HISTOCHEMICAL ANALYSIS OF PAS-POSITIVE SUBSTANCES IN THE RETICULAR CELLS OF THE RABBIT VERMIFORM APPENDIX

(UDC 616.346-018.24-008.9-092.18)

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(Presented by Active Member AMN SSSR G. V. Vygodchikov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 60, No. 7, pp. 115-118, July, 1965
Original article submitted February 11, 1964

We demonstrated in previous works that the cytoplasm of the reticular cells of the lymph follicles of the vermiform appendix of the mature rabbit contains a lipogenic pigment in appreciable quantities [1-2]. At the same time there are indications in the literature that phagocytosis of PAS-positive microbes penetrating into the wall of this organ is accompanied by the accumulation, in the reticular cells, of their products of destruction in the form of agregations of PAS-positive bacterial polysaccharides [1, 4, 5]. Since this conclusion was made on the basis of an analysis which actually did not take into account the presence in the cytoplasm of phagocytes of PAS-positive material—lipochrome, having a chemical nature differing from carbohydrates, it became necessary to reexamine the previously expressed assertions with consideration of this latter circumstance.

The present investigation is devoted to a study of the possible relationship within macrophages between two groups of PAS-positive substances (polysaccharides and lipogenic pigments—lipochromes).

METHOD

Permanent mounts were prepared from cryostat and paraffin sections. Staining was done by the PAS method and 0.01% solution of toluidine blue at various pH values (1.9-6.6), some of the sections being preliminarily sulfated. Sections stained with toluidine blue were examined flooded, and also after passing through alcohol-acetone and embedding in Canadian balsam.

RESULTS

1. PAS-reaction. PAS-positive microbes in the lymph follicles of the rabbit appendix were found between lymphocytes and also within large reticular cells-microphages where they are most frequently arranged chaotically. However, in certain cases the microbes in the macrophages proved to be clumped and surrounded, so to speak, by a common vacuole which was also PAS-positive stained.

In the middle and basal parts of the lymph follicles in the cytoplasm of the macrophages we detected polymorphous granules of a PAS-positive pigment which made it difficult to recognize the phagocytized microbial cells against their background. Microbial cells were not seen at all in the reticular cells forming aggregation around the centers of multiplication and which, as a rule, were filled with PAS-positive pigment masses. The lipochromes comprising these masses were unevenly (in intensity) stained PAS-positively. In the aggregations of reticular cells we found individual elements whose cytoplasm was uniformly filled with minute granules of a yellow-brown pigment which did not give a PAS-positive reaction. Sometimes it was possible to detect in them individual PAS-positive microbial bodies.

2. Staining with a 0.01% solution of toluidine blue. With this method the pigment granules were stained different hues of green. The affinity for the stain was severely weakened in an acid medium (pH $\leq$3.0). Only

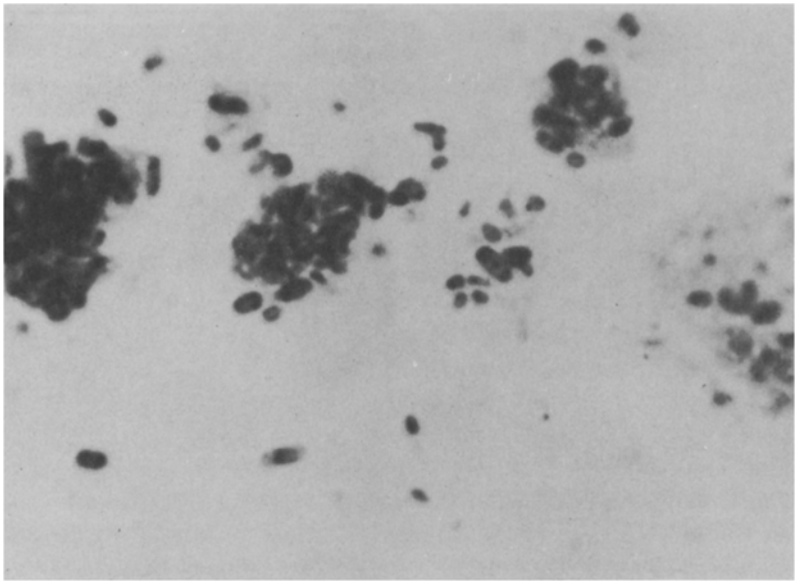

Fig. 1. Microbes in macrophages of the lymph follicle of the rabbit appendix. Various stages of destruction of microbes within the phagocytes. Fixation by Shabadash's method. Sulfating—toluidine blue. Objective 90×, ocular 8×.

minute PAS-negative pigment granules which solidly filled the cytoplasm of certain reticular cells arranged as aggregations around the centers of multiplication, yielded slight metachromasia which was also retained under these conditions (pH $\leq$3.0). It was most noticeable upon examination of the flooded mounts, but for all practical purposes completely disappeared after dehydration of the stained sections in alcohol-acetone and embeddment in balsam. The microbes remained unstained. In the sulfated sections basophilia of the pigment granules was retained, but became more evident at pH $\leq$3.0. The microbial cells upon staining demonstrated distinct metachromasia (Fig. 1). This stain was distinguished by a high alcohol-fastness. An examination of the flooded preparations, while yielding a general idea about the distribution of the areas of metachromasia in the sections, did not enable us to elicit with what cytological elements it was associated owing to the unfavorable optical conditions. On the other hand, in the preparation which passed through the alcohol-acetone and was embedded in balsam the interrelationship between the microbial cells and the pigment granules with such staining were clearly demonstrated (Fig. 2). The microbes (red color) were situated both on the surface and within the granules which were orthochromatically stained green. In this case the morphology of the microbial cells was unchanged in most cases: their outlines, membranes, and internal, markedly metachromatic structures were distinctly seen. Using the described method we were not able to elicit in the cytoplasm of the reticular cells amorphous inclusions which would yield a clear γ-metochromasia. It was clearly seen that the latter is associated only with elements of the microbial cells. Even more demonstrative pictures could be observed when the unfixed cryostat sections before staining were held for 48 h at 55° in a mixture of chloroform and methyl alcohol. Such treatment did not have a noticeable effect on the pigment granules, basophilia of the cytoplasm of the macrophages disappeared, and metachromasia of the microbial cells became even more evident.

The mechanism of histochemical differentiation of PAS-positive bacterial polysaccharides from lipochromes upon staining of sulfated mounts with toluidine blue and the specificity of this method for carbohydrates were examined previously [3]. It is necessary to note that the use of alcohol in this method of staining is a necessary methodological procedure which makes it possible to accurately localize the areas of distribution of the artifactitious ethyl ethers of polysaccharides [6, 12-18]. Here it was found that polysaccharide masses which were not bound with the structures of the microbial cells were absent in the cytoplasm of the reticular elements. This should not be unexpected since it is difficult to assume that the products of the enzymatic degradation of bacterial polysaccharides within the phagocytes would be retained without substantial changes of their original properties and especially their polymeric state which, as is known, is a necessary condition for their histochemical demonstration [11]. An intimate relation between microbes and lipochrome granules in the reticular cells was established. Taking into account

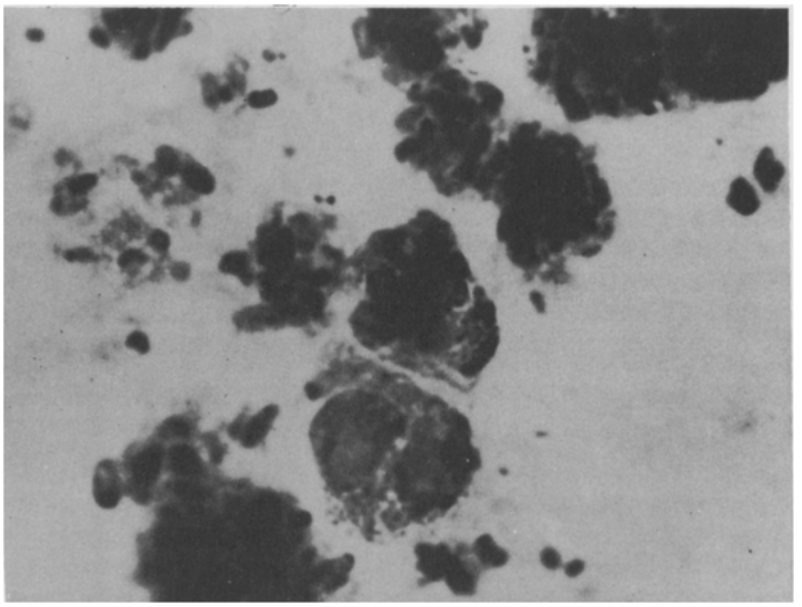

Fig. 2. Macrophages of the lymph follicle of the rabbit appendix. Morphology of phagocytosis of microbes against a background of basophilic cytoplasm of macrophages and pigment granules. Conditions of staining and photomicrography are the same as in Fig. 1.

the indications of the role of surface-active inclusions in the development of autooxidation processes [7-10], this is remarkable since it makes it easier to understand the conditions under which the formation and accumulation of lipogenic pigments apparently occur in the rabbit appendix. These findings indicate that the PAS-reaction cannot be used under the given conditions for the histochemical differentiation of the two types of PAS-positive substances discussed here and show to what extent the interpretation of the observed morphological pictures depends on the correct selection of the staining technique.

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