ON THE NATURE OF SCHIFF-POSITIVE PIGMENT IN THE RETICULAR CELLS OF RABBIT APPENDIX

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It has been shown in several publications dealing with the mechanism of phagocytosis that in the wall of the appendix of adult rabbits [2, 3, 7] and in the cytoplasm of reticular cells in the lymphatic follicles of this organ, there regularly appear certain Schiff-positive inclusions. These inclusions consist of large and small polymorphic granules, some of which stain with Sudan black B in paraffin sections [1].

The present publication presents additional information on the histochemistry of these Schiff-positive cytoplasmic inclusions which, having their own natural coloration, may be regarded as a pigment.

METHODS

Frozen sections of adult rabbit appendix wall, as well as sections of tissue fixed by the method of Shabadash, and embedded in paraffin, were used in this study. Sections were stained by Schiff's method [11, 4]: formic acid—Schiff [9, 14], Kasell's method [13], Schmorl's method [19] and for plasmalogens [5, 14, 20]. Simultaneously the sections were stained with Sudan stains for acid fastness (according to Ziehl-Nielsen) and for argentaffin reaction (according to Masson-Fontana). Some of these methods were used in combinations with each other, and following various blocking methods [14, 17-19], as well as after extraction of lipids with pyridine, methyl alcohol-chloroform, xylol, etc. Frozen sections were used also in a study of primary fluorescence.

RESULTS

1. General morphology of the inclusion bodies. Polymorphic, usually oval granules, yellow or yellow-brown in color measuring 5-20 μ, (some granules are strongly refractile).

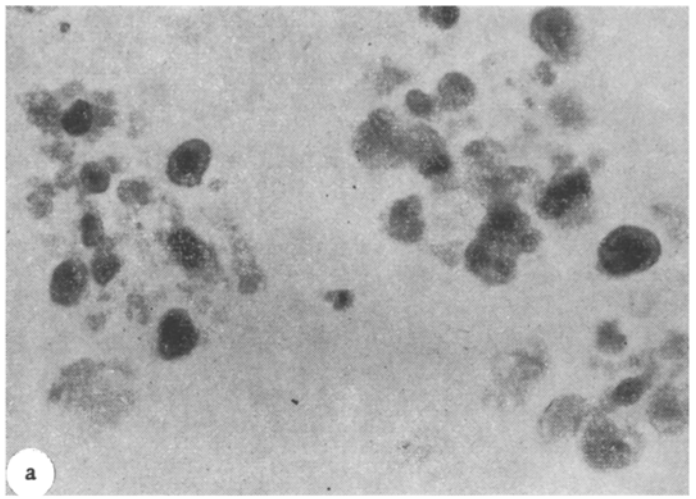
2. Schiff's reaction. Most of the granules stain in different shades of red (pale orange to dark raspberry). A small portion of the granules retain the natural yellowish color. Coloration does not disappear after treatment of the sections with saliva, but is reversibly blocked by acetylization and is considerably weakened by a preliminary treatment with sulfate.

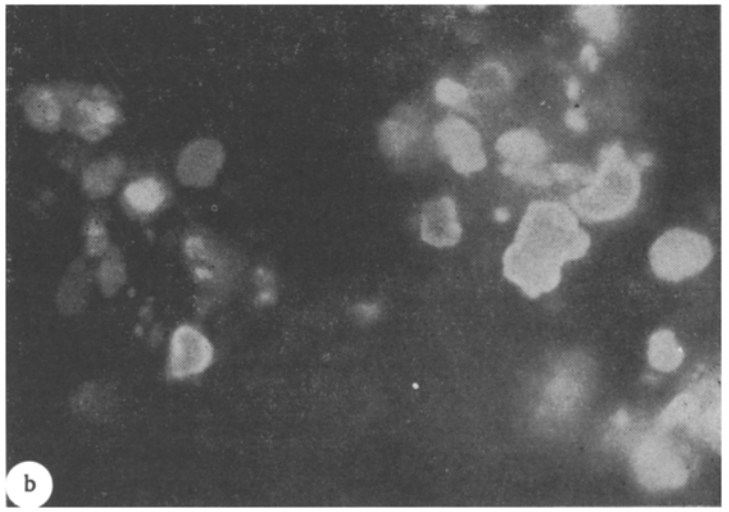
3. Formic acid-Schiff. The majority of the granules are distinctly stained; the remaining ones stain either weakly or not at all. The reaction is blocked by preliminary treatment with bromine.

4. Kasell's reaction. The intensity of staining of granules is variable. The reaction is not blocked by preliminary acetylization or by bromine treatment, but is inhibited by a combination of these two treatments.

5. Plasmalogen reaction. Weak staining was noted in small granules and in the cytoplasm of microphages in which they are contained.

6. Pseudoplasmalogen reaction. No staining. However, a preliminary treatment of sections with tap water for 7 and 14 days, as well as treatment with sulfate, followed by Schiff's reagent, produced a weak staining in some granules.





Morphology of pigment granules in the cytoplasm of macrophages of the lymphatic follicles of the rabbit appendix. a) Unstained frozen section; b) autofluorescence of pigment granules in the same section. Magnification 90x.

7. Sudan IV and Sudan black B. The great majority of the granules in paraffin sections retain a distinct stainability. Additional extraction of lipids has no effect on the staining of granules. However, fat solvents immediately decolorize those granules which are already stained; these granules may be restained.

8. Ziehl-Nielsen's method. All the granules are deeply stained.

9. Masson-Fontana's method. The granules contain argentaffin material.

10. Schmorl's reaction. Distinct staining in small granules and around the periphery of larger ones.

11. Autofluorescence. The granules fluoresce in different shades, from bright yellow-green to orange or dull brown. The fluorescence is stable but of varying intensity. External portions of large granules have a stronger fluorescence (Figure, 1a b).

The above data show that in the cytoplasm of reticular cells in the lymphatic follicles of appendix of adult rabbits there is contained a considerable amount of a material which has morphological and histochemical characteristics of a pigment [8, 15, 16]. While the possibility of the presence of different substances such as heme,

proteins, etc., in this material is not excluded, it is evident that the principal and the most important histochemical properties of this material are determined by the lipid nature of the pigment [21]. It has been determined that the specific peculiarity of this pigment is its well pronounced histochemical heterogeneity, as witnessed by the finding in the cytoplasm of reticular cells of inclusions at different stages of formation, from a certain "precursor" which must be regarded as a lipid [19]. Inspite of the fact that the nature of this "precursor" has not been determined, the histochemical characteristics of the pigment, which had been determined in this study, corresponded to the accepted views regarding the process of formation of lipogenic pigments in general [6, 10, 12, 22], and permit identifying the pigment as a chromolipoid or as a mixture of lipofuscines in statu nascendi. The distinct ability of the great majority of the cytoplasmic inclusions to give a distinct Schiff-positive reaction indicates the predominance of lipofuscines of the ceroid type in their composition. However, the data obtained do not include sufficient information on the relationship of the above-described process with the mechanism of phagocytosis of Schiff positive bacteria, as seen in the lymphatic follicles of the adult rabbit appendix. It must be noted, however, that some earlier observations [2, 3] have indicated that such a relationship is possible.

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