Damage and Repair of Small Intestinal Mucosa in Acute and Chronic Diarrhea

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Damage to the mucosa of the digestive tract frequently results in diarrhea. The variety of etiologies leading to this situation is so great that of necessity we focus this discussion only on a limited number of examples. They have been selected because of their frequency, their repercussions for the patient or the community, or because they serve as models that help to analyze some responses of the intestinal mucosa to injurious agents. Within this framework, alterations of the small intestine are analyzed in relation to different types of etiologies. Some reference is also made to diseases that may interfere with the function and/or structure of the mucosa of the large intestine.

The mucosa of the small intestine is a large interface that absorbs the nutrients of the diet. In addition, it provides an extensive surface for the interaction of the body with the animate and inanimate constituents of the external milieu. For these reasons it is endowed with multiple defensive capabilities. These protect the lumen and the surface of the organ, and in fact the whole body, against antigenic molecules and the myriad of microorganisms that gain access to it with food or drink (1). Macromolecules are digested in the lumen in close contact to the external leaflet of the plasma membrane or in the cytoplasm of the absorptive epithelium by complex arrays of enzymes that destroy their immunologic identity. The smaller, simpler molecules that result from this process are, to a considerable extent, absorbed and fill the nutritional requirements of the body.

The digestive tract is endowed with specific and nonspecific defense mechanisms against living organisms. Acting simultaneously or in succession, these mechanisms more often than not are capable of avoiding the appearance of disease (mainly acute infectious diarrhea). The specific mechanisms are the result of previous contacts with the offending organisms or some of their antigens (2) (Fig. 1). The additive effect of both types of defensive mechanisms is in general highly effective, and only a few species of bacteria, viruses, or parasites are actually capable of causing damage to the mucosa of the digestive apparatus and, therefore, inducing the appearance of symptoms.

For the enteropathogenic capacity of an agent to become evident, an important
FIG. 1. Part of the lamina propria of a normal infant. Two of the main cellular components of the local immune system are present: three lymphocytes (L) in rather close contact with four plasma cells (P). Parts of a macrophage (M) and a Schwann cell (S) are also seen. (×5,400)
requirement is that it gain access to the lumen of the gut in such numbers that it insures its survival despite the presence of the local defensive mechanisms (3). The next stage involves the contact of the microorganism with the surface of the intestine and its attachment to the mucosa. This is a crucial step from which very few species are exempted. An example of these latter is Clostridium botulinum, which is capable of inducing disease without adhering to the mucosa: it synthesizes large amounts of a readily absorbed, very powerful toxin that even in minute amounts induces severe systemic effects (4).

The first structure of the host that comes into contact with a microorganism is the epithelial glycocalyx (5,6). In the case of bacteria and parasites this contact also involves the glycocalyx of the pathogen (4) (Fig. 2). The epithelial glycocalyx is a filamentous layer composed of glycoproteins that form a dense, highly hydrated net with the main axis of the fibrils approximately perpendicular to the outer leaflet of the plasma membrane. Its components are synthesized in the rough endoplasmic reticulum and the Golgi apparatus, and from the latter they migrate to the microvilli (7,8). The mechanisms by which these materials are transferred from the cytoplasm to the outside are not fully understood (9). The glycocalyx is very rich in sialic acid, and this confers on this structure its strongly negative charge (10). Electrical charges of the bacterial surface may therefore play an important role in their attachment to the absorptive cells.

The bacterial glycocalyx is also made of glycoproteins. In the case of gram-negative bacteria, it is located outside the plasma membrane, and in the case of gram positives, outside the peptidoglycans (4). Bacterial glycocalyces are composed of hexoses, dihexoses, uronic acids, polyols, and substituted amino sugars. Thus, bacteria and the cells of the small intestinal epithelium enter into contact through structures that are chemically comparable. It is not known how tight this contact may be. It probably involves chemical receptors in the bacteria that recognize some moieties in the host cells, and this renders the linkage stronger and more specific. Once a pathogen has become attached to the epithelium and begins to proliferate, its glycocalyx probably provides it with a protective environment against the defensive mechanisms of the gut (11,12).

In their approach to and contact with the host surface, enteropathogens encounter an additional, highly hydrated layer composed of mucus secreted by the goblet cells. It is not known how effective this may be as a barrier. When present in rather thick layers, mucus may serve as an obstacle for contact. It may also interfere with the chemotactic capabilities of bacteria and protozoa by providing false receptors. The mechanisms that regulate goblet cell discharge are not fully understood. It has been shown that antigen–antibody reactions in the vicinity of the surface of the absorptive epithelium induce mucus secretion (13). In addition to the factors already described, it is possible that the primary attachment of bacteria, parasites, and viruses to the surface of the intestinal epithelium is influenced by physicochemical characteristics such as the ionic strength of the milieu, the presence of substances with detergent properties, movement and flow of intestinal contents, and secretions (14).
FIG. 2. a: Environmental enteropathy. Ruthenium red slightly counterstained with lead citrate. This image probably represents one of the earliest stages of the contact between bacteria and the epithelium. In this case, both cells are connected by a few fine strands of stainable material that joins both glycocalyces. (×34,000) b: Environmental enteropathy. Same processing as in a. Microvilli are irregular in shape and thickness. One of them appears to result from the fusion of three elements. Two bacteria (arrow) are embedded in the mucus overlying the brush border. Those microvilli facing one of the bacteria are probably bent, giving a distorted appearance to that area of the brush border. (×25,000)
Firm attachment of bacteria to the epithelium is achieved through a complex mechanism in which both the host and the pathogen interact. Pili or fimbriae, nonflagellar, rod-like bacterial organelles, firmly anchor the procariont cell to the outer leaflet of the plasma membrane of the absorptive cell (15,16). Pili are made of subunits of a protein called pilin (17). This is an antigenic material of which, in strains of *Escherichia coli* pathogenic for humans, at least two colonization factor antigens (CFA) I and II have been thus far identified (18,19). Other CFAs are likely to be isolated in the future. These CFAs have affinity for a moiety of the plasma membrane that contains mannose (20). As a matter of fact, the presence of free mannose in a medium in which *Escherichia coli* are incubated with intestinal epithelial cells inhibits bacterial adherence (19). Adhesion of *Escherichia coli*, and probably of any bacteria, to the brush border of the absorptive epithelium may produce by itself the appearance of diarrhea and damage to the enterocytes. This has been clearly shown in piglets infected with strains of *Escherichia coli* that have pili K88 or K99 (which are homologous to the CFA antigens of human strains) but lack all the other pathogenic factors that may cause acute diarrhea (enterotoxins, entropathogenicity, invasiveness). By contrast, strains of *Escherichia coli* that do not have pili but synthesize enterotoxins do not induce disease because they cannot adhere to the epithelium and therefore cannot colonize the host (20).

It is likely that each absorptive cell has a limited number of receptors for each type of pilus (21). The importance of surface receptors in this process is evinced by the observation that *Escherichia coli* do not adhere to isolated intestinal epithelial cells that have previously been incubated with purified pili from the same strain (19). This failure of attachment suggests that it may be possible to prepare vaccines against purified pili and thus prevent diarrhea by hindering attachment and colonization of the surface of the gut.

In addition to this specificity for receptors in the small intestinal epithelium, there is, at least for some species or strains, specificity for certain loci along the organ. Thus, some strains of *Escherichia coli* first attach to the M cells in Peyer’s patches (22). In chicken, different species of *Eimeriae* attach to very specific segments of the small intestine (23). In surgical specimens obtained from patients with malignant tumors of the mucosa of the colon, it is possible to demonstrate that although some bacteria adhere to the surface of the normal tissue, they fail to attach to the surface of the adenocarcinoma (24). Because both tissues are in contact with a comparable luminal milieu, it is possible to postulate that bacterial attachment requires exposure on the part of the host cell of specific membrane receptors.

Adhesion is probably the key step in the genesis of infection and cellular damage in the intestine, and this is the reason we have dwelt at length on this matter. It is not known how viruses attach to the surface of the brush border. It has been claimed that rotavirus may attach to lactase before invading the epithelium, and this would explain the high frequency of lactose intolerance during episodes of diarrhea caused by this agent. This has been denied by others.
(25,26). *Giardia lamblia* probably uses specific receptors to attach to the brush border of the enterocytes in the duodenum and upper jejunum. As with some bacteria, specificity for the upper segments of the intestine suggests the existence of receptors and the influence of unidentified conditions of the luminal milieu.

Once they have attached to the surface of the intestine, some pathogenic bacteria will penetrate into the epithelial cells, whereas others will not. Enterotoxigenic bacteria do not invade the epithelium and cause disease by secreting a complex enterotoxin that in turn becomes attached to a specific receptor in the plasma membrane (27–29). One of the components of the enterotoxin penetrates into the cytoplasm of the enterocyte and produces biochemical changes that induce the crypt epithelium to secrete large amounts of chloride and water or block absorption by the villi and/or the crypts. This is not associated with any changes in the morphology of the mucosa or in its fine structure (30). The classical examples of this pathogenic mechanism are the secretory diarrheas caused by *Vibrio cholerae* and the enterotoxigenic strains of *Escherichia coli*.

When one studies the intestinal mucosa of patients with these conditions under the light microscope, it is important to keep in mind whether these people live in highly contaminated environments, since they may have a “background” histologic alteration that is independent of the actual infection. This alteration has been called chronic environmental (“tropical”) enteropathy (31,32). In its milder forms it consists of some shortening of the villi, with slightly blunted tips, a more basophilic cytoplasm in the epithelium at the apex of the villi, and a rather increased number of teliolymphocytes. The crypts of Lieberkühn may be moderately elongated. The cellularity of the lamina propria is increased. However, asymptomatic individuals who live in highly contaminated environments may have much more severe histologic changes with flattening of the mucosa, damage to the surface epithelium, elongation of the crypts, and heavy infiltration of the lamina propria, giving an appearance comparable to that of tropical sprue or celiac disease (33). These changes have been attributed to repeated interaction of the small intestinal mucosa with bacteria, viruses, and parasites, sometimes resulting in episodes of diarrhea. Environmental enteropathy is also associated with increases in bacterial counts in the upper segments of the bowel, including gram negatives and anaerobes. How this modification in the flora damages the mucosa is not fully understood, although some toxigenic mechanisms have been proposed (34).

Whereas enterotoxigenic bacteria do not cause histological or fine structural alterations to the epithelium, infection with enteropathogenic and enteroinvasive bacteria definitely results in cellular changes of variable severity. Ulshen and Rollo (35), Phillips (36), and, more recently, Rothbaum and co-workers (37) have shown that infection with enteropathogenic *E. coli* is associated with damage to the absorptive epithelium. In each of these reports, the diarrhea was caused by a different strain. Mild or moderate nonspecific morphologic changes of the architecture of the mucosa of the small intestine (comparable to some extent to those seen in environmental enteropathy) were detected in all cases, and they
cleared with treatment. Bacterial colonization of the surface of the epithelium was demonstrated in all biopsies. Studies with the electron microscope revealed close attachment of bacteria to the absorptive cells. At the points of bacterial attachment, the surface of the enterocytes developed cup-like outgrowths. The brush border was damaged, with disappearance of the normal structure including the web area with its complex filamentous apparatus. There were also some signs of damage to cytoplasmic organelles, probably suggesting that the deleterious effect of this infection extended beyond the apical portion of the cytoplasm of the absorptive cells. All of these changes, especially those of the brush border, are remarkably similar to those observed in the ileum of newborn pigs infected with *Escherichia coli* 055:B5:H7 (38).

In enteroinvasive diarrheas, bacteria penetrate into the absorptive cells and cause severe injury to the epithelium (39–43). This is demonstrable by immunofluorescent techniques and particularly with the electron microscope. Some of the data that are presented have been obtained in studies of rectal mucosa of humans and from observations in experimental animals (44,45). The first stages of the approach of the bacteria to the surface of the mucosa are probably comparable to those already discussed for enterotoxigenic and for enteropathogenic *Escherichia coli*. Bacterial penetration probably begins with their strong attachment to the luminal surface of the cells by means of special differentiations (holdfasts) around which a condensed area of cytoplasm is formed (46). At the points where the plasma membranes of the pathogen and of the host come into close contact, the latter may form special structures such as those described by Neutra (47) for spirochetes and flagellated bacteria in the rectal mucosa of humans and monkeys. At the same time, the brush border in the area of attachment disintegrates into vesicles or is severely distorted (43). Those microvilli that remain seem to bend toward the point of bacterial attachment (48).

A subsequent step involves the formation of a cytoplasmic vacuole in which a single bacterium is enclosed. Some of the bacteria may become incorporated into autophagosomes. The epithelial cells show signs of damage characterized by the appearance of multivesicular bodies, autophagosomes, and, finally, disorganization of the cytoplasmic structure and death. The underlying lamina propria becomes packed with inflammatory cells. After 24 hr of infection, salmonellae can be seen within neutrophiles and macrophages. The damaged epithelial cells are desquamated into the intestinal lumen and leave ulcerated areas that enlarge laterally, because the bacteria spread from cell to cell by contiguity (43). The appearance of local immunity probably terminates the infection by blocking the invasion of uninvolved cells while making the bacteria more susceptible to the phagocytic activity of leukocytes and macrophages. Probably the same phenomenon happens in the colonic mucosa during *Shigella* infection (44).

The invasive capacity of some bacterial species goes beyond the lamina propria. They enter the bloodstream and generate systemic illness, even colonizing distant organs. The prototype for this infection is *Salmonella typhi*. The morphology
of the invasion of the mucosa during *Salmonella typhi* infection is not known in detail (40,49).

*Giardia lamblia* is a protozoan whose habitat is the surface of the mucosa of the duodenum and upper jejunum, where it lives in close contact with the brush border of the absorptive cells. Studies with the scanning electron microscope show the imprint of the sucking plate of *Giardia* on the brush border of absorptive cells (50). It is not known what effect this strong attachment of the parasite may have on the contraction of the apex of the columnar cells (51,52), and what the consequences of this latter alteration are on the absorptive capacity of the epithelium, particularly in massive infections.

*Giardia* may invade the epithelium or even the lamina propria (53,54), but whether this is a regular feature in all subjects or whether it represents a failure of the host's defensive mechanisms to confine the parasite is not known. Besides this capacity to invade the mucosa in some individuals, the pathogenic properties of *Giardia lamblia* have been attributed to mechanical blockage of the intestinal surface, its association to an abnormal flora, deconjugation of bile salts, competition for essential nutrients, or cytotoxic effects caused by substances liberated by the parasite (50,55). The brush border of epithelial cells directly in contact with the parasite shows some minor to moderate alterations in implantation (56). As shown by Barbieri et al. (56), the supranuclear cytoplasm of these cells has increased numbers of lysosomes, mainly multivesicular bodies, that probably are the result of the injury caused by the parasite. Other studies have also shown minor alterations of cytoplasmic structure. *Giardia lamblia* stimulates immune responses in parasitized individuals (57). This is probably the reason why, although the first attacks tend to cause an episode of acute diarrhea, subsequent infections seem to induce less symptoms or are asymptomatic, especially in older children or adults (55). In individuals with immune deficiencies, instead, *Giardia* may induce severe lesions of the mucosa of the duodenojejunal junction comparable in magnitude to those observed in celiac disease, with improvement after satisfactory chemotherapy (58,59).

*Isospora belli* is another parasite that is found with some frequency in Chile and other areas of the world and which causes protracted diarrhea with malabsorption and nutritional disturbances (60). This sporozoan invades the epithelium and causes moderate nonspecific damage to the intestinal mucosa. Characteristically, it is possible to observe all phases of the life cycle of the parasite in biopsies of the jejunal mucosa. The lamina propria shows an infiltrate with eosinophils (61). Studies in patients and volunteers with viral diarrheas of different etiologies reveal that the histology of the mucosa shows mild to moderate architectural changes, which are nonspecific: there is some shortening and thickening of the villi, a moderate degree of elongation of the crypts, and disorganization of the absorptive cells (62,63). These cells are more cuboidal, with occasional vacuolization of the supranuclear cytoplasm, and with increased numbers of mononuclear cells among them. Some polymorphonuclear leukocytes may also be
observed in the same location. The cellularity of the lamina propria is increased mainly as a result of the accumulation of mononuclear cells and neutrophils.

The mechanisms by which enteric viruses penetrate into the epithelial cells are not known. Neither is it known with certainty what serves as receptors for them. Worthington and Graney have shown that in suckling rats adenovirus penetrates into the jejunal and ileal epithelium by pinocytosis through the apical tubular system of these cells. These channels lead to lysosome-like structures in the former and to the supranuclear vacuole in the latter (64,65). Penetration of rotavirus into the epithelial cells has never been observed. In biopsies taken from symptomatic subjects, viral particles appear in large numbers in dilated cisternae of the rough endoplasmic reticulum, in autophagic vacuoles, and even in some cells of the lamina propria (36).

In general, the changes induced in the cytoplasm of infected cells are similar for all viral agents thus far studied: the brush border shows greater or lesser degrees of disorganization, the endoplasmic reticulum is dilated, and lysosomes are abundant. Many cells degenerate and die (63,66,67). In cases of diarrhea caused by adenovirus, virions have been observed in the nucleus of infected cells (36). Studies in piglets by Hamilton's group (66) and Takeuchi et al. (67) demonstrated that the mature, infected epithelium of the villi is desquamated and replaced by apparently immature cells rather similar in appearance to those of the crypts: they are more cuboidal, with sparse microvilli, many free ribosomes, and a poorly developed endoplasmic reticulum and Golgi apparatus. The replacement of the well-differentiated epithelium by crypt-like cells may explain some of the clinical characteristics of rotavirus diarrhea, e.g., low lactase activity associated with the passage of liquid, acidic feces when patients ingest milk (68). This crypt-like epithelium probably has limited absorptive capacity.

Invasion of the epithelium by a diarrhea-causing virus is a complex phenomenon. It is possible that at the beginning scattered cells are infected and that from these, the virus contaminates the neighbors by passing through the intercellular space or because sloughed cells disintegrate and release viral particles, which may in turn invade intact cells. The fact that individuals with immune defects tend to have more prolonged episodes of rotavirus diarrhea suggests that the appearance of specific immunity to the infecting serotype ends the episode of diarrhea by blocking further invasion of new cells (69). It is not known whether the action of the antibodies takes place within already infected cells or in the extracellular environment. Desquamation of the diseased cells and interruption of reinfection probably result in termination of the episode of diarrhea and hinder the appearance of the carrier state.

As previously discussed, the vast majority of the acute diarrheas are of short duration, either because they are self-limited or because appropriate treatment eradicates the etiologic agent(s). The damaged cells are replaced by healthy elements arising from the crypts, and absorptive function is rapidly restored to its (normal) previous levels. However, for reasons that are not well understood and that are explored in this volume, in some individuals the diarrheal episode
persists, does not respond to well-conducted treatments, and becomes associated with profound alterations of the hydroelectrolytic balance, malabsorption, and deterioration of nutritional status. Neither the reasons for this undesirable change, which is called prolonged or intractable diarrhea, nor its histological or fine structural substrate has been well characterized.

So far, this discussion has centered mainly on acute infectious episodes as one of the possible antecedents for prolonged, intractable diarrhea. There are a number of other conditions associated with damage to the intestinal mucosa, chronic diarrhea, and malabsorption and whose histology and fine structural features have been more extensively studied. The severity of these depends probably on two factors: the intensity of the damage to the mucosa and the length of the involved segment (70). The most frequent of these conditions are celiac disease, tropical sprue, dermatitis herpetiformis, some forms of milk intolerance, and malnutrition. When intestinal biopsies are examined under the light microscope, the severe forms of these diseases have a histological lesion that is nonspecific. Surprisingly (or perhaps not surprisingly), when examined under the electron microscope, epithelial cells of patients with these diseases share a common set of fine structural alterations even when, as is the case in marasmic malnutrition, the architectural integrity of the mucosa may be rather well preserved (73,74). When the epithelium of the flat areas of the mucosa or that near the openings of the "elongated" crypts is studied, this similarity of cellular damage becomes evident. This suggests that under the impact of different injurious stimuli, and once these reach a certain intensity, the epithelium has a common, basic pattern of response at the fine structural level.

The brush border may appear with sparse, shorter or longer microvilli, irregularly implanted, sometimes bi- or trifurcated from a common base of implantation (Fig. 3). The glycocalyx that coats the altered brush border loses its "compactness," with its appearance ranging from a loose mesh of filaments to complete absence in the extended zones. Not infrequently, especially when alterations of the brush border are severe, the web area also becomes distorted (33,75,76). Disturbances of this area of the cell are probably associated with absorptive defects. The endoplasmic reticulum is frequently dilated, and fat droplets accumulate in many cases (33). Fat secretion into the intercellular space of the epithelium requires the synthesis of VLDL in the Golgi apparatus. Abnormal accumulation of fat in the liver and in the intestinal epithelium has been shown to be associated with defects in the synthesis of VLDL, specifically of some of its constituent peptides (77–79). Therefore, the deposits of fat in the endoplasmic reticulum may reflect abnormalities in epithelial protein metabolism.

The finding of numerous free ribosomes and the break-up of polysomes also suggest alterations of protein synthesis. Large numbers of free ribosomes are observed in conditions in which the mature epithelium desquamates rapidly and is replaced by cells from the crypts of Lieberkühn, which are not fully mature (67). Since enzymes are protein or glycoprotein molecules, these changes
FIG. 3. a: Celiac disease. Brush border of a cell of the flat surface of the mucosa. Microvilli are irregular in size, shape, and implantation. The web is disorganized. (×22,000) b: Environmental enteropathy. Same processing as Figs. 2a and b. Fusion and bifurcation of microvilli are prominent. See also Fig. 2b. (×19,000) c: Tropical sprue. Microvilli are extremely shortened, and their thickness is irregular. (×16,000) d: Marasmic malnutrition. Bi- and trifurcation of microvilli are a common feature. Stretches of plasma membrane appear devoid of microvilli (arrow). (×20,000) Most of these appearances can be seen intermixed in different proportions with stretches of normal brush border in a variety of clinical conditions.
in ribosomal distribution may explain the alterations in enzyme activity and, in general, the metabolic disturbances demonstrated in the mucosa in these diseases (80,81).

Voluminous lysosomes, many of them autophagosomes, are another indication of the nonspecific response of the epithelial cells to injury (Fig. 4). They have been described not only in tissue samples from the abovementioned diseases but also in conditions such as radiation enteritis or treatment with methotrexate (82,83). They have also been described in involuting tissues (84,85). The appearance of autophagosomes may also be an indication of alterations of microtubule function (86,87). As microtubules are involved in the translocation of particulate components of the cytoplasm, this may explain in part the accumulation of fat within the cells (88). The basement lamella remains unchanged in thickness and overall appearance. However, immediately beneath it, there may be accumulations of collagen fibers, filaments, and finely granular materials of variable density in which fat droplets are sometimes embedded (33,75) (Fig. 5). This may be an indication that the complex barrier function of the epithelium towards the luminal content is altered and that antigen–antibody reactions are taking place in this area (89). This is supported by the finding that in many of these diseases it is not uncommon to detect circulating antibodies against dietary proteins (90). Furthermore, immune reactions occurring in the vicinity of the epithelium may in turn further damage the absorptive cells. Deposits with rather similar morphological characteristics have been observed in entities associated with immune reactions in close proximity to different epithelia (74,91).

MUCOSAL REPAIR

Repair of damage to the mucosa of the small intestine in acute or chronic diarrhea depends basically on the interplay of three factors: (a) the possibility of removing the offending agent, (b) the severity of the damage that has resulted, and (c) the potential of the epithelium to divide and replace the cells that have been lost or damaged.

In the case of some bacteria or parasites, removal of the causative agent may require the administration of pharmacological treatments. The appearance of specific immunity is one of the main factors in the elimination of pathogens from the gastrointestinal tract and accounts for the self-limited nature of the vast majority of uncomplicated episodes of acute diarrhea. In the case of chronic diarrhea, elimination of the etiologic agent may involve measures such as elimination of gluten or other components of the diet (milk, soy, or other proteins), the administration of folate, zinc, vitamin B₁₂, improvement of nutritional status, etc.

Once the cause of diarrhea has been eliminated or at least neutralized, improvement of the intestinal mucosa depends on replacement of the damaged epithelium by healthy cells originated by proliferation and maturation of undifferentiated elements from the crypts of Lieberkühn.
FIG. 4. a: Marasmic malnutrition. A large autophagosome that contains amorphous material and rough endoplasmic reticulum. Two residual bodies are seen in this picture. The brush border was on the right. (X25,000) b: Celiac disease. Two voluminous residual bodies slightly over 2 μm in diameter and containing heterogeneous material are present in the supranuclear cytoplasm of an absorptive cell in the flat surface of the mucosa. The brush border was on the right. (X20,000) c: Dermatitis herpetiformis. Three autophagosomes (arrows) are present in this frame. The largest measures 2.5 μm in length and contains mitochondria in various stages of structural disintegration. (X11,000)
FIG. 5. a: Tropical sprue. Base of epithelium (EP). A basal lamella of normal thickness is visible (arrow), and underneath it there are deposits of finely granular material in which collagen fibers are embedded. These appear as negative images. (×32,000) b: Celiac disease. Lamina propria underlying the surface epithelium of a flat area of the mucosa. The base of the epithelium is visible in the left upper corner. Collagen fibers are prominent, finely granular material is abundant, and numerous fat droplets (F) are embedded in it. (×15,000)
FIG. 5. c: Marasmic malnutrition. All the cytologic elements described in the previous illustration—basal lamella (arrow), finely dense material, and collagen (in negative images)—are present. (×24,000) d: Dermatitis herpetiformis. The image is comparable to a and b. Collagen is unstained and appears as negative images. The arrow points to the basal lamella. The cell with the large, heterogeneous inclusions is a macrophage (M). (×15,000)
Many years ago, Bizzozero (92) concluded that the cells at the crypts divided and that the daughters resulting from this process ascended along the walls of the crypts and replaced those that were lost through desquamation. Studies using colchicine to induce mitotic arrest, counts of cells pulse-labeled with tritiated thymidine, and calculations of the ratio of labeled to unlabeled cells resulted in the demonstration that the epithelium of small intestine was constantly undergoing rapid renewal and that this was completed every 3 days in the rat and about every 5 days in humans (93–97). It was also shown that this velocity is not even along the small intestine but that it is significantly faster in the ileum (97).

Cheng and Leblond (98–102) stated that within the population of dividing cells in the crypts of Lieberkühn it was possible to postulate the existence of two compartments. One of these is located at the bottom of the crypts, in contact with the Paneth cells; it is formed by 20 to 30 cells, which divide very slowly. These cells have the potential of differentiating into any of the four main lines of cellular components of the epithelium: absorptive, goblet, Paneth, and enteroendocrine. These slowly dividing cells may be considered true stem cells. The evidence about the size of the stem cell compartment and its renewal velocity is under discussion as a result of studies of their behavior when the second compartment, whose cells divide rapidly, is destroyed by radiation or by drugs that block DNA synthesis or mitosis (103,104). It has been proposed that under these circumstances the stem cells are capable of dividing more rapidly and thus replenishing the compartment that has been damaged (105,106). Because under normal circumstances these stem cells are dividing at a slow rate, they are relatively resistant to the deleterious effects of drugs or radiation. It is not known whether some cells of the rapidly dividing compartment of the crypt retain the capability to dedifferentiate and thus revert into stem cells.

The mechanisms that regulate the commitment of a stem cell into cell(s) of the dividing compartment of the crypt are not known. The rapidly dividing compartment is formed by cells located in the middle third of the crypt. At any given moment, one or both of the daughter cells may not again enter into mitosis but rather initiate a period of differentiation in the course of which they will acquire the organelles and enzymatic complement of mature enterocytes. This occurs at or near the boundary between the crypt and the villus. The factors that control this transition from dividing, immature cell to maturing and eventually fully mature absorbing cell in the villus are not known. Although cell desquamation and renewal are delicately balanced, they are not absolutely linked because, for example, while cell division is arrested, shedding of enterocytes at the apex of the villi continues uninterrupted (107).

Dividing crypt cells complete a cycle in which different phases can be defined by labeling with tritiated thymidine (108). These phases are the M phase, (mitosis proper), which lasts about 1 hr and is followed by the G₁ phase (postmitotic, presynthetic), the S phase (replication of nuclear DNA), and, finally, the G₂ phase (postsynthetic, premitotic). Phases G₁, S, and G₂, which form the inter-
phase, last from 8 to 23 hr in the rat. The S phase occupies about half of the interphase. These processes are probably slower in humans. However, because measurements of these parameters in humans have been carried out in individuals who are affected by malignant diseases, it is not known whether they truly represent what happens under physiologic conditions. As a consequence, their validity is open to some discussion (6,108).

Renewal of the epithelium may be investigated by other methods. To calculate the mitotic index, the proportion of crypt cells that are unmistakably undergoing mitosis is expressed as percentage of the total cell population of the crypt. Normal values in humans are in the vicinity of 3% (109).

Another way of measuring epithelial cell renewal in animals includes the use of colchicine or other drugs to induce arrest of mitosis in metaphase. Groups of animals given one of these drugs are killed at intervals, and the number of mitoses arrested in metaphase is plotted as a function of time. According to this technique, cell production occurs at a rate of 13.6 to 21.5 cells per crypt and per hour in mice compared to 32.4 to 39.7 cells per crypt and per hour in rats (93).

Besides the use of a pulse of intravenous tritiated thymidine to measure the percentage of labeled cells and determine the length of the phases of the cell cycle, this technique may be used to measure the speed with which cells migrate from the crypt to the villus. The migration rate is the time that it takes the front edge of labeled cells to reach certain predetermined, clearly identifiable landmarks: openings of crypts, junction of lower and middle third of villi, tip, etc.

The measurements obtained by the methods discussed in the preceding paragraphs provide a statistical approach to the normal and abnormal parameters of cell renewal. Based on this information, it is possible to infer how some of the mechanisms that control cell division and epithelial renewal operate in health and in disease. It should be kept in mind that most of this information is lacking for humans and that the data available in experimental animals does not necessarily apply to man, although it may provide a reasonable approximation.

REGULATION OF RENEWAL OF THE ABSORPTIVE EPITHELIUM

Development of the small intestine during embryogenesis is associated with considerable changes in cell renewal and in mucosal morphology. During the early embryonic stages in rats, the surface of the mucosa is flat and it is covered by a pluristratified layer of epithelial cells (110,111). In later stages, the epithelium changes into a single layer of prismatic cells with a complex supranuclear vacuolar and tubular system. At the same time villi become apparent. At birth, the mucosa resembles morphologically that of axenic animals: villi are slender and long, the lamina propria is populated by rather low numbers of mononuclear cells, and the renewal rate in the crypts is rather slow (112).
After birth, factors such as the appearance of the bacterial flora and bile and pancreatic flow accelerate the renewal and migration rates of the epithelium (113–115). Food in the intestinal lumen is another factor that regulates cell renewal after birth (116). The bulk of the food ingested and the discharge of hormones by the endocrine glands and the enteroendocrine cells may be of importance in this phenomenon. Starvation decreases DNA synthesis, cell proliferation, and migration in mice (117). The cell renewal cycle is prolonged in starved rats, and mitotic counts in the crypts of Lieberkühn are decreased (118). Gastrin, discharged by the cells of the enteroendocrine system in response to the presence of food in the intestinal lumen, has a trophic effect on the mucosa and stimulates renewal of the epithelium (119). This effect of gastrin on cell renewal is reversed by secretin. The effect of gastrin and its terminal pentapeptide, pentagastrin, is tissue specific, since it is not observed in the esophagus or the gastric antrum of rats (120,121).

The effect of intraluminal food on mucosal trophism is further supported by the observation that total parenteral nutrition, although it may provide all the nutritional requirements, is associated with decreases in DNA and protein content of the small intestinal mucosa and in its weight, thickness, and disaccharidase activities (122,123). This effect stabilizes to a plateau. This is in contrast to total starvation, in which all of these parameters decrease steadily until the death of the animals (124,125). Other hormones that modulate renewal of the epithelium are thyroxine, growth hormone, and perhaps prolactin (126–128). Thyroxine and growth hormone decrease in infantile marasmic malnutrition, and this may explain, at least in part, the decreased mitotic index observed in these patients (109,129). Corticosteroids decrease epithelial cell renewal in the stomach and the small intestine, and this may be one of the explanations for the increase in the incidence of peptic ulcer in individuals undergoing prolonged treatment with these hormones (130).

Epinephrine, isoproterenol, and α-adrenergic blocking drugs in general inhibit cell renewal, perhaps through the liberation of chalones or similar substances (131). Surgical or pharmacological sympathectomy also decreases cell renewal in the crypt of Lieberkühn. By contrast, α-adrenergic stimulation with norepinephrine or β-adrenergic blockade with drugs such as propranolol accelerates cell renewal (132). This indicates that the catecholamine system intervenes in the regulation of the cell population of the intestinal mucosa. Stimulation of the innervation that supplies jejunal loops also accelerates proliferation of the crypt epithelium (133). The substance(s) responsible for this effect has (have) not been identified.

Resection of segments of the small intestine constitutes a powerful stimulus for crypt cell proliferation. This is associated with the hypertrophy of the mucosa, which, after extensive ablations, allows survival of patients or laboratory animals (134). Changes in cell renewal appear early, after 48 hr, and are associated with changes in DNA content, in the activity of the enzymes that participate in the de novo synthesis of pyrimidine bases, etc. (135,136). As simultaneous increases
in proliferative activity occur in the epithelium of other segments of the digestive tract, an effect of blood-borne or neural factors has been postulated (137).

Food in the lumen of the small intestine also has a clear-cut effect in promoting compensatory hypertrophy following segmental resections (138). This is evident in the ileum of rats with extensive resections of the proximal intestine. A possible explanation for this observation may be that when the upper segments of the gut are resected, increased amounts of food and secretions arrive in the ileum. Under normal conditions the ileum receives less stimuli from food than the jejunum, and its morphological and biochemical parameters have lower values. In consequence, it may appear that the ileum has greater potential for response to increased stimuli. The requirement for the presence of food in the lumen is further supported by observations that total parenteral nutrition after extensive resections in animals is not associated with significant hyperplasia or hypertrophy of the remanent segment (139,140). Some of the "messengers" that induce proliferation of the epithelium are transported in the blood since increases of mitotic activity are observed in parabiotic animals. The fact that the proliferative response is proportional to the length of the segment amputated also lends support to the idea that there is some mechanism of control that is released into the blood and carried to the remaining loops (136-140).

That biliary and pancreatic secretions also participate in the regulation of cell proliferation was shown by Altmann in elegant experiments (114,115) in which the ampulla of Vater was transposed to the ileum. This was associated with an increase in the size of the villi in this segment of the intestine and increases in the weight of its wall, of the scraped mucosa, and of its DNA and protein. This effect is potentiated by resection of the jejunum (141). These changes may respond to the interplay of different factors, including the presence of biliary and pancreatic secretions (as shown by Altmann) and the presence of significant amounts of food in the lumen of a segment of intestine that under normal circumstances has a rather limited absorptive load.

REPAIR OF THE EPITHELIUM UNDER PATHOLOGICAL CONDITIONS

Studies carried out by our group demonstrated years ago that the architecture of the mucosa of the small intestine of infants with severe marasmus was rather normal, although thin (109). At the same time, the mitotic index was significantly decreased. During nutritional rehabilitation, the thickness of the mucosa and the mitotic index in the crypts of Lieberkühn increased. The reason for this decrease in mitotic activity may be explained in part by the decreased stimulation provided by the limited food intake and by the multiple endocrine and neural disturbances demonstrated in this condition. In kwashiorkor, instead, the architecture of the mucosa was distorted, its thickness was preserved, the surface epithelium was damaged, and, surprisingly, the mitotic index was only moderately reduced. Why a global, intense decrease in calorie and protein intake resulting
in marasmus is associated with sparing of mucosal architecture, whereas a more normal calorie intake, as in kwashiorkor, induces important histologic damage remains unanswered. Protein intake does not appear to control mucosal morphology in these cases, whereas calories seem to modulate cell proliferation. In rats fed different amounts of calories and protein, changes of cell renewal have been demonstrated (124).

Vitamin B\(_{12}\) appears to be another factor that modifies crypt cell proliferation. Foroozan and Trier (142) showed that in pernicious anemia there are megalocytic changes in the epithelium of the crypts with decreased mitotic counts. Villi exhibited mild to moderate nonspecific architectural changes, and their epithelium was also megalocytic. The changes in cell renewal improved in about 2 weeks after treatment with parenteral vitamin B\(_{12}\), but it took somewhat longer for the architectural modifications to revert to normality.

Folate-deficient alcoholics develop some histologic changes, which are mostly nonspecific (143). Rats fed alcohol-containing diets exhibit increased numbers of cells and mitotic counts in the crypts of Lieberkühn (144). Pulses of tritiated thymidine disclose increased numbers of labeled cells in the crypts, where the activity of thymidine kinase is also increased. All of this is suggestive of an increased turnover rate of the epithelium. However, although villus size and cell numbers remain unchanged in the ileum, they are decreased in the jejunum. One possible explanation for this may be the stimulation of cell turnover provided in the ileum by the increases in luminal bacterial population that have been demonstrated in alcoholics (145).

Celiac disease is a condition in which severe alterations of epithelial cell renewal are suggested by the visualization of increased numbers of mitotic figures in the crypts of Lieberkühn, including some that appear in rather unusual places, such as close to the opening of the crypts (146). Enzymatic (147) and electron microscopic histochemical (148) characteristics of the epithelial cells of the celiac flat surface suggest that they resemble immature crypt-like cells. The recovery of increased amounts of DNA from washings of the intestinal lumen of celiac patients has been considered suggestive of increased desquamation of the damaged epithelium (149). However, part of this DNA may originate from nonepithelial cells such as the large numbers of lymphocytes present among the absorptive cells of the surface of the mucosa. Studies in samples of cultured mucosa also suggest increased proliferation of the crypts in untreated patients compared to treated individuals (150). However, although organ culture may provide some important clues about the characteristics of the mucosa in celiac disease, cultured biopsies, by being severed from the body, are not subjected to the many controlling mechanisms and stimuli provided by the luminal contents, the mass of the mucosa, and the rest of the organism.

Mitotic counts are also increased in the mucosa of patients with tropical sprue (151). Mathan et al. (152) have shown that in addition to desquamation in the surface, some cells die and are sloughed off from the crypt of Lieberkühn.

Ionizing radiations reduce the mitotic activity in all rapidly dividing cells,
including those of the crypts. The magnitude of this decrease may be about 50% of the controls and occurs within 24 hr (153). The epithelium of the crypts, and later that of the villi, exhibits many cytological and fine structural changes. Villi decrease in height, probably because, as already stated, although desquamation continues at a normal rate, cell replacement is severely curtailed (154). Within 72 hr of irradiation, the mitotic activity in the crypts recovers (155). Replenishment of the rapidly dividing cell population may occur from the stem cells. Cells in the G₁ stage of interphase appear to be most sensitive to radiation damage, whereas those in the S phase are least susceptible. Cells in the G₂ phase show degrees of sensitivity intermediate between those of the abovementioned phases (156).

Methotrexate alters epithelial cell renewal in the small intestine. This is associated with disturbances of enterocyte ultrastructure: the Golgi apparatus and the endoplasmic reticulum are dilated, mitochondria seem to break down into fragments, and huge autophagosomes become a common finding (82). Although some of these changes may be attributed to a direct effect of methotrexate on cell structure, others may result from damage to the microtubules or to disturbances in intracellular protein metabolism in addition to derangements in DNA synthesis. The abnormal aggregation of chromatin in the nucleus of the epithelial cells may be interpreted as morphological evidence of the latter (82).

In most of the diseases mentioned in the preceding paragraphs there is obvious damage to the epithelium of the crypts, the villi, or the surface of the flattened mucosa. It is highly probable that this damage to the epithelium constitutes the *primum movens* of the mucosal lesions. On the other hand, it is important to keep in mind that changes in mucosal architecture to the point at which villi apparently disappear and the surface becomes flat cannot occur without the participation of the lamina propria. Padykula et al. (147) and Reid and Brunser (157) have presented evidence, on the basis of histochemical reactions, that suggests that villi or comparable structures are “incorporated” into the thickness of the flat mucosa of untreated celiac disease. This modification of mucosal architecture would result from an “expansion” of the lamina propria. The lamina propria of the intestine has not received all the attention it is due from histologists and pathologists (158). This may be because of its “unstructured” nature and the fact that it is composed by variable proportions of so many different cell types that quantitation becomes difficult. As a result, the demonstration of changes in different diseases requires sophisticated techniques (159,160). The subepithelial fibroblastic sheath is an exception to the preceding statement and has been carefully studied during intestinal development, in health and in disease (161–164). The reason for this may be its characteristic location, which allows easy identification and exploration.

The evidence discussed indicates that the small intestinal mucosa is able to react and to adapt to stimuli and requirements originated in the body and/or from the intestinal lumen. To answer why, at a given moment, the structure and/or the function of the small intestinal mucosa alters in such a way and in
such a magnitude that it is no longer able to maintain homeostasis will require not only extensive multidisciplinary studies of well-characterized patients but also the development of suitable animal models.

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