American Journal of Pathology, Vol. 131, No. 3, June 1988

Melanosis Coli

A Consequence of Anthraquinone-Induced Apoptosis of Colonic Epithelial Cells

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A condition closely resembling human melanosis coli was induced in the guinea pig large intestine by daily oral administration of the anthraquinone danthron. Each treatment caused a transient, dose-related wave of apoptosis of the colonic surface epithelial cells. Most of the resulting apoptotic bodies were phagocytosed by intraepithelial macrophages and carried by them through fenestrae in the epithelial basement membrane to the lamina propria. Here, the apoptotic bodies were transformed into typical lipofuscin pigment in macrophage heterolysosomes. Continued danthron administration caused progressive accumulation of pigmented macrophages in the bowel wall, whereas ongoing migration of pigmented macrophages to regional lymph nodes resulted, after danthron was ceased, in sequential loss of the pigmented cells from the superficial and deep lamina propria. Examination of colonic biopsies from patients with melanosis coli shows increased numbers of apoptotic bodies in the surface epithelium and lamina propria, suggesting implication of the same cellular processes in the formation of the pigment in man. (Am J Pathol 1988, 131: 465–476)

THE DEVELOPMENT of melanosis coli has been linked to the chronic ingestion of anthraquinone purgatives.[1–5] The pigment present in macrophages in the lamina propria in this condition shows the histochemical reactions and ultrastructural appearances of lipofuscin[5–8] and is currently believed to originate from the organelles of epithelial cells[7,8] or macrophages[9] damaged by the anthraquinones. The details of the processes involved in its genesis have, however, not been defined.

Recently we noticed numerous apoptotic bodies in the epithelium and superficial lamina propria in colonic mucosal biopsies from patients with melanosis coli. Derivation of macrophage lipofuscin from degraded phagocytosed apoptotic bodies of epithelial origin has been demonstrated by one of us during studies of atrophy of exocrine glands induced by duct ligation[10,11]; in these experiments, the pigment was found to appear in macrophages that had phagocytosed apoptotic bodies within the epithelium and subsequently migrated to the interstitial tissues. It seemed possible that a similar sequence of events might be implicated in the pathogenesis of melanosis coli.

Apoptosis is a distinctive form of cell death characterized morphologically by cytoplasmic shrinkage, margination of chromatin in sharply defined masses adjoining the nuclear envelope, and nuclear and cellular fragmentation to produce rounded, membrane-enclosed apoptotic bodies that are rapidly phagocytosed by adjacent cells. It accounts for cell loss occurring under physiologic conditions but can also be induced by pathologic stimuli, including certain chemicals and toxins.[12–15]

The present experimental studies in guinea pigs were undertaken to assess the role of epithelial apoptosis in producing melanosis coli. The synthetic anthraquinone danthron (1,8-dihydroxyanthraquinone) was found to induce a transient wave of apoptosis of colonic epithelial cells; the resulting apoptotic bodies were phagocytosed by macrophages

Supported by Research Funds from the University of Queensland.

Accepted for publication January 26, 1988.

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within the epithelium and carried by them to the lamina propria, where they were degraded to lipofuscin.

Materials and Methods

Animal Studies

Experimental Induction of Melanosis Coli

Outbred male English shorthair guinea pigs weighing 400–600 g were used. They were fed a pellet diet containing 22% protein, 5–10% fat, and mineral and vitamin supplements and were allowed free access to water containing 500 mg/l ascorbic acid. To induce melanosis coli, danthron (Sigma Chemical Company, St. Louis, MO) was suspended in 40% aqueous sucrose solution and administered orally in 2 ml daily doses[16,17] to two separate groups of experimental animals. The first group received 25 mg/kg danthron for periods ranging from 1 to 10 days.[16,17] One or 2 animals were killed 1, 2, 3, 4, 6, 12, and 24 hours after 1 dose; 6 hours after daily dosing for 3 and 7 days; and 24 hours after daily dosing for 3, 6, and 10 days. Other animals receiving 10 daily doses were killed 3, 6, and 10 days and 2, 3, 4, and 8 weeks after completion of the course of danthron for assessment of pigment loss during the recovery period. The second group of animals received smaller doses of danthron (2.5 mg/kg) that were nearer the recommended dosage range for man.[18] Two animals were killed 4, 6, 12, and 24 hours after a single dose; 6 hours after daily dosing for 3 and 7 days; and 24 hours after daily dosing for 3 days and 1, 2, 4, 8, and 12 weeks. Normal animals and animals given 2 ml 40% sucrose 6 hours previously were used as controls.

Light and Transmission Electron Microscopy

After lethal intraperitoneal injection of pentobarbitone, ileum, cecum, colon, and ileocecal lymph nodes were removed *en bloc* from the experimental animals and the intestine washed with normal saline prior to fixation. For light microscopy, 5 μ paraffin sections of formalin-fixed tissue were prepared and stained with hematoxylin and eosin (H&E) or by the periodic acid–Schiff (PAS), long Ziehl-Neelsen (ZN), or Schmorl's methods.

For electron microscopy, cecum from control animals and cecum and ileocecal lymph nodes from animals given the high dose of danthron were diced and fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2. The tissues were then washed in cacodylate buffer, postfixed in 1% osmium tetroxide, washed in distilled water, stained *en bloc* in 5% aqueous uranyl acetate, dehydrated through a series of graded alcohols, cleared in propylene oxide, and embedded in an Epon/Araldite mixture. Sections 1 μ in thickness were cut on an LKB Ultratome V and stained with toluidine blue for use in selecting areas for detailed study. Ultrathin sections were picked up on uncoated copper grids, stained with lead citrate for 1–2 minutes, and examined in an Hitachi H300 electron microscope.

Scanning Electron Microscopy

Scanning electron microscopy was performed for determination of the size and frequency of fenestrae in the epithelial basement membrane of the cecal mucosa, because it seemed likely that intraepithelial macrophages containing apoptotic bodies would need to pass through these fenestrae to reach the lamina propria. Fresh samples of cecum from normal guinea pigs and from animals given danthron (25 mg/kg) 6 hours previously were immersed in 1% aqueous boric acid for 8 hours, rinsed in buffer, dehydrated in ascending percentages of acetone (25, 50, 75, 95, 100), and stored in 100% acetone for 16 hours. The basement membrane was exposed by tissue microdissection with ultrasonic vibration (50,000 cycles/sec) for 5 minutes.[19] Specimens were then critical point dried, coated with gold and observed in a JEOL JSM 35CF scanning electron microscope. The diameters and density (number per square millimeter) of fenestrae in the cecal surface epithelial basement membrane were calculated from scanning electron micrographs with the aid of a Mop-Videoplan.

Quantification of Cell Death

Counts of apoptotic bodies[12–15] were made in H&E-stained histologic sections of cecum from each of 2 normal animals; 2 control animals given sucrose solution 6 hours previously; and 2 high- and 2 low-dose animals killed 4, 6, 12, and 24 hours after a single danthron dose and 6 hours after the third and seventh daily dose. In each section, the number of chromatin-containing apoptotic bodies was counted in 1) 40 crypts with lumens extending the full length of the crypt, 2) 40 areas of lamina propria lying between adjacent crypts, and 3) the surface epithelium overlying the areas of lamina propria counted in 2. The means and standard errors of the mean (SEM) of the counts were then determined for each pair of animals. Statistical significance of the differences between means of corresponding counts in experimental groups and untreated control animals was determined by the two-tailed, nonpaired Student t test.

Melanosis Coli in Man

Histologic studies were performed on 5-μ H&E-stained paraffin sections. Twenty consecutive biopsies

showing histologically normal colonic mucosa taken from the files of the Department of Pathology, Royal Brisbane Hospital, and 20 similarly selected biopsies showing evidence of melanosis coli were examined. In sections of each biopsy, the number of chromatin-containing apoptotic bodies was counted in 1) 20 areas of lamina propria lying between adjacent crypts and 2) the surface epithelium overlying the areas of lamina propria counted in 1. The results are expressed as the mean ±SEM of the counts in the normal and melanosis coli groups. Statistical significance of the differences between the means of corresponding counts in the two groups was determined by the two-tailed, nonpaired Student *t* test.

Results

Patterns of Accumulation and Disappearance of Pigment in Experimental Animals

Cecum

In normal guinea pigs and those given sucrose only, cecal surface epithelial cells were free of pigment, and only small numbers of vacuolated and occasionally lightly pigmented macrophages were found in the superficial lamina propria. Twenty-four hours after high doses of danthron, fine brown pigment granules had formed in the supranuclear cytoplasm of surface epithelial cells, and coarser brown granules were found in the cytoplasm of the lamina propria macrophages. Continued dosing increased the number and size of pigmented macrophages (Figure 1a), and many of them had vesicular nuclei, prominent nucleoli, and increased numbers of cytoplasmic pigment granules. By Day 7, the pigmented macrophages extended to the muscularis mucosae; and by Day 11, some had entered the submucosa. In superficial macrophages, pigment was in the form of small, widely dispersed granules that stained with PAS and Schmorl's methods. In cells near the muscularis mucosae, pigment occurred as closely packed, large, variably sized granules that stained with the ZN, PAS, and Schmorl's methods. The different appearances were in accord with lipofuscin of varying age, with the older pigment lying deep in the mucosa.[6]

Low-dose danthron induced only minor pigmentation of surface epithelial cells, and daily dosage for 4 weeks was required before pigmented lamina propria macrophages regularly extended to the muscularis mucosae. The intensity of macrophage pigmentation increased from superficial to deep lamina propria, but each macrophage was more lightly pigmented and contained smaller pigment granules than comparable cells in high-dose animals.

After completion of the 10-day, high-dose regimen, the number of pigmented epithelial cells and lamina propria macrophages steadily decreased. After 3 days, pigmented surface epithelial cells were confined to small areas centrally placed between adjacent crypts; and by 6 days, none remained. Residual pigmented lamina propria macrophages progressively moved toward the muscularis mucosae and by 4 weeks were confined to a narrow zone adjacent to the muscularis. After 8 weeks only a few heavily pigmented cells remained (Figure 1b).

Colon and Ileum

The patterns of pigmentation in colonic mucosa in high- and low-dose animals were similar to those in cecum. However, the amount of pigment formed was less and showed much greater regional variation. In the ileum, high-dose danthron produced small brown pigment granules in the supranuclear cytoplasm of epithelial cells of the villus tip, and macrophages in the adjoining lamina propria showed increase in size and brown granular pigmentation. Low-dose danthron induced very mild pigmentation of the same cells.

Ileocecal Lymph Nodes

In high-dose animals, pigmented macrophages were first found in lymph nodes on Day 7; they progressively increased in number (Figure 1c) until 8 weeks after completion of the 10-day regimen. They were located in subcapsular and cortical sinuses, in paracortical areas, and in the margins of germinal centers. Small numbers appeared in the medulla in the later stages of the study. In low-dose animals, pigmented macrophages, first seen in the nodes on Day 15, were similarly distributed. They increased in number throughout the 3-month period of continued danthron administration.

Apoptosis of Epithelial Cells and Pigment Formation

Guinea Pig Cecum

Light Microscopy

In normal animals and those given sucrose only, apoptotic bodies were present in only small numbers (Figure 2). They appeared as small, round, eosinophilic globules, often containing crescents and specks of condensed chromatin, and were located within the surface epithelium, the proliferative zone of the crypts, and the superficial lamina propria. In the epithelium, the apoptotic bodies had mostly been phagocytosed by basal pale cells identified ultrastructurally as macrophages. They were also found in macro-

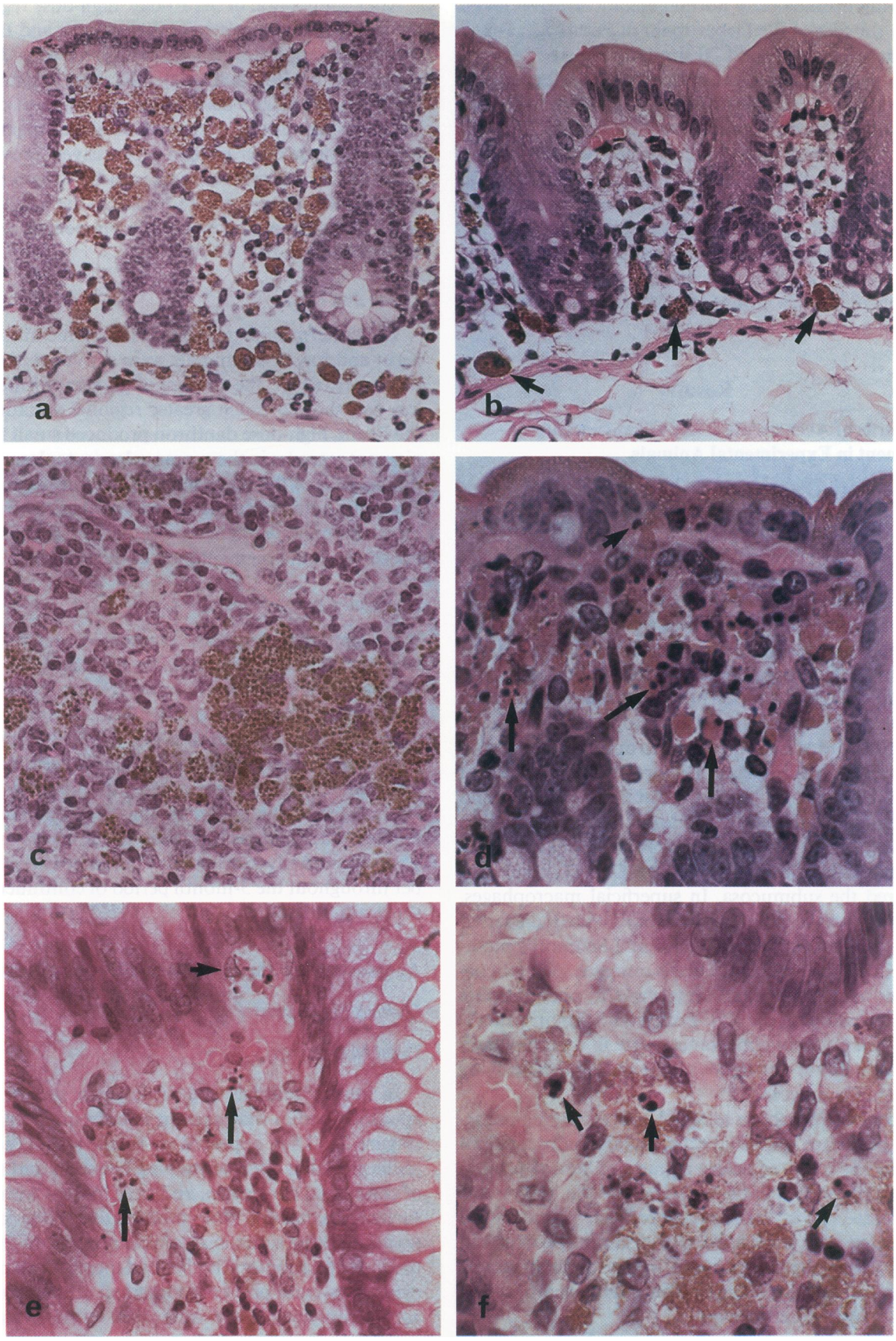
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Figure 1a—Pigmented macrophages in lamina propria of guinea pig cecum after 10 daily doses of danthron, 25 mg/kg. (×280) **b**—Pigmented macrophages (*arrows*) adjacent to muscularis mucosae of cecum, 8 weeks after administration of a 10-day course of danthron (25 mg/kg) to guinea pig. (×350) **c**—Pigmented macrophages in cortex of ileocecal lymph node of guinea pig, 3 weeks after a 10-day course of danthron (25 mg/kg). (×420) **d**—Apoptotic bodies in epithelium (*short arrow*) and lamina propria (*long arrows*) of guinea pig cecum 6 hours after a single dose of danthron (25 mg/kg). Shortened surface epithelial cells contain supranuclear pigment granules. (×660) **e**—Apoptotic bodies in macrophages in epithelium (*short arrow*) and lamina propria (*long arrows*) in colonic biopsy from patient with melanosis coli. (×450) **f**—Same biopsy at higher magnification. Apoptotic bodies (*arrows*) occupy the zone between epithelium and deeper pigmented macrophages. (All H&E, ×700)

phages lying within the superficial lamina propria and crossing the surface epithelial basement membrane.

The yellow danthron suspension reached the cecum 3–4 hours after oral administration. Almost immediately in high-dose animals, greatly increased numbers of apoptotic bodies were found in the surface epithelium and superficial lamina propria (Figures 1d and 2), but no increase occurred in the crypt epithelium (data not shown). The numbers of apoptotic bodies were significantly increased in the lamina propria at 4 ($P < 0.01$) and 6 hours ($P < 0.001$) and in the epithelium at 6 hours ($P < 0.05$) when compared with levels in untreated control animals. Most of the apoptotic bodies were located in macrophages in the superficial lamina propria (Figures 1d and 2); smaller numbers lay within intraepithelial macrophages, surface epithelial cells, or the gut lumen; a few were found in macrophages crossing the basement membrane. Simultaneously, there was a marked reduction in the height of residual surface epithelial cells, and this was associated with the appearance of fine brown pigment granules in their supranuclear cytoplasm (Figure 1d) and focal denudation of the basement membrane. By 24 hours, however, substantial recovery of surface epithelium had occurred. It was accompanied by increased mitoses in crypt epithelium, disappearance of apoptotic bodies (Figure 2), and the appearance of pigment granules in macrophages in the superficial lamina propria.

After repeated high-dose danthron administration, surface epithelial damage recurred, and large numbers of apoptotic bodies were once again found in the cecal mucosa at 6 hours (Figure 2). When compared with the levels in untreated control animals, the numbers of apoptotic bodies were significantly increased in the lamina propria 6 hours after the last of 3 ($P < 0.001$) and 7 ($P < 0.02$) consecutive daily doses of danthron and in the epithelium 6 hours after the last of 3 daily doses ($P < 0.01$). As after a single dose, the apoptotic bodies were most numerous in macrophages in the superficial lamina propria, heavily pigmented macrophages lying at a deeper level being devoid of apoptotic bodies.

Six hours after low-dose danthron, damage to surface epithelium was less evident, with only a slight reduction in the height of the epithelial cells being observed. The numbers of apoptotic bodies, compared with levels in untreated control animals, were significantly increased in the epithelium 6 hours after the first ($P < 0.01$), third ($P < 0.02$), and seventh ($P < 0.02$) daily danthron administration and in the lamina propria after the first ($P < 0.01$) and third ($P < 0.05$). The increases, however, were less than those in high-dose animals (Figure 2). Changes in the number of apoptotic bodies with time ran parallel to those in high-dose animals (Figure 2).

Electron Microscopy

Apoptosis of epithelial cells was seen rarely in normal guinea pig cecum. Most of the resulting apoptotic bodies were found to have been phagocytosed by cells

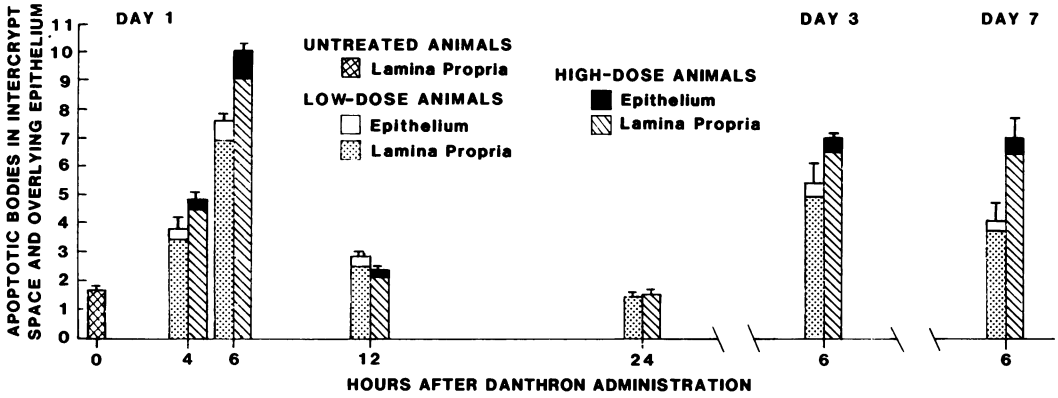


Figure 2—Mean number of apoptotic bodies in cecal lamina propria between pairs of crypts and in each overlying segment of surface epithelium in guinea pigs given oral danthron in low (2.5 mg/kg) and high (25 mg/kg) dosage. Counts in normal, untreated animals are shown at 0 hours on Day 1. The other bars under Day 1 indicate the counts at four time intervals after a single dose. The bars under Days 3 and 7 indicate the counts 6 hours after the last dose of courses of daily doses of these durations. Counts in the epithelium in normal animals and animals 24 hours after a single danthron dose are too small to appear as separate categories. Error bars indicate the SEM of combined counts in epithelium and lamina propria.

with morphologic features of macrophages that were basally situated within the epithelium. These cells had abundant, electron-lucent cytoplasm containing heterolysosomes, residual bodies, and sparse organelles and showed long cytoplasmic processes that often bridged gaps in the basement membrane. Heterolysosomes containing partly degraded cellular material and residual bodies were also found in macrophages in the superficial lamina propria in normal animals.

Four and 6 hours after danthron ingestion, there was abundant apoptosis of cecal surface epithelium. Some affected cells showed sharply defined masses of chromatin lying against the nuclear envelope, convolution of nuclear and cellular outlines, and increased electron density and crowding of cytoplasmic organelles (Figure 3a). Others had fragmented to form membrane-bounded, electron-dense apoptotic bodies containing crowded organelles and nuclear fragments with sharply circumscribed, marginated, condensed chromatin (Figure 3b). Most apoptotic bodies were phagocytosed by intraepithelial macrophages (Figure 3c), but small numbers were taken up by epithelial cells. Macrophages laden with heterolysosomes and residual bodies were sometimes seen passing through the epithelial basement membrane (Figure 3d).

Macrophages were quite numerous in the lamina propria 4 hours after danthron administration and contained many apoptotic bodies, most with structurally intact organelles (Figure 4a). The origin of these apoptotic bodies from epithelial cells was suggested by the simultaneous apoptosis and depletion of the surface epithelium, the type and abundance of organelles in the apoptotic bodies, and the presence of occasional goblet cell fragments. By 6 hours, considerable degradation of the apoptotic bodies had occurred in the macrophages, and individual organelles within them could no longer be identified (Figure 4b). After 24 hours, only large, loose, membranous whorls, lipid globules, and granular electron-dense material remained (Figure 4c). Thereafter, the contents of macrophage heterolysosomes progressively condensed, forming tight membranous whorls within an electron-lucent amorphous and granular matrix at 3 weeks (Figure 4d) and dense lysosomal granules at 8 weeks.

Four and 6 hours after danthron ingestion, autolysosomes containing altered mitochondria and short segments of rough endoplasmic reticulum and residual bodies filled with granular electron-dense material and myelin figures were found in the supranuclear cytoplasm of surface epithelial cells. The latter corresponded with small pigment granules seen by light microscopy. Autolysosomes and residual bodies of similar appearance were also found in the apoptotic bodies in the lamina propria. Rare surface epithelial cells showed severe cellular and mitochondrial swelling, amorphous densities in mitochondria, and rupture of plasma and organelle membranes, manifestations of cell death by necrosis.[20] Where epithelial cell loss was particularly extensive, areas of basement membrane were covered by only attenuated epithelial cell processes.

Guinea Pig Colon and Ileum

By light microscopy, the colonic mucosa in danthron-treated animals showed changes that were qualitatively and quantitatively similar to those observed in the cecum. In the ileum, relatively small numbers of apoptotic bodies were found in the surface epithelium and lamina propria at the villus tip 6 hours after high-dose danthron. Apoptotic bodies in the epithelium were phagocytosed by macrophages and epithelial cells or shed into the gut lumen. In the lamina propria, their presence preceded pigment formation in macrophages. After low-dose danthron, apoptotic bodies were found infrequently, and only scant pigment resulted.

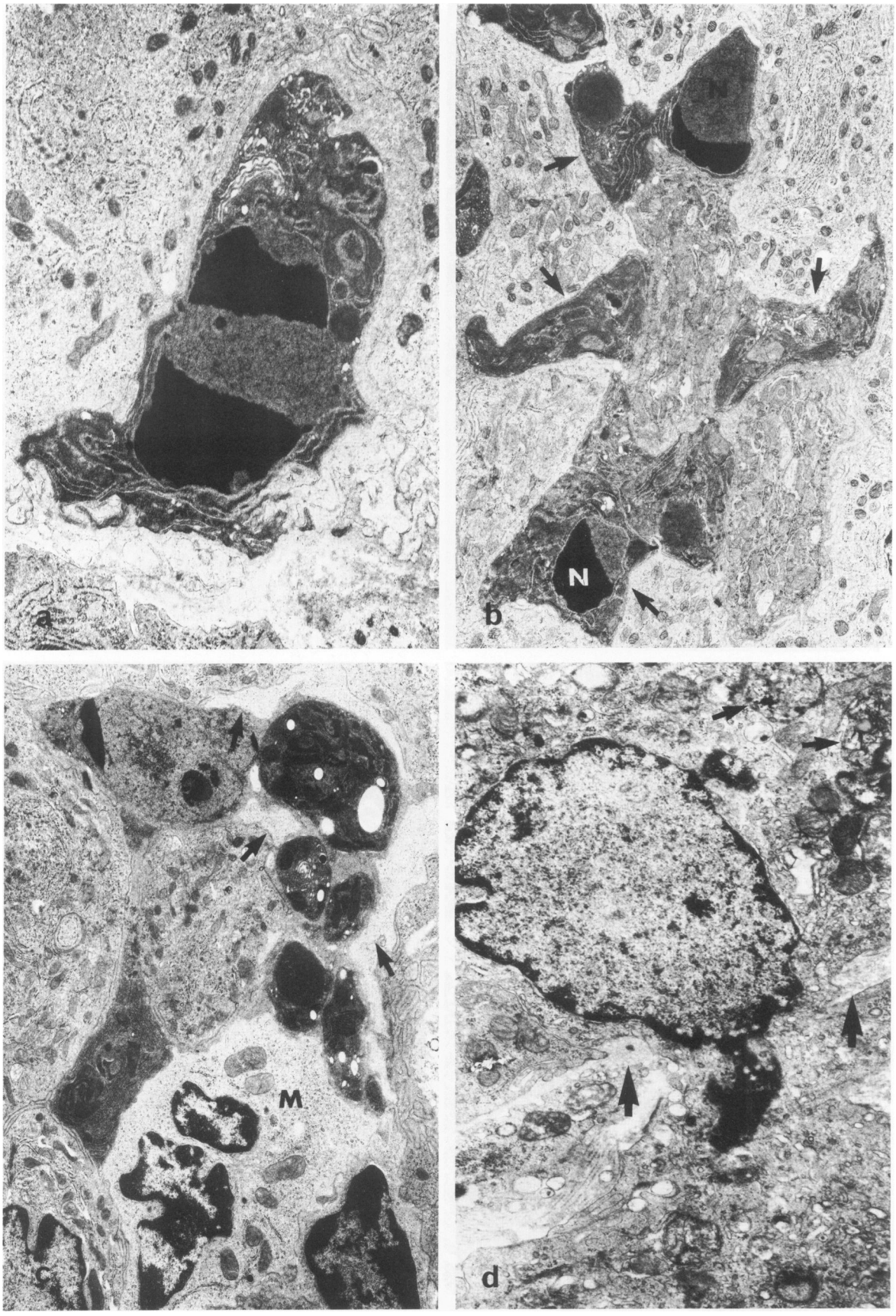
Human Colon

Light microscopy of normal mucosa rarely revealed macrophages containing apoptotic bodies within the surface epithelium and crossing the epithelial basement membrane. In melanosis coli, significantly increased numbers of apoptotic bodies were found within macrophages in the epithelium (Table 1 and Figure 1e) and superficial lamina propria (Table 1, Figure 1e and f). The mean number of apoptotic bodies in different biopsies of this condition, however, showed wide variation, ranging from 0 to 9.3 per intercrypt segment of surface epithelium and from 0.6 to 10.3 per intercrypt space. The variability in the number of apoptotic bodies in the melanosis cases may reflect the length of the time interval between anthraquinone ingestion and biopsy.

Size and Frequency of Fenestrae in Surface Epithelial Basement Membrane

Scanning electron microscopy of the denuded cecal surface epithelial basement membrane showed that in normal guinea pigs it was penetrated by well-defined

Figure 3—Surface epithelium of guinea pig cecum 4 hours after single oral dose of danthron (25 mg/kg). **a**—Early apoptosis of an epithelial cell. (×11,200) **b**—Cluster of apoptotic bodies (*arrows*), some containing nuclear fragments (*N*). (×7500) **c**—Apoptotic bodies partly enveloped by pale cytoplasmic processes (*arrows*) of an intraepithelial macrophage (*M*). (×7500) **d**—Intraepithelial macrophage with cytoplasmic process containing part of nucleus protruding through the epithelial basement membrane (*large arrows*). Its cytoplasm contains several heterolysosomes (*small arrows*). (×13,600)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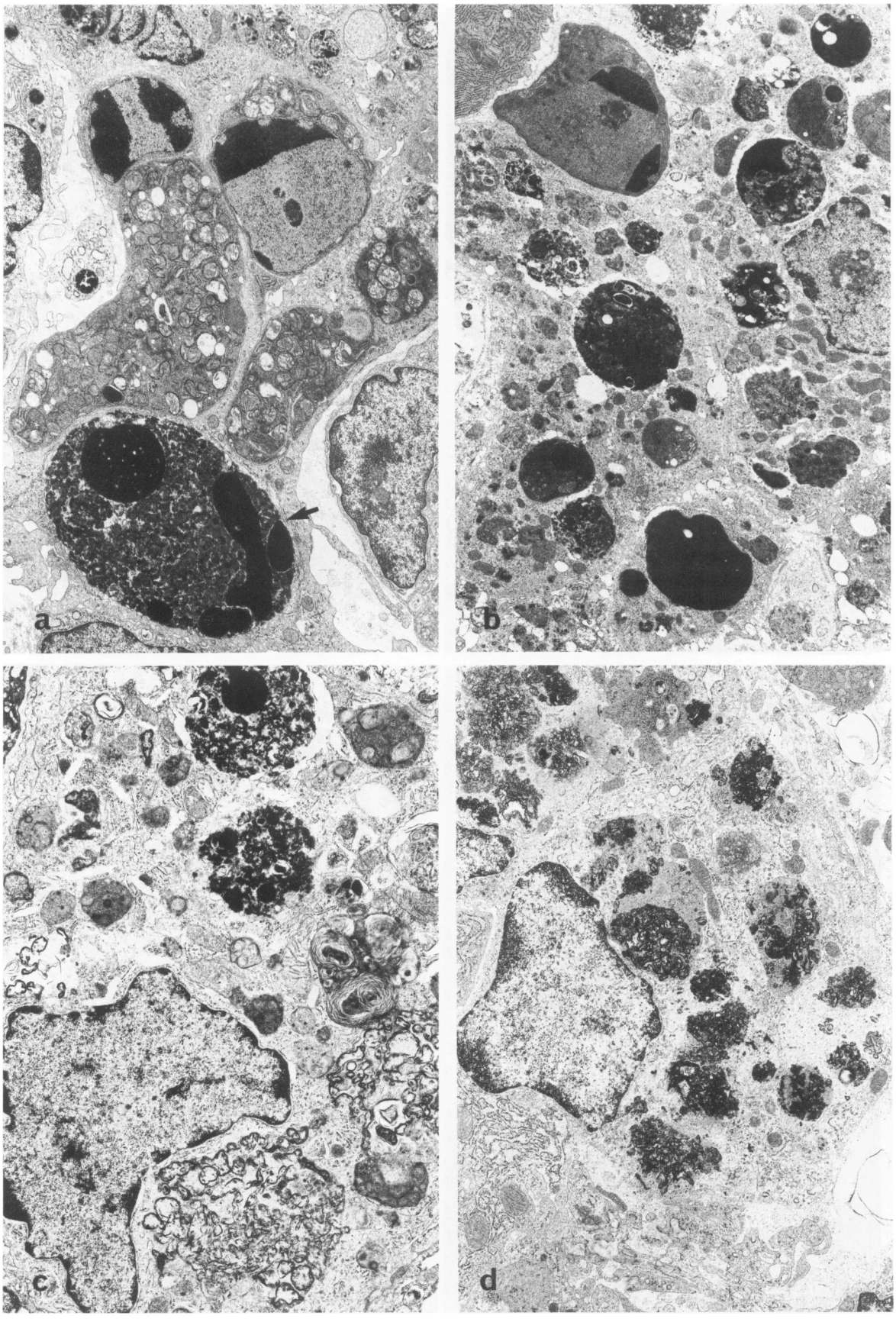
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Figure 4—Development of lipofuscin pigment in macrophages in guinea pigs after high-dose danthron. **a**—Cecal lamina propria 4 hours after single dose. Most apoptotic bodies in macrophages are well preserved. One (*arrow*) is partly degraded. (×6400) **b**—Cecal lamina propria 6 hours after single dose. Most apoptotic bodies are now partly degraded. (×4800) **c**—Cecal lamina propria 24 hours after a single dose. Macrophage contains heterolysosomes and residual bodies. (×8000) **d**—Pigmented macrophage in ileocecal lymph node 3 weeks after completion of a 10-day course. (× 6400)

round to oval fenestrae (Figure 5a). These measured 1–9 μ in diameter (mean, 2.5 μ) and had a density of 6000/sq mm. Similar fenestrae were visible in the epithelial basement membrane of the crypts. Six hours after high-dose danthron, the density of fenestrae in the surface epithelial basement membrane was unchanged, but the mean diameter had increased ($P < 0.01$, two-sided t test) by 30%, and ragged defects were found in the basement membrane at crypt margins (Figure 5b). Large intraepithelial macrophages containing apoptotic bodies were often seen by light microscopy to overlie the latter sites.

Discussion

The distribution of pigment in melanosis of the guinea pig large intestine induced by danthron closely resembled that seen in melanosis coli in man.[21] Pigmented macrophages progressively accumulated in the lamina propria, entered the submucosa, and migrated to the regional lymph nodes. The pigment granules were first evident in macrophages in the superficial lamina propria and became larger and more closely packed as the macrophages migrated to deeper layers of the gut wall. When treatment was discontinued, the pigmented macrophages disappeared sequentially from the superficial and deep lamina propria but continued to increase in number in the regional lymph nodes throughout the period of the experiments.

The first event in the sequence leading to the accumulation of pigment in the lamina propria of the large intestine was induction by the danthron of a transient wave of enhanced apoptosis of surface epithelial cells. The selective involvement of the large intestine may merely reflect conversion of glucuronidated drug to its active form by cecal bacteria.[22,23] On the other hand, the failure of anthraquinones to induce melanosis in human colonic tumors[3] raises the possibility of a specific interaction with normal colonic epithelial cells. Selective enhancement of apoptosis in intestinal epithelial cell populations has been reported in a number of conditions.[24–33] In the case of apoptosis in small intestinal villous epithelium induced by Shiga toxin, the basis of the selectivity is believed to be attachment of the toxin to specific cell membrane receptors.[28] Certain enteric chemical carcinogens have also been shown to selectively enhance apoptosis at particular levels of the gut which correspond to the sites of subsequent tumor formation[34,35]; the reasons for the selectivity in this latter case are uncertain. In the context of the present studies, it is of interest that danthron has been shown to bind strongly to DNA *in vitro*,[36] to be mutagenic and genotoxic in various cell culture systems,[23,37–39] and to cause epithelial tumors of the colon in rodents.[40,41] It has been suggested that the induction of apoptosis by carcinogens and other agents affecting DNA may represent the controlled elimination of cells whose survival might be damaging to the host.[13–15,42]

Most of the apoptotic bodies derived from surface intestinal epithelial cells in both the normal and danthron-treated animals were phagocytosed by intraepithelial macrophages. The occurrence of such cells in normal intestinal epithelium has been described previously,[43–46] and they have also been recorded under pathologic conditions in the mouse intestine in *Giardia muris* infection,[47] in the guinea pig cecum after iota carrageenan ingestion,[48] in the rabbit ileum exposed to Shiga toxin,[28] and in man in hemosiderosis[44] and Whipple's disease.[43] Moreover, they have been shown to phagocytose apoptotic bodies of epithelial origin in small intestinal crypts in zinc-deficient mice.[24] In other glandular epithelia, phagocytosis of apoptotic bodies by intraepithelial macrophages has been demonstrated repeatedly.[10–11,49]

Movement of macrophages through the intestinal epithelial basement membrane, seen previously in rats, mice, and man,[43,47,50] was found in the normal human colon and in the normal and danthron-exposed guinea pig cecum in the present study. Fenestrae in the surface epithelial basement membrane, similar to those caused by migrating mononuclear cells in small intestine and skin,[50–52] were demonstrated by scanning electron microscopy in the cecum of both normal and treated animals. However, after danthron, the fenestrae were larger, and irregular defects appeared in the basement membrane at crypt

Table 1—Apoptotic Counts in Surface Epithelium and Lamina Propria in Normal Large Intestinal Mucosa and Melanosis Coli in Man*

	Normal mucosa	Melanosis coli
Epithelium	0.62 ± 0.15	3.41 ± 0.57
Lamina propria	0.84 ± 0.14	4.21 ± 0.61

* Means ± SEM of counts per intercrypt segment of surface epithelium and lamina propria. Results in melanosis coli are significantly increased above corresponding values in normal biopsies ($P < 0.001$).

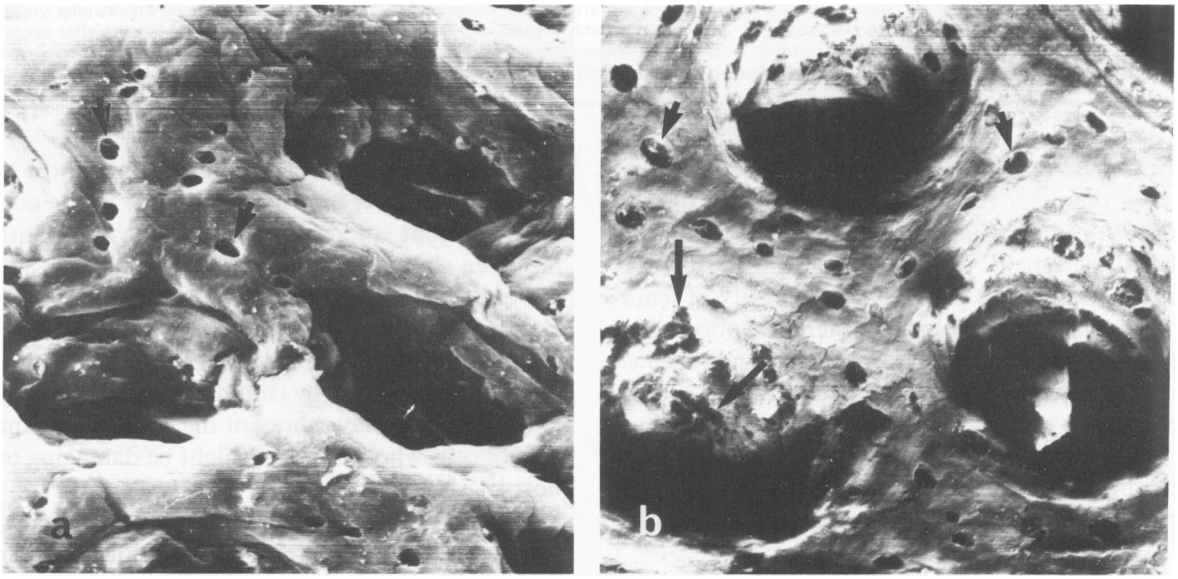

Figure 5—Fenestrae (*short arrows*) in cecal surface epithelial basement membrane demonstrated by scanning electron microscopy. **a**—Normal guinea pig. (×650) **b**—Guinea pig 6 hours after oral danthron (25 mg/kg). Note irregular defects at crypt margins (*long arrows*). (×650)

margins. These changes were possibly caused by migration of increased numbers of mononuclear cells or of larger cells containing phagocytosed apoptotic bodies. The progressive movement of pigmented macrophages from superficial to deep lamina propria and regional nodes during the 2-month period following cessation of danthron administration was similar to that seen in Peyer's patches in mouse ileum after latex and carbon particle ingestion.[53,54]

There is evidence that most and possibly all of the apoptotic bodies observed in the lamina propria soon after danthron administration were derived from epithelial cells, which had been carried there by macrophages. Thus, their incidence paralleled that of apoptosis in the overlying epithelium, their ultrastructure indicated an epithelial origin, and they invariably lay within macrophages, rather than free in the extracellular space. The parallel occurrence of apoptotic bodies in both epithelium and lamina propria has previously been observed in the human rectum in AIDS,[31] graft-versus-host disease,[32] and cytomegalovirus infection (unpublished observation). Cell fragments and chromatin particles, not specifically identified as apoptotic in origin, have been noted in macrophages immediately beneath the epithelium in normal guinea pig small intestinal villi and colon.[17,55] The presence of radiolabel in both the colonic surface epithelial cells and the lamina propria nuclear fragments 3 days after injection of tritiated thymidine in one of these experiments[55] suggests to us that the latter originate from epithelial cells. However, whether apoptosis contributes significantly to normal intestinal epithelial cell turnover is currently unknown.

The pigment produced in guinea pig lamina propria macrophages after danthron treatment showed histochemical reactions and ultrastructural appearances similar to those observed in melanosis coli in man.[5-9] It also closely resembled lipofuscin arising in cellular autolysosomes induced by various stimuli.[6,56] The small supranuclear pigment granules in surface epithelial cells in the danthron-treated guinea pigs resulted from autophagic degradation of organelles, as has been described previously in man.[7] The abundant pigment in lamina propria macrophages, on the other hand, resulted from the degradation of apoptotic bodies in heterolysosomes. The rapid disappearance of the apoptotic bodies correlates with the relatively short time required for digestion of cell organelles and apoptotic bodies by lysosomal enzymes.[13,57] The progressive accumulation of pigment in the guinea pigs given daily doses of danthron resulted from repeated waves of apoptosis in the surface epithelium. The observation of increased apoptosis in both the epithelium and superficial lamina propria in patients with melanosis coli suggests that a similar sequence of events follows repeated ingestion of anthraquinone purgatives in man. The variability of the apoptotic counts in biopsies from different patients presumably relates to varying time intervals between anthraquinone ingestion and biopsy. Our clarification of the mechanism of pigment formation in melanosis coli leads us to support a previous proposal to rename this condition "lipofuscinosis coli."[58]

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Acknowledgments

The authors thank Prof. J. F. R. Kerr for helpful criticisms of the manuscript and Dr. G. R. Walker for statistical advice.