ALKALINE PHOSPHATASE AND PERIODIC ACID-SCHIFF REACTIONS IN THE THYMUS OF *CALOTES VERSICOLOR* (DAUD.)

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CONTENTS

INTRODUCTION

Histological and histochemical studies on the thymus of mammals (Dearth, 1928; Kingsbury, 1928 and 1941; Smith and Parkhurst, 1949) have shown that the multicellular Hassall's corpuscles resemble to a large extent the keratinizing cells of the stratified squamous epithelium. The application of Gomori's (1939) technique for alkaline phosphatase and the periodic acid-Schiff reaction of Hotchkiss (1948) has led to the conclusion that in two widely different cell elements such as these, the same essential processes of the production and deposition of keratin are taking place. It is well known that in reptiles (Jordan and Looper, 1928) these complex type of concentric corpuscles of Hassall, so characteristic of mammalian thymus, are wanting, but instead, there are unicellular Hassall's corpuscles and a group of interesting cell elements known as multinucleated plasmodial masses. From histogenetic point of view the origin of multicellular Hassall's corpuscles of mammals is not clear. It is also not clear which cell elements in the mammalian thymus represent the unicellular Hassall's corpuscles or multinucleated plasmodial masses, though the plasmodial masses resemble to some extent the thymic canals of the mammalian thymus.

It was felt that application of histochemical techniques to the thymus of a reptile would perhaps yield information useful in establishment of a histogenetic relationship of these diverse cell elements in the thymus. The lizard, *Calotes versicolor*, served as the animal of choice in this study.

MATERIAL AND METHODS

The thymus of *Calotes versicolor* was dissected out and fixed in chilled 80% ethyl alcohol for alkaline phosphatase and ice-cold Rossman's fluid for periodic acid-Schiff reaction. Paraffin sections of 6 μ thickness were cut and the technique of Gomori (1939) for demonstration of alkaline phosphatase was employed. No counter-stain was used and control sections were not incubated in the substrate. A few slides were

also stained in Heidenhain's haematoxylin and Mallory's triple stains. Deparaffinized sections of material fixed in Rossman's fluid were stained by the periodic acid-Schiff method according to the procedure of Hotchkiss (1948). For histological studies the material was fixed in Zenker's formol-acetic and Bouin's fluids. Sections 10 μ thick were cut and stained in Heidenhain's haematoxylin, Mallory's triple and Shorr's differential stains.

Observations

(*a*) *Alkaline phosphatase activity.*

In the thymus of *Calotes versicolor* the multinucleated plasmodial masses arise as fusion products of reticular cells. Dissolution of their cytoplasm takes place at the periphery and extends inwards forming lacunae lined by flattened reticulum with central degenerating masses (Figs. 1, 4 and 5). In these degenerating masses granules of irregular shape appear, stained dark in Heidenhain's haematoxylin, dark blue in Mallory's triple and deep green with Shorr's differential stains. It is hard to determine the cells in which these granules appear, as cell boundaries are indistinct. Many a time the central portion of plasmodial mass is compact and appears as a solid homogeneous mass stained heavily in all stains (Figs. 4 and 5).

An examination of sections prepared according to Gomori's (1939) method for alkaline phosphatase shows that the capsular wall, trabeculae, endothelia and plasmodial masses are positive to this reaction. In the plasmodial masses, where degeneration has not yet set in, the reaction is almost negative. As soon as degeneration starts, dark granules appear indicating the sites of alkaline phosphatase activity (Fig. 6). Gradually, as degeneration progresses, black patches of cobalt sulphide appear, indicating increased amounts of this enzyme. It is interesting to note that the wall of lacunae containing these degenerating plasmodial masses does not indicate any phosphatase activity (Figs. 2 and 6).

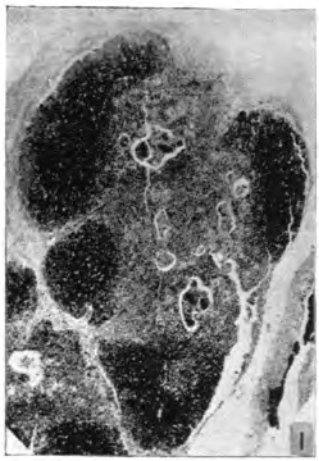
This enzyme is absent in unicellular Hassall's corpuscles and basophilic granulocytes. The thymocytes, however, offer a different picture. Those that are in the proximity of blood vessels and plasmodial masses are positive, whereas those that are away from them show faint or negative reactions. It is difficult to provide an adequate explanation for this fact where morphologically the same type of cell, the thymocyte, gives two different reactions depending on its location with reference to other cells showing a positive reaction. It leads me to the conclusion that perhaps a diffusion from blood vessels and plasmodial masses which show a high concentration of this enzyme, takes place, and that the thymocytes in close proximity to them give a positive reaction. Doyle (1950) has found a similar phenomenon in the lymphocytes of the appendix of irradiated rats.

(*b*) *Periodic acid-Schiff reaction* (PAS).

The capsular wall, trabeculae, endothelia, basophilic granulocytes and plasmodial masses are positive to this reaction. The plasmodial masses, however, show a particularly intense one. Here too, as in alkaline phosphatase, the reaction varies with the progressive degeneration of cell masses. Newly formed plasmodial masses present a pale reaction, while in those where degeneration has advanced considerably the reaction is deep red (Figs. 3 and 7). The walls of the lacunae of the plasmodial masses always present a negative reaction, while the endothelium of the blood vessel is positive.

The basophilic granulocytes are generally in groups and many are multinucleated (Figs. 8 and 9). They are filled with refringent granules. In preparations stained with Mallory's triple, they are coloured pale blue and appear light green when stained with Shorr's method. These cells show a strong positive reaction to the PAS technique (Fig. 9) and appear to have coarse red granules in their cytoplasm.

The unicellular Hassall's corpuscles and thymocytes are negative to this reaction.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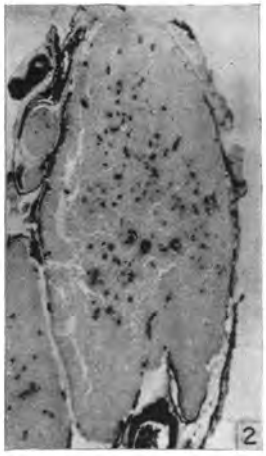
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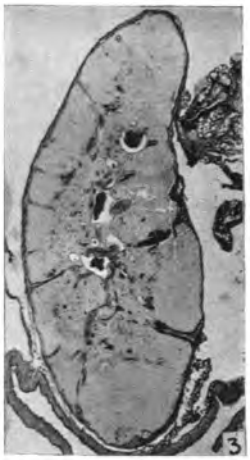
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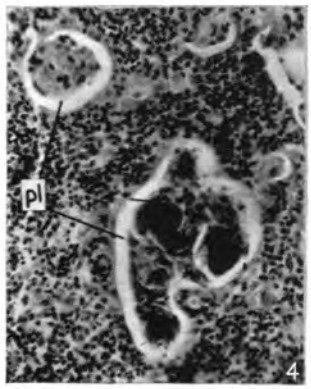
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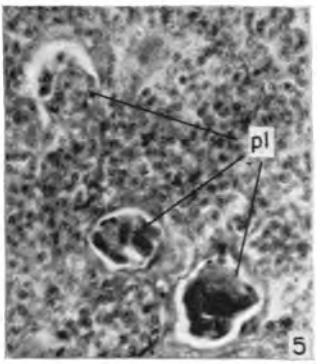
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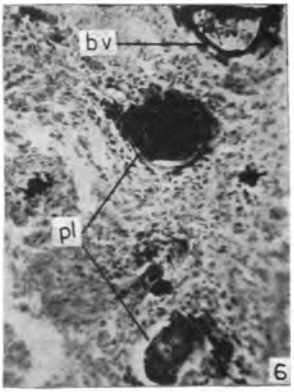
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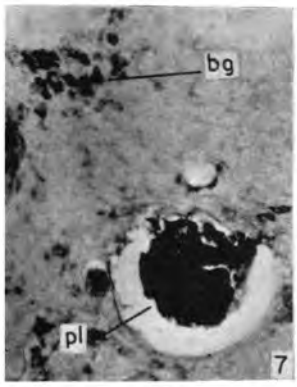
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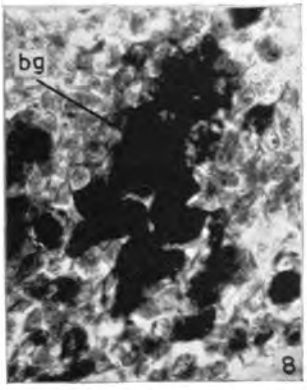
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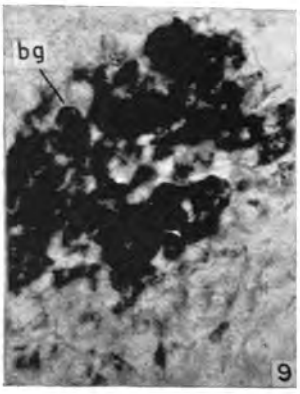
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Discussion

The mammalian thymus differs from that of reptiles in the presence of the complex type of Hassall's corpuscles. These are large cell groups which lie in the medulla and which display a concentric arrangement of fusiform cells (Jordan and Horsley, 1927; Dearth, 1928; Kingsbury, 1928; Smith and Parkhurst, 1949). Such corpuscles are absent in the thymus of amphibians and reptiles, but in their place occur unicellular Hassall's corpuscles, much smaller in size and consisting of a single cell element. In addition to the unicellular Hassall's corpuscles there are in the thymus of these vertebrates, groups of cells fused to form plasmodial masses which are derived from reticular cells (Jordan and Looper, 1928; James, 1939; Fabrizio and Charipper, 1941).

The relationship between these different cell elements is a subject of much interest and an understanding of the homology becomes clear on the employment of specific histochemical techniques. The work of Smith and Parkhurst (1949) has shown that the concentric corpuscles of the mammalian thymus are positive for alkaline phosphatase and PAS reactions. It has been interpreted that during degeneration of these corpuscles there is production and deposition of keratin. Many investigators (Dempsey and Wislocki, 1945; Bradfield, 1950; Doyle, 1951; Ring, 1952) are of the opinion that alkaline phosphatase is probably related to the elaboration of keratin. Comparative studies on the Hassall's corpuscles of the thymus and skin of guinea-pig have shown that in both cases the production and deposition of keratin are closely associated with alkaline phosphatase activity and PAS reaction (Smith and Parkhurst, 1949).

The present studies reveal some interesting differences between the Hassall's corpuscles of the mammalian thymus and those of reptiles. The multicellular Hassall's corpuscle of the mammalian thymus is strongly positive to alkaline phosphatase (Smith and Parkhurst, 1949), whereas the unicellular Hassall's corpuscle of the thymus of *Calotes versicolor* is completely negative for this enzyme. Thus these two cell elements—the concentric corpuscles of Hassall of the mammalian thymus and unicellular Hassall's corpuscles of the reptile—though resembling externally in the concentric system of striations (Jordan and Looper, 1928), are widely different in their reactions to alkaline phosphatase. The absence of this enzyme from the unicellular Hassall's corpuscles of reptiles shows that there is probably no deposition of keratin in these cells.

The above conclusion is supported by the results of PAS reaction. Recent work of Moog and Wenger (1952) has shown that PAS reactive substance and alkaline phosphatase are physiologically related and that wherever the latter is present the PAS reaction is positive. It is known from the studies of Smith and Parkhurst (1949) that the concentric corpuscles of mammalian thymus are positive to PAS reaction, just as they are positive for alkaline phosphatase. On the other hand, the unicellular Hassall's corpuscles in the thymus of *Calotes versicolor* are negative to both PAS and alkaline phosphatase reactions. This indicates that these thymic cell elements in the two vertebrate classes are different in function.

The plasmodial masses in the thymus of *Calotes versicolor*, however, react differently. In contrast to the unicellular Hassall's corpuscles, these masses show intense alkaline phosphatase and PAS reactions (Figs. 2, 3, 6 and 7). The significance of the positive alkaline phosphatase and PAS reactions in the plasmodial masses and negative reactions in the unicellular Hassall's corpuscles is interesting. Jordan and Looper (1928) have tried to establish a genetic relationship between the unicellular Hassall's corpuscle of the thymus of the box turtle (*Terrapene carolina*) and the mammalian Hassall's corpuscle, because of the superficial resemblance in these two cell elements. They consider the multinucleated plasmodial masses, which do not show any characteristic concentric arrangement of cells, as the progenitors of thymic canals of mammals. The alkaline phosphatase and PAS reactions in the thymus of

Calotes versicolor indicate that the multinucleated plasmodial masses resemble the complex type of Hassall's corpuscles of mammals as both show intense positive reactions, whereas the unicellular Hassall's corpuscles are negative and hence cannot be compared as far as these reactions are concerned. It is suggested that the multicellular Hassall's corpuscles of the mammalian thymus have probably been derived from the reptilian thymic plasmodial masses.

It was observed that another group of cell elements in the thymus of *Calotes versicolor* behaved in an interesting manner to these two reactions. The basophilic granulocytes are positive to PAS but are negative to alkaline phosphatase. It is well known that in tissues where the PAS reaction is positive, the interpretation is that large amounts of lipids and polysaccharides are being produced (Hotchkiss, 1948; Moog and Wenger, 1952). The fact that these granulocytes are strongly positive to PAS but negative to alkaline phosphatase indicates the presence of compounds in their cytoplasm which can be oxidized to aldehydes, probably of a lipid nature. That is also the conclusion of Loewenthal and Smith (1952) in the foamy cells of the thymus of mouse.

Summary

1. The unicellular Hassall's corpuscles of the thymus of *Calotes versicolor* are negative to alkaline phosphatase and periodic acid-Schiff reactions and are not comparable with the multicellular Hassall's corpuscles of the thymus of mammals, which are positive to these two reactions.

2. The plasmodial masses of the thymus of *Calotes versicolor* resemble to a very great extent the concentric corpuscles of Hassall of the mammalian thymus, because of the striking resemblance of these two cell elements in their reactions to Gomori's treatment and PAS technique. The intensity of alkaline phosphatase and PAS reactions in these multinucleated plasmodial masses of the thymus of *Calotes versicolor* probably depends upon the degree of degeneration and keratin formation.

3. The basophilic granulocytes are generally in groups. They are large, multinucleated and contain refringent granules. An intense reaction of these cells to PAS treatment is probably due to elaboration of lipids. Generally they are negative to alkaline phosphatase.

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Explanation of Photomicrographs

Key to Lettering

bg.—Basophilic granulocytes.

bv.—Blood vessel.

pl.—Plasmodial mass.

Plate XXI

Fig. 1. Sagittal section of thymus of *Calotes versicolor* showing degenerating plasmodial masses in the medulla. Bouin: Heidenhain's haematoxylin. ×40.

Fig. 2. The thymus showing the distribution of alkaline phosphatase. Gomori's technique. ×30.

Fig. 3. The thymus showing the regions positive to PAS reaction. ×33.

Fig. 4. Enlarged view of sagittal section of thymus showing degenerating plasmodial masses. Bouin: Heidenhain's haematoxylin. ×210.

Fig. 5. A portion of the thymus enlarged to show the progressive degeneration of multinucleated plasmodial masses. Zenker's formol-acetic: Mallory's triple. ×360.

Fig. 6. Enlarged view of sagittal section of thymus. Degenerating plasmodial masses and endothelia of blood vessels show high concentration of alkaline phosphatase. Thymocytes near regions of high concentration are also heavily stained. Gomori's technique. ×216.

Fig. 7. A portion of the thymus enlarged. Only the degenerating portion of the plasmodial masses and basophilic granulocytes are positive to PAS reaction. ×225.

Fig. 8. A portion of thymus enlarged to show the grouping of the basophilic granulocytes. Bouin: Shorr's differential stain. ×570.

Fig. 9. Enlarged view of a portion of thymus to show the positive reaction of the basophilic granulocytes to PAS technique. ×570.

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