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Intestinal Ceroid Deposition – "Brown Bowel Syndrome"

A Light and Electron Microscopic Study

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Summary. Thirteen cases classified in our files as ceroid pigmentation of the intestinal wall ("brown bowel syndrome") are described. Clinically, all patients in this series had some symptoms of chronic bowel disease. The gut in this condition grossly demonstrates a variable orange-brown coloration. The ceroid pigment is difficult to identify in routine hematoxylin-eosin sections but may be demonstrated by a variety of special stains. In addition, ceroid may be identified by its golden-yellow autofluorescence under ultraviolet light. By electron microscopy, the deposited granules resemble ceroid deposits described in experimental animals. In addition to its occurrence in cases of chronic bowel disease, ceroid pigment was also found in 36 of 90 cases of cystic fibrosis (40%) and in 7 of 13 cases of congenital biliary atresia (54%). On the basis of pigment distribution and staining characteristics, brown bowel syndrome may be differentiated from melanosis coli and rectal ceroid histiocytosis.

Key words: Ceroid pigment – Bowel disease – Malabsorption

Introduction

Ceroid pigment is a brownish waxy substance of variable chemical composition, most commonly thought to be produced in cells as the result of peroxidation of unsaturated fatty acids. Ceroid deposition in the intestine was first described by Goebel (1894), and Pappenheimer and Victor (1946) first called attention to a relationship between ceroid deposition in smooth muscle and low clinical levels of vitamin E. Their studies have been elaborated upon over the years, the largest number of patients having been described by Nye and Chittayasoth-

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orn (1967) in a postmortem study of the Thai-Lao ethnic group. In Western countries the disease appears to be sporadic, however, and usually is secondary to a systemic disease rather than the direct result of dietary vitamin E deficiency. In the present study, material from thirteen patients with intestinal ceroid pigmentation is described, and an attempt is made to demonstrate the relationship between this condition and various other diseases.

Materials and Methods

Material used in this study includes cases received in consultation from military, Veterans Administration, and civilian sources. Cases coded as brown bowel syndrome, ceroid pigmentation, or malabsorption were studied initially.

For comparison, slides of bowel sections from 90 autopsy cases diagnosed as cystic fibrosis and 13 cases diagnosed as congenital biliary atresia were studied to determine the frequency of ceroid pigmentation. Finally, five cases each of melanosis coli and ceroid histiocytosis of the rectum were subjected to multiple stains to determine differences, if any, between material in them and the ceroid in intestinal smooth muscle. Sections were stained with the following stains: H & E, PAS with and without diastase digestion, alcian blue, the Ziehl-Neelsen acid-fast stain with a picric acid counterstain, oil red 0, Fontana-Masson, Warthin-Starry (pH 3.3), Giemsa, methenamine-silver, Prussian blue, and melanin bleach (Luna 1968). Where necessary, additional portions of formalin-fixed wet tissue were embedded and sectioned. In incident ultraviolet light, autofluorescence was observed in unstained sections mounted in polyvinyl alcohol under a coverslip. Small portions of formalin-fixed tissue from five cases were processed for electron microscopy by a soak in 0.3 M cacodylate-sucrose buffer, followed by fixation in 10% glutaraldehyde and postfixation in osmium tetroxide. Following dehydration, the tissue was embedded in Epon 812. Thick sections were cut at one micron and stained with methylene blue and basic fuscin. Thin sections were cut at 100 A, stained with uranyl acetate and lead citrate, and examined in a Zeiss model EM9S2 microscope.

Results ("Brown Bowel Syndrome" Cases)

Clinical Data. Eleven cases coded as deposition of ceroid-lipofuscin pigment in smooth muscle of the bowel were present in our files. Four of these have been reported (Nye 1967; Schnitzer 1968). Two additional cases out of forty-four diagnosed as intestinal malabsorption were also found to have heavy deposits of ceroid pigment. These thirteen cases form the basis of this investigation.

Patients ranged in age from 22 to 77 years, the average age being 51. Ten were male and three female. Demographically two were Thai, one was Indian, and one German. The remainder were North Americans of European origin. Clinical symptoms ranged from epigastric pain to mild diarrhea but almost always included some degree of abdominal pain and/or frequent bowel movements. For three patients the clinical diagnosis was regional enteritis, and in a fourth one, results of an X-ray study of the small bowel were abnormal and suggestive of regional enteritis. Duration of symptoms was not stated in most instances, but they had persisted in one instance for as long as 9 years.

Gross Findings. There were remarks as to the coloration of the bowel in several of the surgical and autopsy protocols. Typically it was described as "dark" or "dark brown". In one case the entire small bowel was "orange-brown"

Fig. 1. Region of bowel with heavy ceroid deposition. The longitudinal arrangement in smooth muscle cells is apparent. Several large rounded cells (*lower right*) represent macrophages with large amounts of cytoplasmic pigment. (Fontana stain, AFIP Neg. 78-7443)

and the stomach was "orange". In another case, discoloration was limited to a single loop of jejunum. In the majority of cases, however, gross description of the bowel was cursory, and the pigment was noted only microscopically.

Light Microscopic Findings. Pigment was present in numerous smooth muscle cells of the bowel wall in all thirteen cases (Fig. 1). Ceroid was visible as grey-to-brown unstained material in routine sections. In lightly involved areas, it was almost indiscernible. The pigment appeared as round to ovoid granules clustered in the central portion of the cell. When the nucleus was visible, deposits could be seen in a perinuclear location. The most characteristic appearance was observed when the cell was sectioned longitudinally in which case pigment was visible as a column of granules extending toward both poles from the nucleus. Changes were also seen in the muscle fibers, varying from a variegated pale coloration to fraying of fibers to actual fiber loss and replacement by edematous collagen in one severe case. A number of large rounded cells, presumably macrophages, were scattered throughout the muscle layer and contained large amounts of ceroid. Pigment deposition in other cells was quite variable. Only a rare lesion demonstrated pigment in vascular smooth muscle. Other frequent findings included edema of the submucosa, mild blunting of intestinal villi, and in two cases, collections of foamy histiocytes in the subserosal area focally. These latter cells did not contain ceroid.

Table 1. Special staining characteristics of brown bowel syndrome (BBS) melanosis coli (MC), and ceroid histiocytosis (CH)

Stain	BBS	MC	CH
PAS	4+	4+	4+
PAS post-diastase	4+	4+	4+
Giemsa	3+	3+	3+
Alcian blue	0	0	0
Methenamine silver	4+	4+	4+
Acid fast[a]	3+	−/+	0
Fontana	4+	4+	4+
Prussian blue	0	0	0
Warthin-Starry	0	0	0
Oil red O	1–2+	0	0
Melanin bleach[b]	0	−2	0

[a] Must be performed with a nonmasking counterstain, such as picric acid
[b] Amount of pigment removed

Distribution of pigment throughout the gastrointestinal tract was quite variable. The small bowel was usually the most heavily laden, followed by the colon, with the stomach least affected. No example of esophageal pigmentation could be found. Within any portion or segment of the bowel the ceroid frequently was distributed in a patchy or localized pattern, so that heavily and lightly involved areas might be adjacent. Not infrequently a discrepancy was noted in pigmentation between the longitudinal and radial muscle fibers, even within the same microscopic field.

Results of staining of ceroid pigment by various means are summarized in Table 1. Several points regarding the stains should be noted. First, it is necessary to perform the acid-fast stain with a picric acid counterstain because methylene blue will stain the ceroid and mask the fuchsinophilia. Also, the oil red 0 stain was variably successful, even in heavily involved cases. Storage of tissue for prolonged periods in paraffin was apparently associated with a decreased affinity for oil red 0, though other stains were not affected.

There have been varying results reported with the use of the Fontana-Masson stain in brown bowel syndrome, some (Schnitzer 1968; Fox 1967) reporting positive staining, others (Ansonelli 1957; Foster 1979) negative. We found the stain to be not only positive but also the most sensitive one for ceroid, as well as the one easiest to screen for small amounts of pigment. While it did have the disadvantage of staining dirt and debris, it nevertheless was capable of delineating cells with very sparse deposits (Fig. 2).

Among patients with either cystic fibrosis or congenital biliary atresia there were many examples of ceroid deposition, confirming the findings of previous investigations (Kerner 1960; Toffler 1963). Thus 36 of 90 cases of cystic fibrosis (40%) and 7 of 13 cases of biliary atresia (54%), contained discernible pigment. These figures may underestimate the true incidence, as a maximum of only two paraffin blocks per case was examined. The chief discernible difference between patients with pigmentation and those without it was age. The average age for positive cases of cystic fibrosis was 5.0 years and for biliary atresia, 2.3 years. In cases without pigment the average age of the patients was 10 months for cystic fibrosis and 9 months for biliary atresia. No correlation was apparent with the severity of disease clinically. The degree of pigmentation was quite variable, ranging from only a few cells to many being involved.

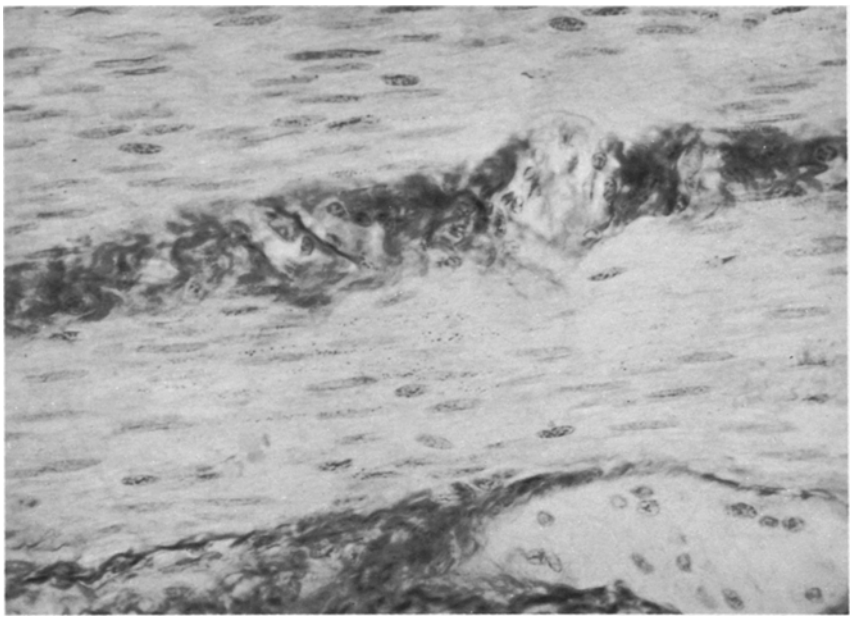

Fig. 2. Slight deposition of ceroid pigment seen in a 12-year-old with cystic fibrosis and malabsorption. The "chain-of-dots" pattern extending in both directions from the nucleus is very distinctive in contrast to randomly scattered debris. (Fontana stain, AFIP Neg. 78-7451)

Fig. 3. Autofluorescence of pigment in ultraviolet light. The distribution pattern of the pigment corresponds to that as seen on routine staining. (Unstained section photographed in incident ultraviolet light, AFIP Neg. 79-2794)

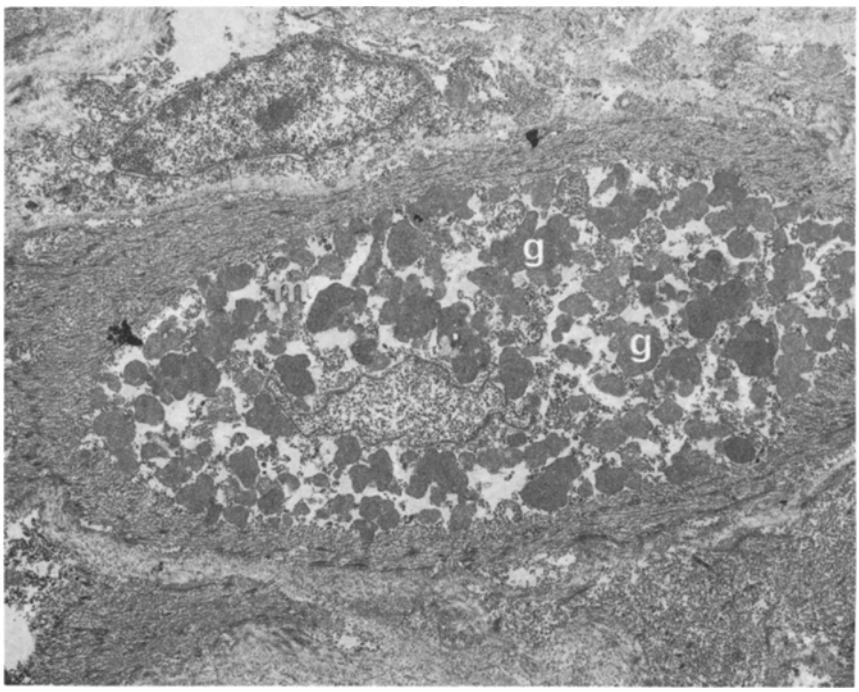

Fig. 4. Smooth muscle cell in bowel wall with ceroid pigment granules (*g*) deposited around nucleus. Clumps of granular material (*m*) may represent degenerating myofibrils. (×4500, AFIP Neg. 79-2866-2)

Findings on Fluorescence and Electron Microscopic Studies. Results of fluorescence microscopy were generally as described by other authors (Fig. 3) (Fisher 1969). The distribution of pigment revealed by this method was identical to that seen by routine light microscopy. The densest accumulations were in the large macrophages within the muscle layers.

The appearance of the bowel by electron microscopy correlated well with the light microscopic appearance. Within the cytoplasm of a smooth muscle cell irregularly shaped globules of electron-dense material were observed (Fig. 4). These were concentrated about the nuclei, the periphery of the cells being largely spared. As a result of the absence of normal cytoplasmic components in the region of the ceroid granules, these gave the appearance of a "swarm" of particles within a large perinuclear clear zone. In addition to the ceroid there were irregular collections of amorphous granular material. At the periphery of the clear zone the smooth muscle cell myofilaments either ended abruptly (Fig. 5) or were stretched around the ceroid deposit. Many cells were swollen by the granules which severely marginated the myofilaments. Extracellular material appeared normal.

The granules had smooth, irregular outlines with no discernible substructure. No limiting membrane was visible, so that the granules did not appear to be within lysosomes. They were similar in appearance to material described

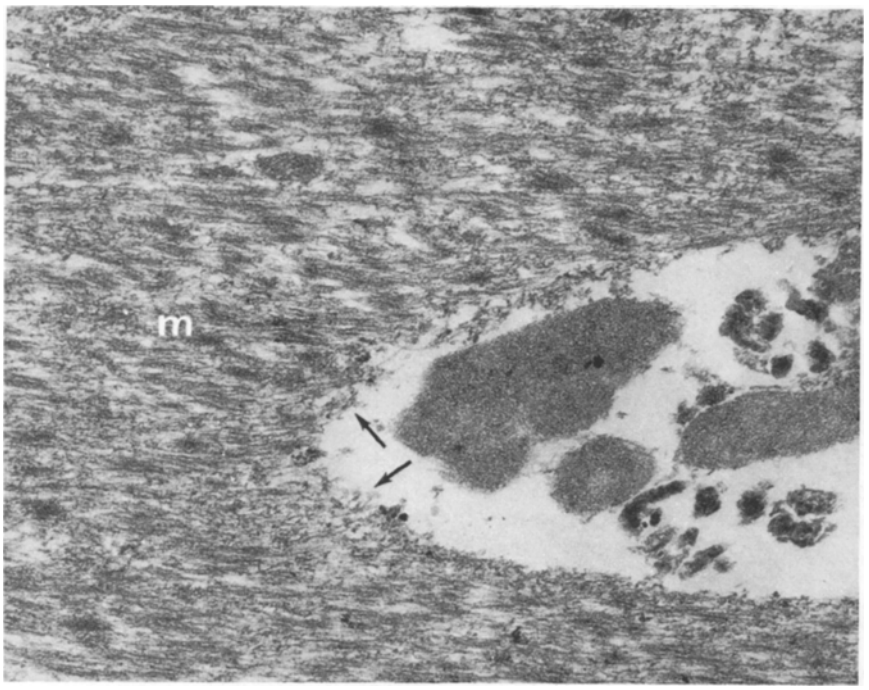

Fig. 5. Affected smooth muscle cell demonstrating bulbous projections (*arrows*) of myofibrils (*m*) adjacent to granular material in the clear zone, suggesting damage to the cell as a result of pigment deposition. Note that the pigment granules do not have a limiting membrane. (×9000, AFIP Neg. 79-2866-1)

as either lipofuscin or ceroid in experimental studies (Bourne 1973; Brownstein 1961; Ghadially 1966; Hall 1978).

Results (Melanosis Coli and Ceroid Histiocytosis)

The histologic appearance of ceroid histiocytosis and melanosis coli were distinctly different from that of ceroid in smooth muscle. In cases of ceroid histiocytosis the cells involved were in the lamina propria of the bowel and were not pigmented. In melanosis coli, pigmentation was intense and was present inside and outside cells in the lamina propria. Results with various special stains are listed in Table 1. It is apparent that the cytoplasmic materials present in these two conditions are similar to the ceroid of brown bowel syndrome but not identical. Thus, the acid-fast picrate stain is nearly specific for brown bowel syndrome. Although all materials are stained by the Fontana stain, the Warthin-Starry stain at pH 3.3, which is more specific for melanin, is negative for all cases of brown bowel syndrome. Despite the bleaching effect of permanganate, therefore, the pigment in melanosis coli would appear not to be true melanin (Morson 1979).

Comment

There is some confusion in the literature as to the chemical differences between lipofuscin and ceroid. Kajihara et al. (1975) separate ceroid from lipofuscin pigment by classifying lipofuscin as being collected in parenchymal cells (eg., liver, muscle) by lysosomal phagocytosis of endogenous lipid. Ceroid is described on the other hand as accumulating in macrophages by phagocytosis of exogenous unsaturated fatty acids. By such a classification, the lipid in brown bowel syndrome would be termed "lipofuscin", whereas that in melanosis coli would be called ceroid. Most authors, however, would probably agree more readily with the system used by Bourne (1973). He classifies lipofuscin as a pigmented peroxidized fatty acid residue occurring normally in many organs as a result of aging, and ceroid as a pigment accumulating as the result of an abnormal physiologic state.

Pappenheimer and Victor (1946) were the first to comment on the possibility of an etiologic role of vitamin E (tocopherol) deficiency in ceroid smooth muscle pigmentation. This has been amplified by many authors in clinical and experimental studies of several organs (Blanc 1958; Kerner 1960; Toffler 1963). One study (Underwood 1972) of patients with cystic fibrosis demonstrated the tocopherol levels of plasma and erythrocytes to be one-fourth or less than the levels in control subjects. There was a depletion of unsaturated fatty acids, particularly linoleic acid, which returned toward normal following tocopherol supplementation. The details of the mechanism by which unsaturated fatty acids are peroxidized to ceroid are not fully known, but a role for vitamin E in preventing this is definitely indicated.

With the exception of the absence of a limiting membrane, the electron microscopic appearance of the pigment granules in brown bowel syndrome is similar to that of ceroid in other conditions. There is evidence to suggest that muscle cell injury may occur as a result of the pigment accumulation. In cases of heavy deposits, there is a large volume of the cell devoid of myofilaments, as well as ultrastructural evidence for actual loss of myofilaments, presumably as a result of granule accumulation. No degenerated cells were seen by electron microscopy; some of the large rounded cells seen by light microscopy, however, might have been dying smooth muscle cells. Although there was no evidence of scarring seen by electron microscopy, our light microscopic findings support the statement of Fox (1967) that in heavily involved cases structural and functional impairment of the bowel might be expected, and that this could be permanent. On the other hand in one case of Lee and Nicholson (1976), for example, pigment apparently disappeared from the bowel (as observed both grossly and microscopically) during a seven-month course of steroid therapy. This is the only example in the literature describing a successful attempt at reversal of pigmentation.

It appears that in most cases of ceroid pigmentation secondary to systemic disease (e.g., cystic fibrosis or biliary atresia), especially in children, the pigment is slight in amount and not widely distributed. Several interpretations are possible from this: Tocopherol deficiency in most of these affected children may not be as severe as it is in adults with chronic malabsorption, thus resulting in

less severe bowel involvement. On the other hand, and as suggested by some of our data, the amount of pigment visible may simply be in proportion to the duration of disease.

It is unusual that as many as four patients in this series had clinical evidence of regional enteritis. The association between this disease and significant ceroid deposits has not been reported previously, the majority of cases reported having been associated with pancreatic malabsorption.

Several authors (Brownstein 1961; Kerner 1960) have commented on the irregular distribution of pigment throughout the gastrointestinal tract in brown bowel syndrome. Despite statements to the contrary (Foster 1979; Lee 1976), pathologists should not feel tempted to comment on the presence or absence of ceroid pigment deposits on the basis of inspection of a small biopsy specimen.

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