CEROID, THE PIGMENT OF DIETARY CIRRHOSIS OF RATS

Its Characteristics and Its Differentiation from Hemofuscin *

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A peculiar pigment occurring in rats fed choline-deficient diets has been reported from several laboratories. Lillie, Daft, and Sebrell [1] described a coarsely globular pigment which occurred in phagocytes in the cirrhotic livers of rats fed low protein (4 per cent), low fat (5 per cent) diets. In subsequent papers [2-4] they reported its occurrence in liver cells, lung, spleen, lymph nodes, bone marrow and adrenal cortex. They were able to prevent its production by supplementing the basal diet with choline, methionine, and casein singly or in combination. They named the pigment "ceroid" [4] because of its wax-like appearance and behavior. Blumberg and McCollum [5] and Blumberg and Grady [6] reported a similar pigment in the cirrhotic livers of rats fed low protein, high fat diets. Edwards and White [7] reported a similar pigment in the livers of rats fed low protein diets supplemented with the carcinogen, p-dimethylamino-azobenzene (butter yellow). In a subsequent paper, White and Edwards [8] reported the pigment in rats fed the basal diet without butter yellow. Smith, Lillie and Stohlman [9] produced cirrhosis but no ceroid in rats fed high protein diets and butter yellow. György and Goldblatt [10] found a similar pigment in the cirrhotic livers of rats fed low protein, high fat diets.

In view of the specific occurrence, peculiar properties and possible metabolic significance of this pigment (henceforth referred to as ceroid), it seems proper to characterize it more fully. The following observations were made in this laboratory upon liver fixed in formaldehyde and embedded in paraffin.

(1) As previously reported,[4] ceroid occurs as globules varying in diameter from 1 to 20 μ. These are seen occasionally in liver cells but ordinarily in large rounded phagocytes. The phagocytes usually contain several globules and are seen in greatest number in the liver. In minimal cases a few phagocytes are seen between liver cell cords or surrounding central veins, or rarely in portal areas. In moderate to marked cases, numerous phagocytes form broad sheets and trabeculae which divide and encircle nodules of liver cells. Ceroid has been seen in phagocytes in alveolar septa of the lung, in splenic pulp, bone marrow, lymph nodes and adrenal cortex. A substance with similar staining re-

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actions occurs as rims or halos about large fat vacuoles in the liver. The large vacuoles are presumed to lie in liver cells.

(2) In sections stained in hematoxylin and eosin, van Gieson's connective tissue stain, Masson's trichrome stain, and in sections immersed for 1 hour in 1/1000 aqueous acid dyes: naphthol green B, orange G, congo red, trypan blue, tartrazine [C.I. no. 640], acid fuchsin, eosin Y, eosin B, erythrosin bluish, phloxine B, rose bengal, nigrosin W.S., alizarin rubinol [C.I. no. 1091], alizarin saphirol [C.I. no. 1054], indigo carmine, and orcein, and in paraffin or celloidin sections mounted unstained, the ceroid appears as pale yellow globules.

(3) Eosin and polychrome methylene blue stain ceroid yellowish green to greenish blue. It is stained slowly by a number of basic dyes when immersed for 1 hour in 1/1000 aqueous solutions. The following dyes all stained ceroid: Bismarck brown, malachite green, brilliant green, pararosanilin, basic fuchsin, new fuchsin, methyl violet, crystal violet, methyl green, safranin O and D, methylene violet RRA, brilliant cresyl blue, Nile blue A, methylene blue, toluidine blue, and cresyl violet. The slowness with which ceroid is stained by basic aniline dyes may explain Edward and White's[7] failure to stain it in fuchsin and in phloxine methylene blue.

(4) It is strongly acid-fast. When stained with steaming Ziehl's carbolfuchsin for 10 minutes, the bright red globules resist decolorization in 3 per cent hydrochloric acid in 70 per cent alcohol for at least 2 days.

(5) It gives several reactions for fat. It stains brownish orange to orange-red with sudan IV in frozen sections. With sudan brown, it stains brownish orange. In paraffin sections the sudanophilia is retained provided the sections are mounted in the usual fat-stain mounting media. In the Lorrain Smith Nile blue sulfate stain for differentiating neutral fats, many ceroid globules stain reddish purple while a few are blue, but if such sections are mounted in xylol clarite, most of the globules turn blue in a day or two (paraffin sections). In frozen sections, some are blue while most are reddish purple. Ceroid is black after treatment of paraffin sections or formaldehyde-fixed blocks with osmic acid, with or without antecedent chromation.

(6) It is stained red in Mallory's stain for hemofuscin. In this connection it was noted that 1/1000 aqueous basic fuchsin failed to stain ceroid in 5 minutes, stained a few globules in 10 minutes and stained almost all globules in 30 minutes.

(7) It is negative for iron with both the acid ferrocyanide test of Perls and the ammonium sulfide-Turnbull blue method.[11]

(8) Ceroid is gram-negative in Weigert's stain for fibrin and bac-

teria, in Lillie's [12] acetone technic, and in Lillie's [13] thiosulfate technic. When stained for 2 hours in hot crystal violet and then treated with iodine and acetone, ceroid stains violet rather than the blue-black of gram-positive bacteria.

(9) It stains gray to black in Weil's modification of Weigert's stain for myelin.

(10) It reduces silver nitrate very slowly and irregularly. Foot's [14] diamino-silver carbonate solution is reduced in 48 to 96 hours, giving brown to black ceroid globules.

(11) In paraffin sections, ceroid does not reduce ferric chloride-ferricyanide in Schmorl's [15] test for "Abnutzungspigmente."

(12) It is stained a light brown by Gram's iodine.

(13) In ultraviolet light under the fluorescence microscope, the globules of ceroid display a greenish yellow fluorescence which changes slowly to a yellowish white fluorescence. The fluorescence is displayed by frozen sections and by paraffin sections dry, in water, or in paraffin, but not in xylol or xylol clarite.

(14) It is not removed from paraffin sections by prolonged treatment in water, alcohols (methyl, ethyl, propyl, or isopropyl), acetone, ether, chloroform, benzene, xylene, gasoline, propylene glycol, hydrogen peroxide, chlorine water, potassium permanganate, dilute acids (hydrochloric, sulfuric, acetic, nitric), or dilute alkalies (sodium hydroxide, sodium carbonate, ammonium hydroxide).

(15) In autolyzed material, where basophilia is poor, this staining quality of ceroid is improved by post-formaldehyde block mordanting with picric acid, or in paraffin sections by treating with potassium permanganate followed by oxalic acid, by treating with picric acid, or by treating with iodine followed by thiosulfate.

(16) When a formaldehyde-fixed liver is ground with mortar and pestle, suspended in distilled water and allowed to stand overnight, the ceroid settles out in a sharply defined layer of bronze-brown viscid material just above the liver débris from which it may be separated in a separatory funnel. When extracted with chloroform, ether and acetone and evaporated to dryness this material yields a coarsely granular, dark brown residue. The residue, when crushed on a slide in a drop of water and examined microscopically, is seen to consist of ceroid globules and a little cellular débris. This crude concentrate reacts to stains and solvents in the same manner as ceroid in paraffin sections. It is decomposed by concentrated mineral acids and strong alkalies. It chars but does not melt on heating. It gives a negative Gmelin reaction and produces no color reaction in the chloroform-sulfuric acid test for carotenoids. It is slowly dissolved in boiling 10 per cent sodium hydroxide

from which a fatty substance is precipitated on neutralization with hydrochloric acid.

Discussion

The chemical nature of ceroid cannot be fully stated at present. Several theories as to its composition have been advanced by other laboratories and will be discussed briefly.

Blumberg and Grady [6] regarded the pigment as hemofuscin. However, von Recklinghausen,[16] who first described hemofuscin, characterized it as a gall brown, very finely granular, iron negative pigment which occurs in hemochromatosis along with large amounts of hemosiderin. He found it in smooth muscle of the intestines and blood vessels, in mast or connective tissue cells of the vascular supporting tissue (Glisson's capsule, etc.) and in certain cells of the salivary and lacrimal glands. Hemofuscin, as previously described and as further characterized by Mallory, Parker and Nye,[17] has been studied in this laboratory in material from cases of hemochromatosis, some of which was furnished us by the Curator of the Army Medical Museum. It was found to differ from ceroid in appearance, location and staining. It is not acid-fast, stains readily with dilute basic aniline dyes and is not sudanophilic after paraffin embedding.

Edwards and White [7] suggested that it may be a conjugated lipoid. György and Goldblatt [10] advanced the theory that it may represent a lipoid conjugated with a protein and may arise from necrotic remnants of liver cells. Available evidence lends some support to these theories. Ceroid gives several reactions for fat. It is not, however, soluble in ordinary fat solvents and, furthermore, demonstrates staining reactions not shared by neutral fat, ordinary fatty acids and the simpler lipoids.

In this laboratory it has been observed in cases of progressive cirrhosis as small globules within apparently surviving and often fat-free liver cells, suggesting that it may be a product of altered liver cell metabolism, but it also occurs as acid-fast, basophile rims about obvious coarse fat globules probably located in distended liver cells, suggesting origin also from what, in earlier stages of the process, appears to be neutral fat. Similar acid-fast material originating from foreign fat has been reported in cod liver oil pneumonia,[18, 19] but the further agreement of this material with ceroid has not been fully established.

Ceroid has shown no properties which would justify classing it with anthocyanins, sterols, carotenoids, porphyrins, or the hemoglobin derivatives.

Material furnished this laboratory by Blumberg and by Goldblatt contains a pigment indistinguishable from the ceroid in our own material.

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