Effect of Daily Ethanol Ingestion on Intestinal Permeability to Macromolecules

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The effects of regular ethyl alcohol ingestion on morphological and permeability characteristics of the small intestine were assessed in mature rats using the tracer protein, horseradish peroxidase. Thirty adult rats were divided into two groups and provided a standard commercial diet in pellet form. Each morning, after an overnight fast, every animal in the experimental group was administered by gavage an aliquot of 20% ethanol; animals in the control group were provided aliquots of 20% sucrose in water by the same method. After 4 and 8 weeks on the gavage routine (and 10 days and 4 weeks after gavage cessation), jejunal permeability to horseradish peroxidase was examined in animals from each group. Using a routine ligated-loop procedure and light and electron microscopy, ethanol-exposed rats demonstrated increased intestinal permeability to horseradish peroxidase by 4 weeks; sucrose-exposed animals revealed little alteration in mucosal integrity. It is proposed that regular ingestion of sizable amounts of alcohol alters morphological characteristics of the gut and increase the permeability of the mucosa to "undigested" macromolecules.

The mucosal surface of the small intestine has traditionally been considered impermeable to macromolecules. We now know, however, that the barrier is incomplete and that "leakage" of toxins and antigens through the gut wall occurs to varying degrees in most mammals (1, 2). Of particular significance is the recognized problem of *increased* gut permeability to macromolecules under circumstances of intestinal damage. Such conditions of damage have been demonstrated in animals and in man and include surgical trauma (3), gastrointestinal disease (4–6), and protein–calorie malnutrition (7–9).

In view of the fact that chronic ethanol ingestion has recently been found to damage the lining of the small intestine (10, 11), it seemed plausible that it might also produce an increased permeability of the gut. The purpose of this study, therefore, was to determine morphologically if intestinal permeability to macromolecules is increased by chronic ethanol ingestion. Intestinal loop studies were performed in rats using the electron-opaque tracer protein, horseradish peroxidase.

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MATERIALS AND METHODS

Female Sprague-Dawley rats weighing approximately 100–150 g were obtained from a local distributor (Tyler Laboratories, Bellevue, Washington) and housed individually in an air-conditioned room with lighting regulated to provide equal hours of light and dark. During the first week in our laboratory, the rats were fed a commercial ration (Purina Rat Chow, Ralston Purina Co., St. Louis, Missouri) *ad libitum* and observed for normal weight-gain progress. Those animals whose body weights did not increase at the expected rate during this period were eliminated from the study and the remaining animals were assigned at random (using a table of random numbers) to either the control or the experimental group, such that each group contained 15 rats.

At the beginning of the study, all animals were placed on the commercial diet used during the adjustment period (Purina Rat Chow). The food was made available to the rats between 9:00 AM and 5:30 PM six days per week and

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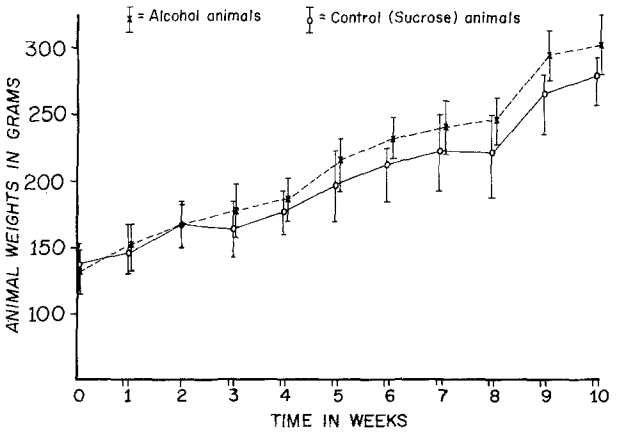


Fig 1. Average weight gains and standard deviations of rats in each group taken at weekly intervals.

all day on the seventh day. Each morning following the overnight fast, each rat was given a 3-cc gavage of either 20% "sugar water" (sucrose dissolved in water) or 20% ethanol; control animals received "sugar water" and experimental animals received ethanol. The dosage of ethanol provided to the experimental rats was sufficient to produce lethargy and disorientation for approximately 1 hr following administration. Body weight and gross appearance were recorded weekly and gross estimates of food intake were maintained.

At 4 and 8 weeks after gavage initiation, five animals from each group were evaluated for small-intestine permeability to horseradish peroxidase. Each rat was anesthetized intraperitoneally with sodium pentobarbital (0.4 mg/g body weight), a laparotomy was performed to expose the small intestine, and thread ligatures approximately 4 cm apart were placed *loosely* around the circumference of the small bowel in the region of the upper jejunum (approximately 3 cm distal to the ligament of Treitz). After irrigation of the lumen of the gut with saline, horseradish peroxidase (Sigma type VI), 5 mg/ml normal saline, was introduced into the ligated segment of jejunum. Each loop preparation was retained *in vivo* for approximately 30 min, after which time it was excised and processed for light and electron microscopy. Adjacent jejunal loops that had been exposed to normal saline only were collected and prepared in a similar manner.

The five animals remaining in each group after 8 weeks of daily gavage were withdrawn from the gavage regimen and allowed to feed normally. After 10 days or 4 weeks of "rehabilitation," intestinal loop procedures were performed again using the same tracer material (horseradish peroxidase) and the same methods employed earlier.

Tissue Processing

Excised jejunal tissue was immersed in a cold solution of 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, diced into 1-mm cubes, and placed in fresh fixative for 1.5 hr at 0° C. The small tissue cubes were rinsed in 0.1 M cacodylate buffer, pH 7.4, for approximately 18 hr and then sectioned into slices 40–75 μm in thickness, using a Smith-Farquhar tissue chopper. Slices were then incubated for 30 min at room temperature in a solution containing 5 mg diaminobenzidine tetrahydrochloride (Sigma Chemical Co.) dissolved in 10 ml Tris-HCl buffer, pH 7.6, after which they were transferred to a fresh batch of the diaminobenzidine solution containing 0.1 ml of 1% H_2O_2 and incubated for an additional 15 min. The slices were then washed three times in 0.1 M cacodylate buffer and postfixed for 1 hr in 1% osmium tetroxide in cacodylate buffer pH 7.4, at 0° C. Tissue slices were then stained with 1.5% uranyl acetate for 1.5 hr, dehydrated in ethanol and propylene oxide, and embedded in Epon 812 (12). Thick and thin sections were cut on a Sorvall MT2 ultramicrotome with glass and diamond knives, respectively. Thin sections were examined in an AEI-801 electron microscope. Procedural controls consisted of segments of jejunum which had received no peroxidase, but were incubated in solutions of diaminobenzidine and H_2O_2 prior to fixation and embedding. At least ten sections were examined from each animal with the observer assessing blindly the amount of tracer within the mucosa, the number of peroxidase-containing pinocytotic vesicles in each absorptive cell, and the incidence of breakdown of junctional complexes as determined by obvious separation of apical intercellular membranes.

RESULTS

Weight Gain

All animals gained weight at a consistent rate during the study period, and no significant difference was observed between the two groups (Figure 1). Rate of weight gain was approximately 13 g/week during the first 8 weeks. During the 2-week period following gavage discontinuation, weight gain rose to almost 25 g/animal/week.

Liver Morphology

Light and electron microscopy of representative liver samples from control and experimental animals revealed a limited number of differences between the two. At the light microscopic level, all liver specimens appeared essentially the same with relatively well-organized lobular structure consisting of normal-appearing hepatic parenchymal cells. At the electron microscopic level, however, several major differences were evident; hepatic cells from control rats demonstrated well-organized and intact rough endoplasmic reticulum (RER) and mitochondria, and the cytoplasm was largely devoid of fat droplets. Parenchymal cells from alcohol-treated animals, on the other hand, consistently contained somewhat fragmented RER and distorted mitochondria and depots of stored fat. The number and size of the fat droplets in hepatic cells from the

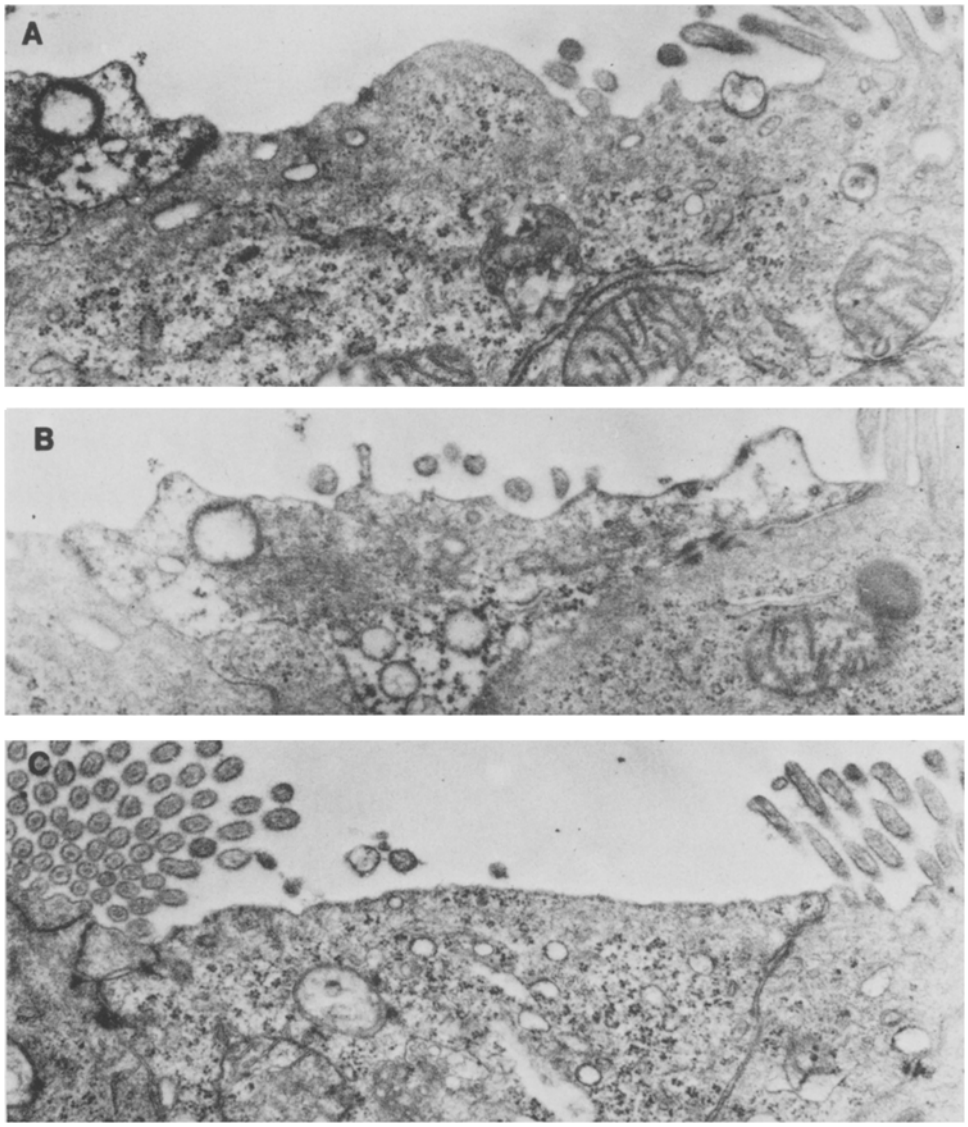


Fig 2. Apical surface of jejunal absorptive cells from experimental animals after 8 weeks of alcohol administration showing distortion or absence of microvilli and degenerative changes in the apical cytoplasm. A (top). Swelling of apical border with loss of microvilli (magnification, 30,000×). B (middle). Swelling and distortion of microvilli (magnification, 30,000×). C (bottom). Absence of microvilli (magnification, 30,000×).

latter group varied from cell to cell and from animal to animal, but in most cells one to three moderately sized lipid droplets were apparent in a standard thin section.

Jejunal Morphology: General

Light microscopic evaluation of jejunal tissue from control and alcohol-exposed animals revealed few distinct differences in morphology at either 4 or 8 weeks after gavage initiation. Intestinal tissue from animals assessed after 10 days or 4 weeks of "rehabilitation" also demonstrated architectural features similar to those of controls. The most obvious and interesting characteristics of the jejunal mucosa from alcohol-exposed rats was the occasional flattening and blunting of intestinal villi. Jejunal tissues from sucrose-exposed animals appeared nor-

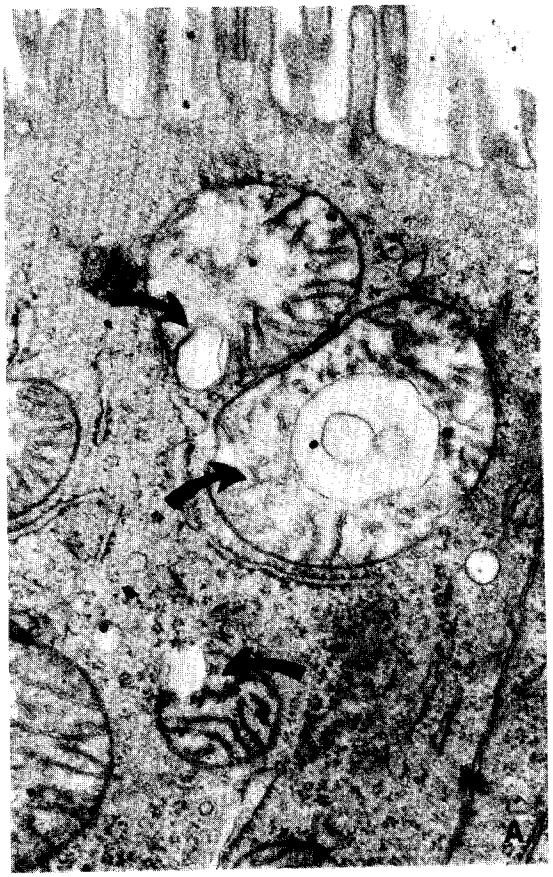


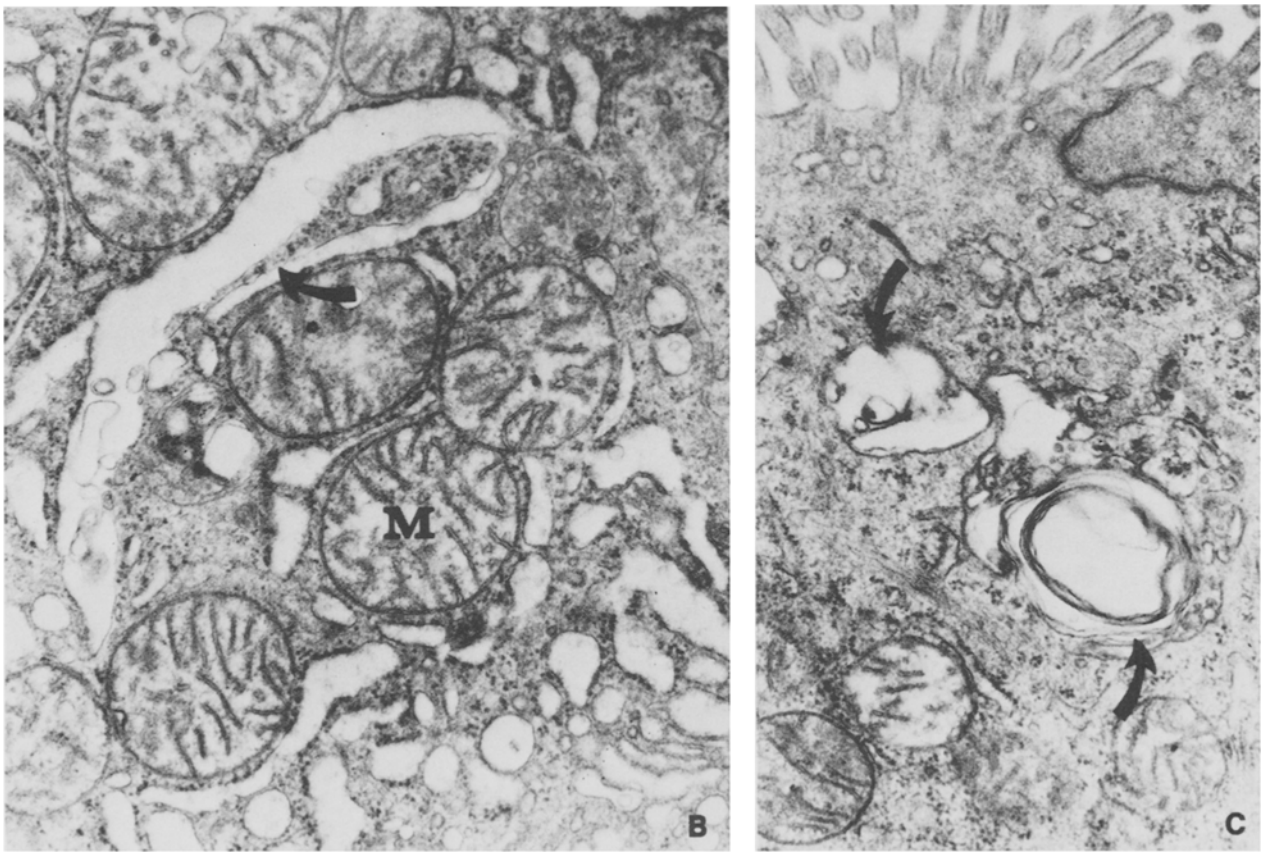
Fig 3. (right and facing page) Supranuclear region of jejunal absorptive cells from experimental rats exposed to alcohol for eight weeks. A (right). Jejunal absorptive cell showing swelling of mitochondria (arrows) (magnification, 30,000×). B (facing page). Jejunal absorptive cell showing swollen portion of rough endoplasmic reticulum; such regions were commonly seen in mucosal cells of rats exposed to alcohol. M = mitochondrion (magnification, 30,000×). C (facing page). Jejunal absorptive cells showing degenerative changes in the apical cytoplasm (arrows) (magnification, 30,000×).

mal in all respects with typical villi appearing long, well-formed, and only occasionally "swollen" at the tips.

Comparison of control and alcohol-exposed jejunal mucosae by electron microscopy revealed significant differences at both 4 and 8 weeks in the alcohol-treated animals. Nearly half of the jejunal absorptive cells from the distal two thirds of each villus in each specimen displayed fusion, distortion, and shortening of microvilli (Figure 2), swelling and deterioration of mitochondria (Figure 3A), and dilation and occasional fragmentation of rough endoplasmic reticulum and Golgi cisternae (Figure 3B). In addition, some cells contained areas of cytoplasmic degradation, especially those located along the upper third of each villus (Figure 3C). Of greatest interest and possibly most significance was the consistent finding of disintegration of apical junctional complexes between adjacent epithelial cells (Figure 4); approximately eight such disrupted complexes were observed to the distal two thirds of each villus. After 10 days and 4 weeks of rehabilitation, mucosal tissue of alcohol-treated animals appeared similar to controls.

Jejunal Morphology: Peroxidase Permeability

Comparison of peroxidase-exposed jejunal mucosa from control and alcohol-treated animals revealed obvious differences in degree of mucosal permeability by light microscopic examination (Figures 5A, B). In both cases, the luminal surface of the distal two thirds of the villi demonstrated adsorption of enzyme reaction product; additionally, however, in alcohol-treated animals, substantially larger amounts of tracer protein appeared to penetrate surface epithelial cells of the villus. In some specimens from alcohol-exposed rats, intercellular spaces of the epithelium as well as the lamina propria appeared loaded with reaction product. In all control samples, horseradish peroxidase penetration was minimal, with only occasional evidence



of tracer material between cells at the tips of the villi.

Electron microscopic examination of tissue from control and experimental animals confirmed permeability findings recorded with light microscopy. In both control and experimental animals, reaction product was observed on the surface of the microvilli and occasionally within pinocytotic vesicles in the apical cytoplasm (Figure 6A). Efforts to quantitate the number of peroxidase-containing pinocytotic vesicles per cell in the upper halves of representative villi revealed fewer such vesicles per cell in the epithelium of representative alcohol-treated animals as compared with controls (0.43 ± 0.37 SD vs 0.98 ± 0.47, $P \leq 0.05$). The amount of tracer protein within the intercellular spaces of the epithelium and lamina propria was substantially greater in the alcohol-treated animals than in the controls (Figure 6B); no evidence of exocytosis at the lateral cell membranes was apparent. In addition, direct penetration of peroxidase through the apical junctional complex region was observed frequently (3.2 times more often than through the villous surface) in the mucosal surface of rats provided daily alcohol (Figure 7A, B); such an event was not observed in control tissue.

The morphological characteristics here described for alcohol-exposed rats were apparent in both the 4-week and 8-week treatment groups. The rats for 8 weeks with alcohol and then "rehabilitated" for 10 days to 4 weeks appeared similar in all respects to the control animals.

DISCUSSION

It has long been known that digestion and absorption are compromised by acute ethanol consumption. This functional impairment is accompanied by structural alterations in the cells of the small intestine as well as those of the liver (13), pancreas (14), and stomach (15, 16). Findings in the present study confirm earlier work by Rubin et

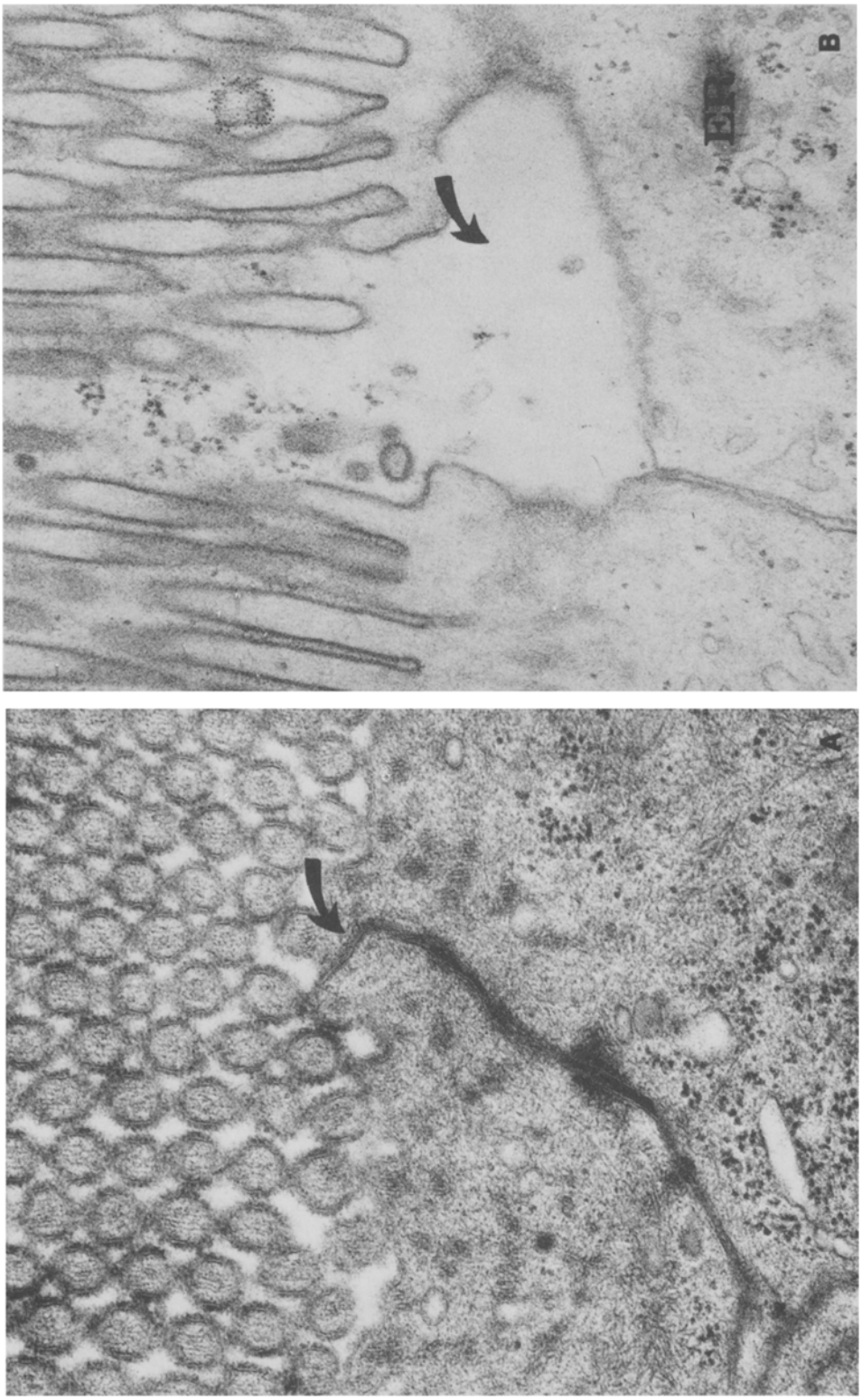


Fig 4. Jejunal mucosa of control and 4-week alcohol-treated rats showing region of the junctional complex. A (left). Control rat showing integrity of junctional complex with adherence of apical tight junction (arrow) (magnification, 40,000×). B (right). Alcohol-treated rat showing separation of intercellular junction at the apex (arrow) (magnification, 35,000×).

al (11) in that electron microscopic assessment of jejunal absorptive cells from ethanol-exposed rats demonstrated degenerative changes in microvilli, mitochondria, and rough endoplasmic reticulum. Abnormalities reported in both studies include mitochondrial distortion, dilation of rough endoplasmic reticulum, and focal accumulation of cytoplasmic "debris." These alterations in fine structure are similar to those produced by ethanol in the livers of rat and man (13).

While loss of gut integrity during chronic ethanol ingestion may significantly reduce the nutritional status of the host by promoting malabsorption, another serious consequence may also develop from intestinal damage. This consequence relates to the possibility that a damaged gut may lose its ability to restrict migration of foreign proteins or other macromolecules into the body. Under normal circumstances, only limited quantities of "undigested" macromolecules are absorbed in the small intestine (17–19); this limited uptake is a desirable condition since continual migration of foreign proteins into an immunologically competent host might promote constant synthesis of antibodies and accumulation of antigen–antibody complexes in body tissues.

Intestinal permeability to one macromolecule (horseradish peroxidase) was assessed in this study in control and alcohol-gavaged rats. Both light and electron microscopic findings clearly revealed that mucosal uptake of horseradish peroxidase was significantly increased in alcohol-treated animals. The observed quantity of tracer material in intercellular spaces of the epithelium and lamina propria was obviously greater in the experimental group. At the same time, it was interesting to note that the degree of pinocytotic activity in the absorptive cells of the alcohol-treated rats was clearly reduced in comparison with controls. Since evidence for apical junctional complex degeneration was also apparent simultaneously in tracer-exposed jejunal preparations from these rats, the possibility exists that direct migration of intact protein between cells actually occurred. Morphological evidence supportive of this concept is provided in this report and summarized in Figure 8. The relative significance of this phenomenon in accounting for mucosal uptake of horseradish peroxidase is a question which cannot be answered with the data in hand.

We propose, as a result of the present work and that of others, that integrity of the mucosal lining of the small bowel is compromised by chronic, intermittent exposure to ethyl alcohol. As a result of this

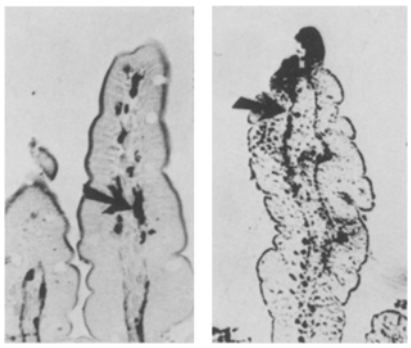


Fig 5. Light micrographs of typical villi from the jejunal mucosa of control and experimental rats. The villi have been exposed to horseradish peroxidase in a gut loop; the tissue has been processed histochemically to show the presence of the black reaction product. The sections are unstained. A (left). Typical villus from control rat showing characteristic coating by peroxidase reaction product but minimal penetration through the surface epithelial cells. Arrow: blood cell in lamina propria with normal dense reactivity due to content of endogenous enzyme (magnification, 300×). B (right). Representative villus from an experimental rat after eight weeks of exposure to alcohol. Note adherence of dense tracer material to villous surface and the significant degree of its penetration of the villous surface cells into the underlying tissue (arrow) (magnification, 300×).

structural alteration in the absorptive surface, digestive and absorptive functions in this region may be impaired with the eventual development of malabsorption and malnutrition. Additionally, however, because of the damage created in the mucosal lining, permeability of the lining to "foreign macromolecules" is also increased with the potential consequence of exposing the internal milieu of the animal to undesirable antigens, toxins, or pathogenic organisms.

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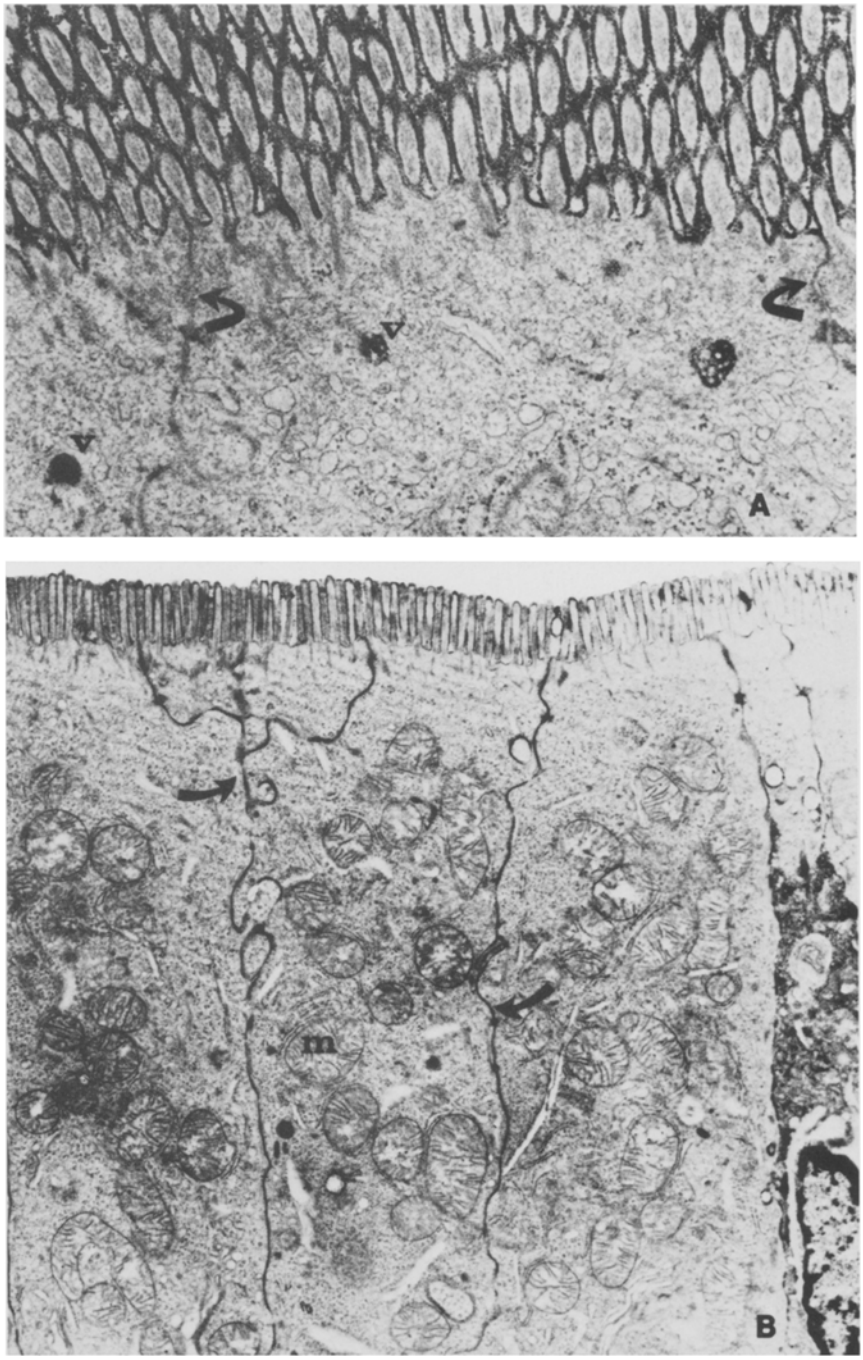

Fig 6. Jejunal absorptive cells from control and experimental animals. A (top). Apical portion of jejunal absorptive cell from control rat showing adherence of tracer material to the microvilli and its presence in several vesicles (v) within the cytoplasm. Note intact nature of the junctional complexes between adjacent cells (arrows) (magnification, 30,000×). B (bottom). Jejunal absorptive cells from experimental rat after 8 weeks of exposure to alcohol. Note intercellular spaces filled with tracer material (arrows) and relative absence of pinocytotic activity; M = mitochondrion (magnification, 12,000×).

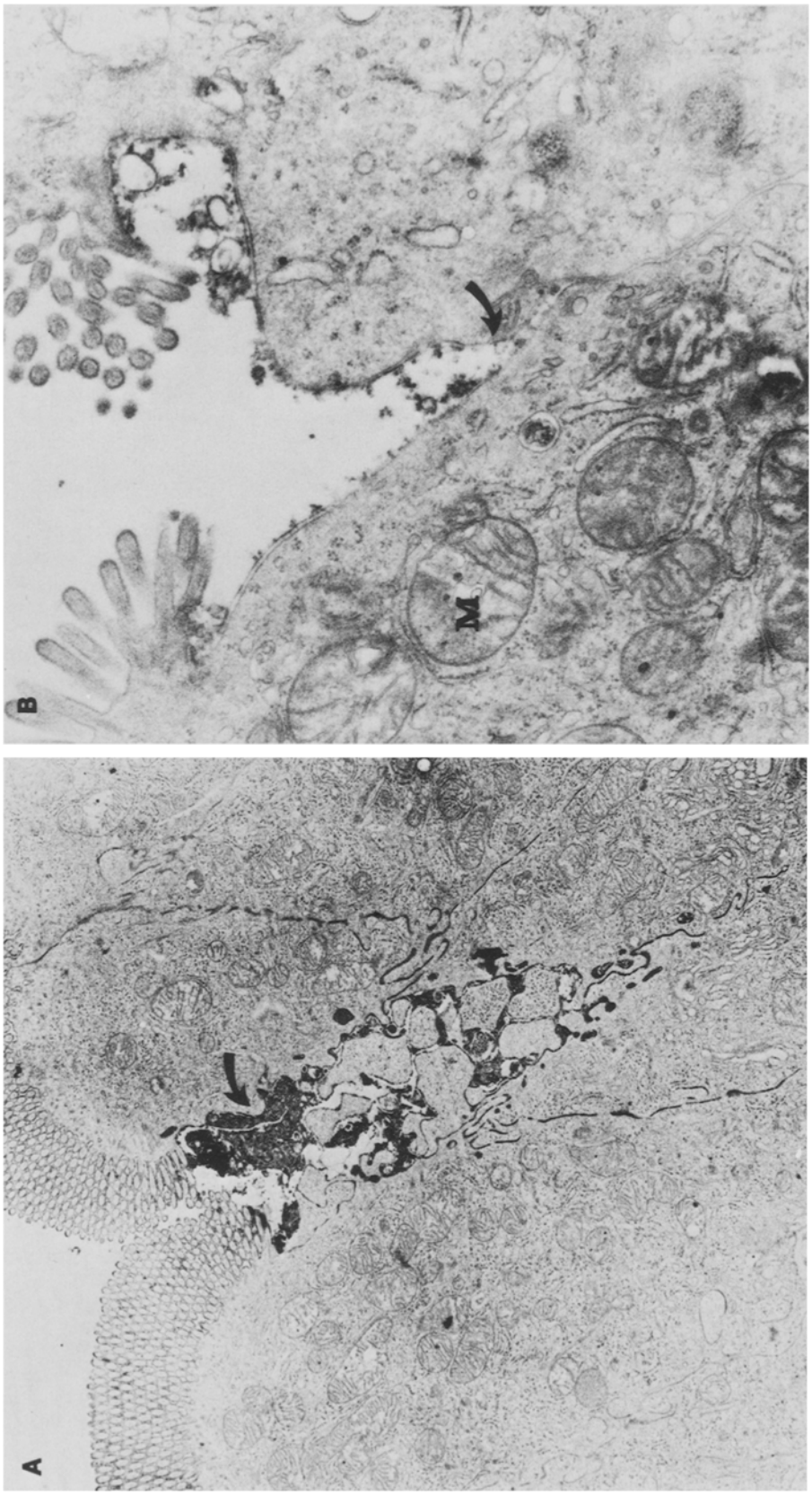

Fig 7. Jejunal mucosal of experimental animals exposed to alcohol for 8 weeks. A (left). Note adherence of tracer material to surfaces of microvilli and its apparent migration into a "defect" in the mucosal lining (arrow) (magnification, 12,000×). B (right). High-magnification view of jejunal mucosal cells showing apparent migration of tracer material into a broken junctional complex (arrow); M = mitochondrion (magnification, 35,000×).

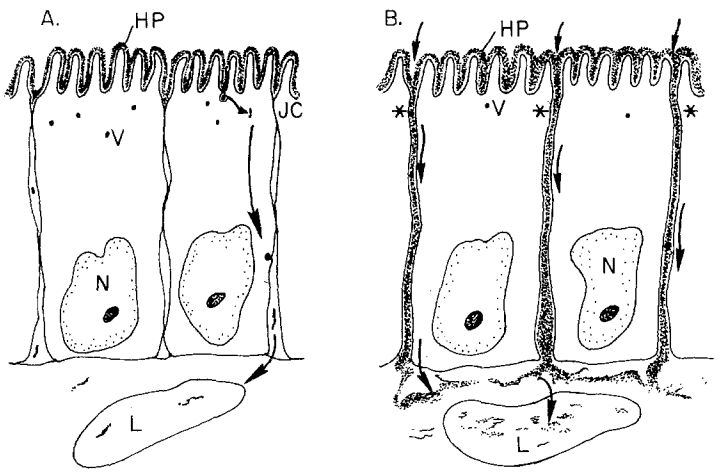

Fig 8. Diagrammatic representation of the proposed fate of horseradish peroxidase in the proximal jejunum of control and alcohol-exposed rats. HP, horseradish peroxidase; JC, junctional complex; v, peroxidase-filled pinocytotic vesicle; N, nucleus; L, lacteal; *, broken junctional complex. A. Jejunal mucosa from control rats showing adherence of peroxidase to microvillous border, its presence in apical pinocytotic vesicles, its occasional movement into intercellular spaces via exocytosis, and its eventual presence in very limited amounts in the lamina propria. B. Jejunal mucosa from alcohol-exposed rats showing adherence of tracer material to the microvillous border, its occasional presence in pinocytotic vesicles in the apical cytoplasm, and its proposed movement directly into intercellular spaces of the epithelium via broken or poorly functional junctional complexes.

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