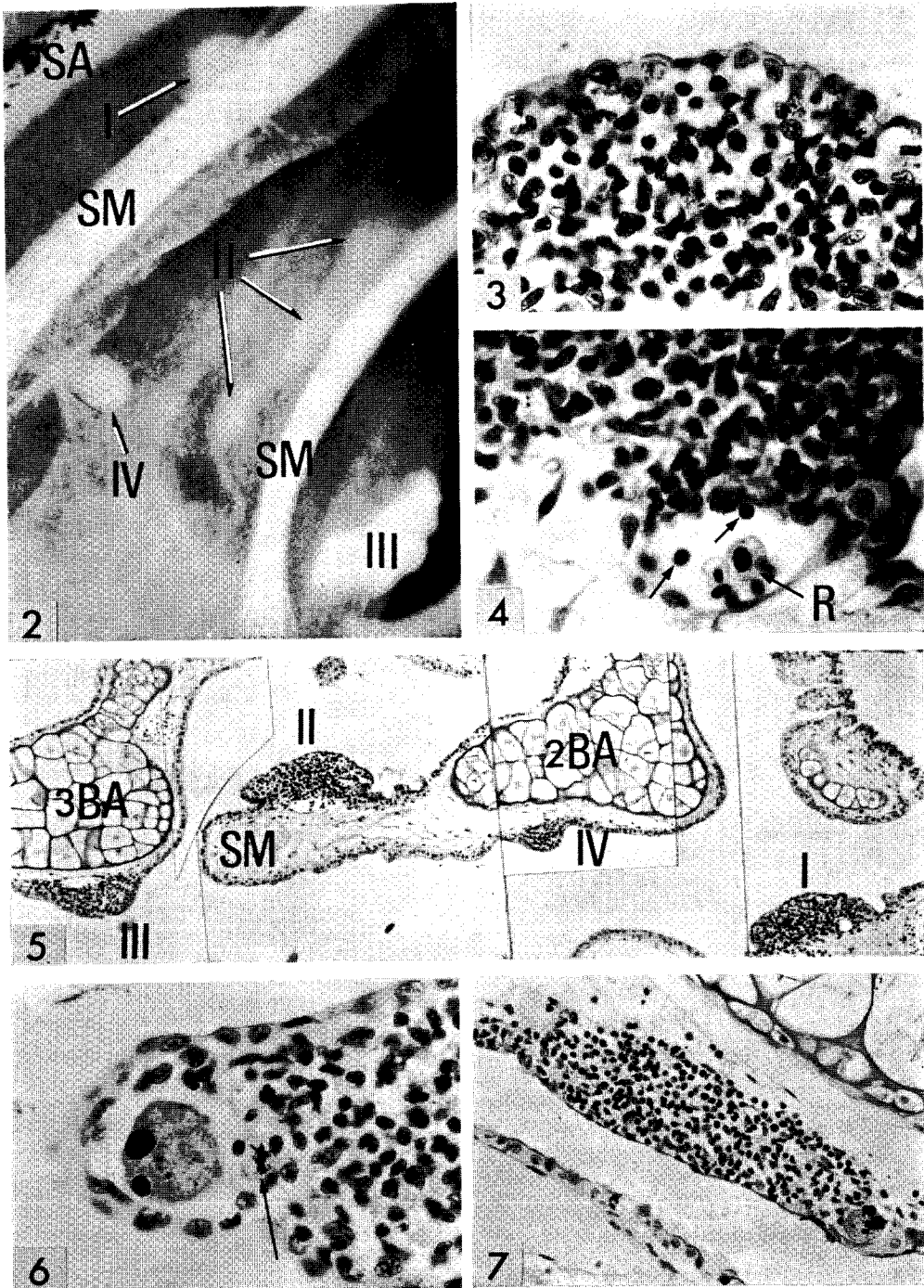
Fig. 2. Ventral view of the right branchial region of a Bouin-fixed larva, showing 4 groups of VCBs (I, II, III, and IV) seen as white masses. The first and second VCBs are seen opaquely through the floor of branchial chamber. Operculum has been removed. Anterior to the upper. SA, systemic arch; SM, subarcual muscle. $\times 80$.

Fig. 3. Apical portion of first VCB, showing accumulation of small and medium lymphocytes together with a loose distribution of connective tissue cells. Note a heavy infiltration of lymphocytes into epithelium (a "dome epithelium"). $\times 500$.

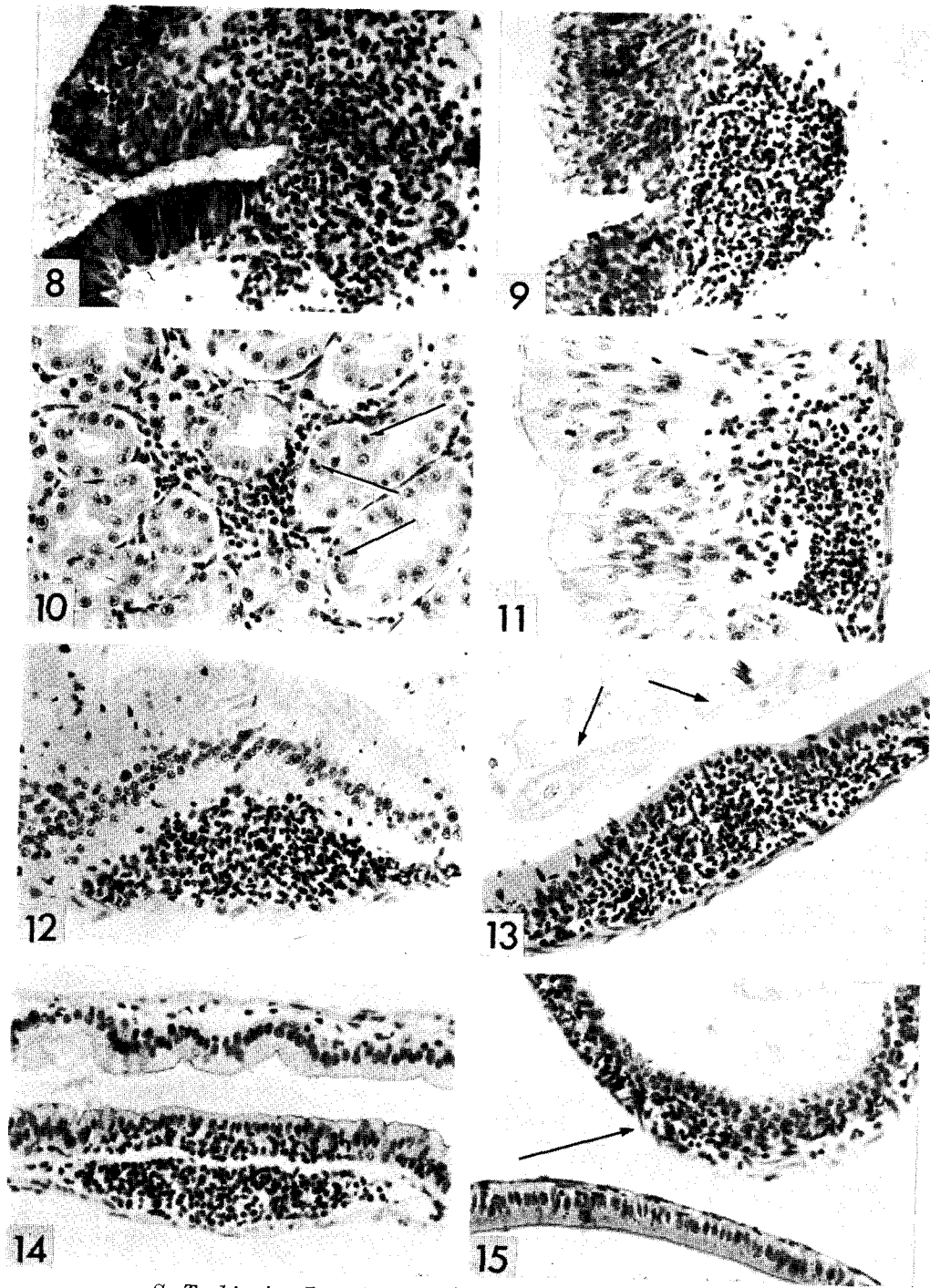
Fig. 4. Basal portion of first VCB, in association with blood vessel containing erythrocytes (R) and small lymphocytes (arrows). Note there is no definite demarcation between lymphoid aggregation and surrounding connective tissue. $\times 500$.

Fig. 5. Low magnification of a sagittal section through the branchial region, showing the situations of 4 VCBs (I, II, III, and IV). Compare this figure with Figs. 1 and 2. Anterior to the right. 2, 3BA, second and third branchial arch; SM, subarcual muscle. $\times 75$.

Fig. 6. Apical portion of third VCB. Note a "giant inclusion" containing basophilic particles of various sizes. Right of the inclusion, a mitotic figure (arrow) is seen. $\times 500$.



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Fig. 7. Sagittal section of an anterior DCB forming a "dome epithelium". Note the presence of a "giant inclusion" similar to that shown in Fig. 6. $\times 200$.

Fig. 8. Cross section of a posterior DCB, showing a heavy lymphoid invasion into ciliated pseudo-stratified columnar epithelium. $\times 200$.

Fig. 9. Section through the pharyngo-esophagus, showing the lymphoid invasion into the epithelium, similar to that found in the branchial region (Fig. 8). $\times 200$.

Fig. 10. Lymphoid accumulation found in connective tissue among the gastric glands. Arrows indicate small lymphocytes occasionally present in gland cells. $\times 200$.

Fig. 11. LET body found in the duodenum, with a lymphoid aggregation between its epithelium and peritoneum. $\times 200$.

Figs. 12, 13 and 14. LET bodies found in the ileum (Figs. 12 and 13) and rectum (Fig. 14), showing different degrees of lymphoid invasion into the epithelium. Lymphoid accumulation in Fig. 13 is so heavy as to form a "dome epithelium". Arrows in Fig. 13 indicate parasiting *Opalinida*. The space in lymphoid accumulation in Fig. 14 is probably an artifact made during the preparation. $\times 200$.

Fig. 15. Gut-associated LET body (arrow) found in a stage 49 larva. Note the intimate relationship of accumulated lymphocytes with the gut epithelium. $\times 200$.
