

Melanosis Coli

— Histochemical and Immunohistochemical Comparison of the Pigments of Melanosis Coli and Dubin-Johnson Syndrome —

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We compared the pigment of melanosis coli with the pigment of Dubin-Johnson syndrome, melanin, and lipofuscin. The pigment of melanosis coli appeared similar to lipofuscin in that it stained positively with periodic acid-Schiff, oil red-O and Victoria blue stains and revealed negative reactions to the immunohistochemical stains for S-100 protein and neuron specific enolase, but had similarity to melanin as shown by the positive reaction to Fontana-Masson stain and negative autofluorescence. The pigment of Dubin-Johnson syndrome showed the same histochemical and immunohistochemical characteristics as that of melanosis coli. The results indicate that the pigments of melanosis coli and Dubin-Johnson syndrome are identical and are variants of lipofuscin.

Key Words: Melanosis coli, melanin, lipofuscin, melanin-like pigment, Dubin-Johnson syndrome

The term melanosis coli was adopted by Virchow in 1847 for a peculiar colonic lesion which had been described first by Cruveilhier in 1830. The lesion is characterized by yellow-brown pigment within large mononuclear cells of the tunica propria in the large intestine, particularly the cecum and ascending colon, and by the association of its development with protracted abuse of laxatives (Bockus *et al.* 1933).

The yellow-brown pigment is known to have the staining characteristics of lipofuscin. The pigment lipofuscin generally represents the indigestible residues of autophagic vacuoles formed during aging or atrophy, and shows positive staining reactions to periodic acid-Schiff for glycogen, oil red-O for lipid, Victoria blue for lipofuscin and toluidine blue for metachromasia and gives autofluorescence. But the origin and nature of the pigment still remain to be determined (Ghadially and Parry 1966), because it stains black with Fontana-Masson stain like melanin. The pigment melanin is formed by the neuroectoderm

derived melanocytes and thus shows a positive reaction to the immunohistochemical stainings for S-100 protein and neuron specific enolase (NSE). Pigment of similar histochemical properties to that of melanosis coli is also found in Dubin-Johnson syndrome, in which it deposits in the perivenular hepatocytes (Baba and Ruppert 1972; Kermarec *et al.* 1972).

Recently we experienced a case of melanosis coli, which has been very rarely encountered in both foreign and domestic literature, and at the same time a case of Dubin-Johnson syndrome. Then we attempted to perform a comparative histochemical and immunohistochemical study on the pigments of melanosis coli and Dubin-Johnson syndrome.

CASE REPORT

The patient, a 59-year-old male, had suffered from indigestion and epigastric discomfort for 1 year, and constipation for the last 2 months. He had taken two tablets of Dulcolax intermittently for 2 months. The outstanding laboratory feature was blood eosinophilia (28%). Because of intractable constipation, a total colectomy was performed. The blood eosinophil count gradually declined to 14% following operation.

Gross Findings

The resected specimen included the terminal ileum

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Fig. 1. Mucosal aspect of the formalin-fixed cecum showing black discoloration



Fig. 2. A cutsection surface of the cecum showing black discoloration limited to the mucosa.

and all segments of large intestine from the cecum to the rectum. The mucosal aspect of all segments of the specimen except for the terminal ileum and the rectum showed brown-black to black discoloration (Fig. 1). There was neither erosion, ulcer nor mass lesion. The discoloration was more intense at the cecum, ascending colon and transverse colon, and tended to become lighter distally. But the discoloration was still abruptly transitioned to the nonpigmented

rectum and terminal ileum. On sections through the colonic wall, the discolored area appeared to be confined to the mucosa (Fig. 2), and the muscular coat was intact.

Microscopical Findings

The pigment was yellow-brown and granular and was confined to the tunica propria of the mucosa (Fig.

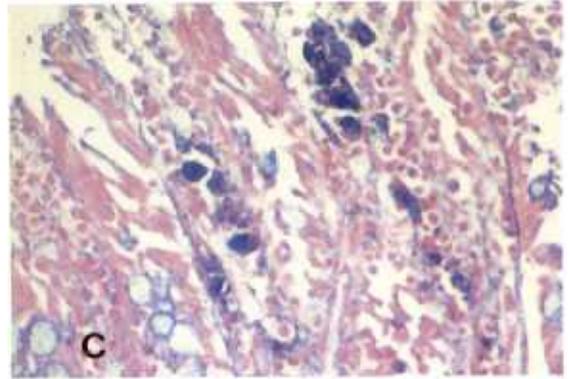
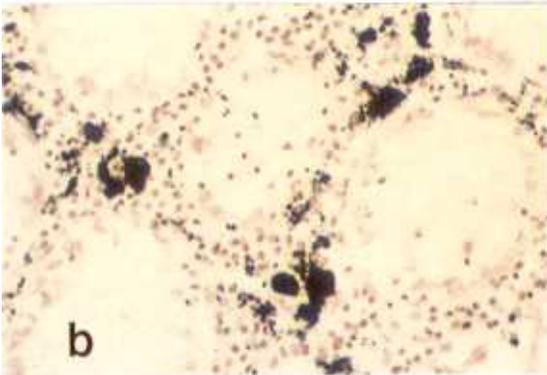
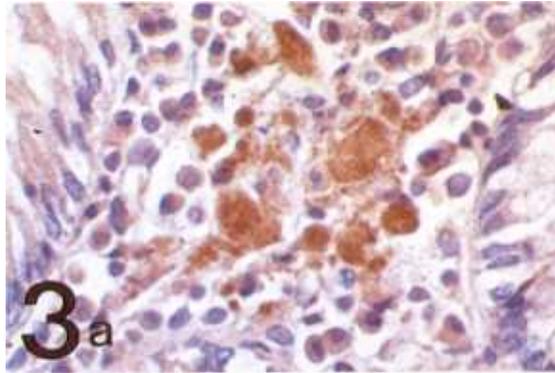


Fig. 3. Light microscopic features of melanosis coli: a) Pigment laden cells are confined to the lamina propria and appear to be macrophages. The pigments are stained dark brown with hematoxylin-eosin ($\times 400$). b) They are black on Fontana-Masson stain ($\times 200$), and c) dark blue on Victoria blue stain. The goblet cells are faintly blue on Victoria blue stain ($\times 200$).

3a). Pigment granules were found within the cytoplasm of large mononuclear histiocytes, but not in the epithelial cells nor in other inflammatory cells. The submucosa appeared slightly edematous but was otherwise not remarkable. There was focal sloughing of the mucosa with an inflammatory exudate at the splenic flexure. Mesenteric lymph nodes showed nonspecific changes consistent with reactive hyperplasia without melanin-like pigment engulfing cells.

MATERIALS AND METHODS

For the histochemical and immunohistochemical studies on the pigment of melanosis coli, multiple serial sections were made from the melanotic colon which had been fixed in 10% neutral formalin and embedded in paraffin. To clarify the histochemical nature of the pigment, Fontana-Masson, periodic acid-

Schiff, oil red-O, toluidine blue and Victoria blue stainings and prussian blue staining for iron were done. Additional unstained sections were examined under ultraviolet light for autofluorescence.

Immunohistochemical stainings were performed on the formal-fixed paraffin-embedded sections for the determination of whether the pigment is of neuroectodermal origin or not. The peroxidase-antiperoxidase method was applied utilizing anti-S-100 protein and anti-NSE (Histogen kit, Biogenex Lab. San Ramon, CA, USA) as the primary antibodies.

The same histochemical and immunohistochemical studies were undertaken on the needle biopsied liver of patient with Dubin-Johnson syndrome. The autofluorescence study could not be done on Dubin-Johnson syndrome. Tissues from malignant melanoma and from subsiding hepatitis were taken as controls for the melanin and lipofuscin respectively.

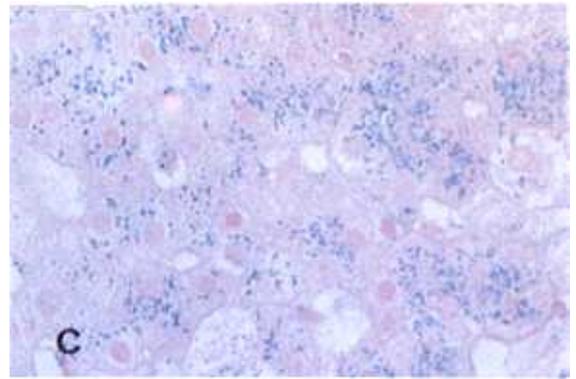
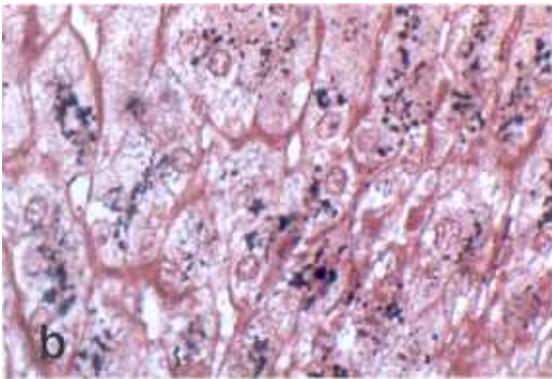
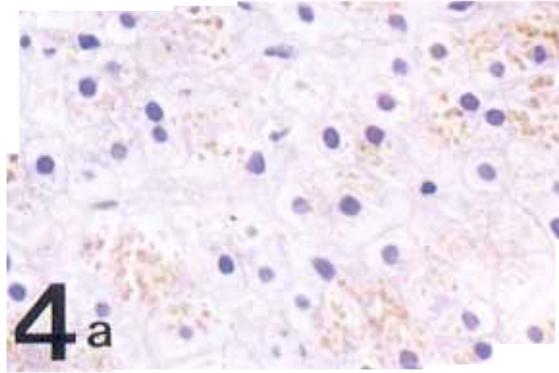


Fig. 4. Stain characteristics of the pigment of Dubin-Johnson syndrome: a) The pigments accumulated exclusively in hepatocytes are yellow-brown on hematoxylin-eosin stain ($\times 200$). Like the pigments of melanosis coli, they are stained b) black with Fontana-Masson stain ($\times 400$) and c) dark blue with Victoria blue stain ($\times 400$).

Table 1. Pigment of melanosis coli in comparison with that of Dubin-Johnson syndrome, melanin and lipofuscin

	Control		Dubin-Johnson syndrome	Melanosis coli (identical to)
	Melanin	Lipofuscin		
Hematoxylin-eosin			Yellow-brown Finely granular	Yellow-brown Finely granular (L)
Fontana-Masson			Pos	Pos (M)
Oil red-O			Pos	Pos (L)
PAS			Pos	Pos (L)
Victoria blue			Pos	Pos (L)
Toluidine blue			Pos	Pos (NC)
Prussian blue			Neg	Neg (NC)
Autofluorescence			NT	Neg (M)
S-100 protein			Neg	Neg (L)
NSE			Neg	Neg (L)

L: lipofuscin, M: melanin, Pos: positive, Neg: negative, PAS: periodic acid-Schiff, NC: not contributory, NT: not tested, NSE: neuron specific enolase

present study by the positive reactions to periodic acid-Schiff, Victoria blue and oil red-O stains.

The pigment granules of Dubin-Johnson syndrome are also yellowish brown and were reported to have the staining characteristics similar to lipofuscin except for the absence of autofluorescence (Johannessen 1978). Dubin-Johnson syndrome is known to be caused by an inherited defect in the hepatocellular secretion of conjugated bilirubin and sulfobromophthalein, and differs from melanosis coli in the pathogenesis. There are no reported cases in which melanosis coli and Dubin-Johnson syndrome developed simultaneously in the same patient. If the pigments of melanosis coli tissues. With respect to the results of histochemical melanin, they should have revealed a positive reaction to the antibodies to S-100 protein and NSE, which is an important property of the neuroectodermal derived tissues. With respect to the results of histochemical and immunohistochemical studies, the pigment of melanosis coli was nearly identical to that of Dubin-Johnson syndrome. They were yellowish brown and finely granular on hematoxylin-eosin stained sections. The pigment of Dubin-Johnson syndrome also revealed positive reactions to Fontana-Masson, periodic acid-Schiff, oil red-O and Victoria blue stains. Both pigment granules of melanosis coli and Dubin-Johnson syndrome were negative for S-100 protein and NSE.

Electron-microscopically, the pigment of Dubin-Johnson syndrome is present in the lysosomes, which are single membrane-bound, electron-dense bodies that vary in size, shape and electron density (Johannessen 1978; Phillips et al. 1987). The pigment of melanosis coli has a complex ultrastructural morphology, generally smooth and occasionally lobulated, with heterogenous electron densities (Lee et al. 1984).

We concluded that the pigment granules of melanosis coli and Dubin-Johnson syndrome share common histochemical, immunohistochemical and ultrastructural characteristics and represent variants of

lipofuscin. Pigmentation in melanosis coli may increase with time or decrease with therapy (Bockus et al. 1933), suggesting that the pigment granules are not a permanent cytoplasmic residue.

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