



8-1989

Cessation of Chronic Ethanol Intake Reverses Ultrastructural Alterations in Ileal M Cells

Robert Rosback Eversole

Follow this and additional works at: https://scholarworks.wmich.edu/masters_theses



Part of the Medical Pathology Commons

Recommended Citation

Eversole, Robert Rosback, "Cessation of Chronic Ethanol Intake Reverses Ultrastructural Alterations in Ileal M Cells" (1989). *Master's Theses*. 1102.

https://scholarworks.wmich.edu/masters_theses/1102

This Masters Thesis-Open Access is brought to you for free and open access by the Graduate College at ScholarWorks at WMU. It has been accepted for inclusion in Master's Theses by an authorized administrator of ScholarWorks at WMU. For more information, please contact wmu-scholarworks@wmich.edu.



CESSATION OF CHRONIC ETHANOL INTAKE REVERSES
ULTRASTRUCTURAL ALTERATIONS IN
ILEAL M CELLS

by

Robert Rosback Eversole

A Thesis
Submitted to the
Faculty of The Graduate College
in partial fulfillment of the
requirements for the
Degree of Master of Science
Department of Biomedical Sciences

Western Michigan University
Kalamazoo, Michigan
August 1989

INFORMATION TO USERS

The most advanced technology has been used to photograph and reproduce this manuscript from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book. These are also available as one exposure on a standard 35mm slide or as a 17" x 23" black and white photographic print for an additional charge.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.



University Microfilms International
A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
313/761-4700 800/521-0600

Order Number 1337011

**Cessation of chronic ethanol intake reverses ultrastructural
alterations in ileal M cells**

Eversole, Robert Rosback, M.S.

Western Michigan University, 1989

U·M·I
300 N. Zeeb Rd.
Ann Arbor, MI 48106

TABLE OF CONTENTS

LIST OF FIGURES	ii
INTRODUCTION	1
MATERIALS AND METHODS	3
Experimental Design	3
Animals and Diet	3
Ethanol Assay	4
Sample Collection and Preparation	4
Section Preparation and Electron Microscopy	5
RESULTS	8
Light Microscopy	8
Electron Microscopy	8
Ethanol Assay	14
DISCUSSION	17
BIBLIOGRAPHY	22

LIST OF FIGURES

1. Example of Section Through the Follicle Dome of the Ileal Peyer's Patch	7
2. M Cells Showing Mitochondrial Profiles in Control and Ethanol-Treated Animals	9
3. Ileal M Cells Demonstrating Alterations in Endoplasmic Reticulum	11
4. Ethanol-Treated Epithelium of the Ileal Peyer's Patch With Vacuolization	12
5. Lateral Plasma Membrane Separation in Ethanol-Treated M Cells	13
6. Recovery-Group M Cell 15 Days After Cessation of Ethanol	15
7. Ethanol Determinations of Serum and Ileal Chyme Samples	16
8. Oxidation of Ethanol in the Hepatocyte	18

INTRODUCTION

The relationship between alcohol abuse and abnormalities of the human immune system has been acknowledged for over 30 years. Increased titers of circulating antibodies is one of the conditions reported. Ethanol-induced changes in the overlying epithelium of mucosa-associated lymphoid follicles have been implicated as a mechanism for elevation of serum antibody titers (Hall, 1985). The increase in serum levels of polyclonal subclasses of IgA, IgG, and IgM is seen in chronic alcoholics where any stage of liver disease is prevalent. In cases where the disease has progressed to cirrhosis, a respective ten-fold and four-fold increase of polymeric and monomeric IgA has been reported (Iturriaga, Pereda, Esterez, & Ugarte, 1977).

Since decreased catabolism of these immunoglobulins was ruled out by Havens, Dickensheets, Bierly, and Eberhard, (1954); a two part theory of increased synthesis, due to excessive antigenic stimulation, was proposed by Hall, (1985, p. 72). One aspect of the theory involves impaired sequestration of antigens by the liver. This may be attributed to reduced Kupffer cell phagocytosis, direct portacaval shunting under portal hypertension, or liver cell necrosis. The other aspect is increased absorption of

macromolecules and microorganisms. The altered absorption being attributed to ethanol-induced changes in permeability of the gastro-intestinal mucosa. In either case, Bjorneboe (1971) has shown that these elevated titers are due, in part, to dietary and enteric bacterial proteins.

The points of entry for many gastro-intestinal antigens are the Membranous (M) cells of the intestinal epithelium. M cells are specialized for the rapid transport of luminal macromolecules and microorganisms to the underlying lymphoid follicle (Owen, 1977). This transport process may be a necessary step in the development of a specific immune response and subsequent antibody production (Sneller & Strober, 1986). We conducted this study as an initial investigation into the relationship between chronic ethanol consumption and modification of M cell function expressed as ultrastructural changes.

MATERIALS AND METHODS

Experimental Design

Six control animals were maintained, ad libitum, on liquid diet alone. Fourteen experimental animals were maintained, ad libitum, on liquid diet plus ethanol. On day 45, three controls and seven experimental animals were terminated by decapitation and tissue samples collected. Ileal Peyer's patches were fixed for transmission electron microscopy (TEM) to evaluate ultrastructural aspects of M cells from both groups. Liver samples were prepared for light microscopy of toluidine blue stained sections. Serum and ileal chyme were collected and assayed for ethanol content. The seven remaining experimental animals were taken off of ethanol on day 45 and maintained on the liquid diet alone. These recovery animals, along with the three remaining controls, were sacrificed on day 60. Identical procedures for sample collection were followed.

Animals and Diet

Male Sprague-Dawley rats from the animal breeding facilities at The Upjohn Company, Kalamazoo, MI 49001, were obtained when approximately 200 grams. The experimental animals were maintained on a nutritionally adequate liquid diet in which ethanol comprised 30% of the total caloric

content. The nutritive base of the diet was the Carnation product "Slender" (Carnation Company, Los Angeles, CA 90036). This diet provided similar nutrient composition as described by DeCarli & Lieber (1967). The ethyl alcohol was 200 proof, punctilious, and obtained from USI Chemicals, Tuscola, Ill 61953.

Ethanol Assay

The ethanol levels of the serum and chyme samples were determined using Alcohol Procedure No. 332-UV from Sigma Diagnostics, St. Louis, MO 63178. This procedure utilizes alcohol dehydrogenase (ADH) to catalyze the conversion of ethanol to acetaldehyde. The reduction of nicotinamide adenine dinucleotide (NAD) is coupled to the oxidation of the ethanol. The increase in absorbance at 340nm when NAD is converted to NADH provides an accurate measure of the ethanol present. The spectrophotometry was carried out on a Gilford "Response" system (The Gilford Company, Oberlin, OH 44074).

Sample Collection and Preparation

Whole blood was collected in plastic centrifugation tubes. Serum was obtained by centrifugation immediately following coagulation. A 20cm segment of ileum was removed within 60 seconds of decapitation and the luminal contents collected. The chyme was centrifuged and the liquid

portion of the sample retained for ethanol determination. The ileal segment was placed in Hank's balanced salt solution (HBSS) for dissection (Gibco Laboratories, Chagrin Falls, OH 44022). The Peyer's patches were excised and washed thoroughly in HBSS (pH 6.0) to remove the glycocalyx on the villar surface. Individual Peyer's patches were sliced into 0.5mm X 3.0mm ribbons for later orientation during embedding. Liver samples were cut into 0.5mm cubes. Primary fixation was performed with 3% glutaraldehyde in HBSS at 4°C for 2 hours. Secondary fixation in 1% osmium tetroxide was followed by ethanol dehydration and BEEM capsule embedment in Polybed 812 (Polysciences, Inc., Warrington, PA 18976).

Section Preparation and Electron Microscopy

After polymerization, the sample blocks were trimmed and 1 micron sections cut, stained with toluidine blue, and examined with the light microscope. Special consideration was given to obtain proper tissue orientation. The dome epithelium of the Peyer's patches was aligned so that sections perpendicular to the ileal lumen were obtained (see Figure 1). Positional information on M cells was noted and only cells located in the first 60% of the epithelium arising out of the crypts were selected. The blocks were retrimmed and ultra-thin sections prepared on a diamond knife. The sections were collected on 200 mesh,

3.05mm, copper grids. Methanolic uranyl acetate and Reynold's lead citrate (Dawes, 1988) were used to stain the ultra-thin sections prior to TEM. Electron microscopy was carried out on a Siemens 101 transmission electron microscope at 80 KV.



Figure 1. Example of section through the follicle dome of the ileal Peyer's patch of a control rat. M cells are indicated (see inset) with arrows. L, lumen; C, intestinal crypt; LF, lymphoid follicle. Bars: 10 micron; (inset) 1 micron.

RESULTS

Light Microscopy

Examination of 1 micron sections of experimental liver samples showed characteristic ethanol-induced pathology described by Lieber and DeCarli (1976). The production of numerous cytoplasmic lipid droplets was noted in all ethanol-treated livers.

Electron Microscopy

The follicle-associated M cells of the ethanol-treated animals had distinct ultrastructural differences from the controls. These included mitochondrial changes and dilation or vesiculation of various intra- and inter-cellular membrane components of the cells.

Changes in mitochondrial appearance were present in virtually every experimental M cell observed. The changes encompassed a range of degenerative profiles (see Figure 2). Most mitochondria showed variable degrees of swelling. This was accompanied by rarefaction of the matrix. This loss of matrix density appears in sharp contrast to the normally dense staining matrix seen in controls. The mitochondria that reached a ballooned state showed diminished cristae organization and areas of complete matrix clearing.

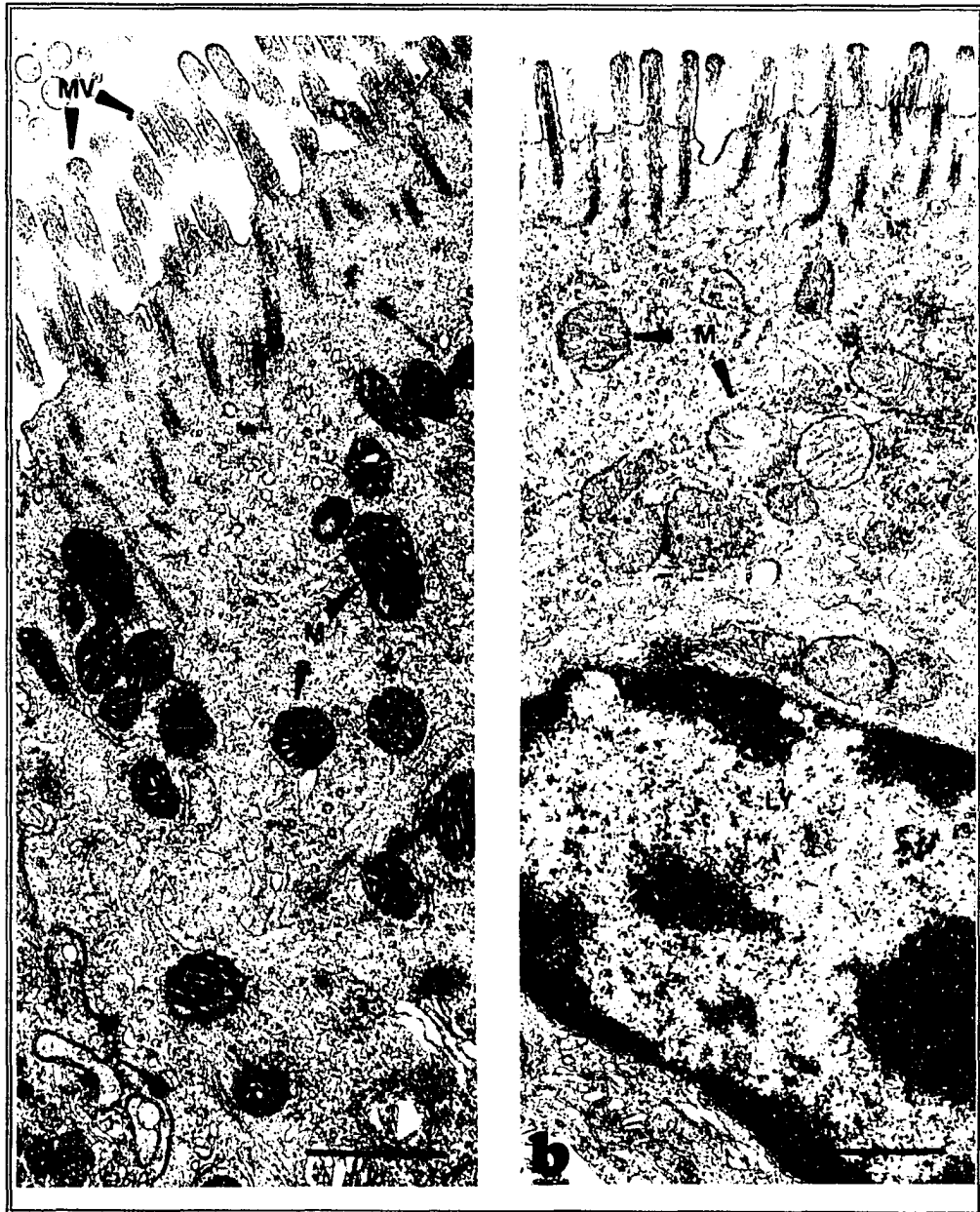


Figure 2. Ileal M cells showing mitochondrial profiles in control (a) and ethanol-treated (b). M, mitochondria; MV, microvilli; LY, lymphocyte. Bars: (a,b) 1 micron.

Most of the experimental M cells displayed abnormal endoplasmic reticulum (ER). Dilation of both rough and smooth ER was observed; however, dilation and vesiculation of the smooth type was predominant (see Figure 3). These changes were not as common in M cells as they were in absorptive cells of the epithelium. The absorptive cells showed much more hypertrophy of the smooth endoplasmic reticulum.

Numerous, large, cytoplasmic vacuoles were present in many of the ethanol-treated M cells. These were bounded by a single membrane and were either filled with sparse granular material or clear in appearance (see Figure 4). A well developed Golgi of similar appearance was noted in both control and experimental M cells. The abnormal vacuolization was usually associated with the lateral plasma membrane but was also found throughout the cytoplasm.

Intercellular junctions between ethanol-treated M cells and adjacent absorptive cells were different than in controls (see Figure 5). The apical region of the epithelial sheet shows intact zonula adherens but the plasma membranes located between the macula adherens or desmosomes are distinctly separated in the ethanol group. This region of adherens-type junctions serves an adhesion function (Drenckhahn & Franz, 1986). Extensive intercellular separation was not observed in any of the

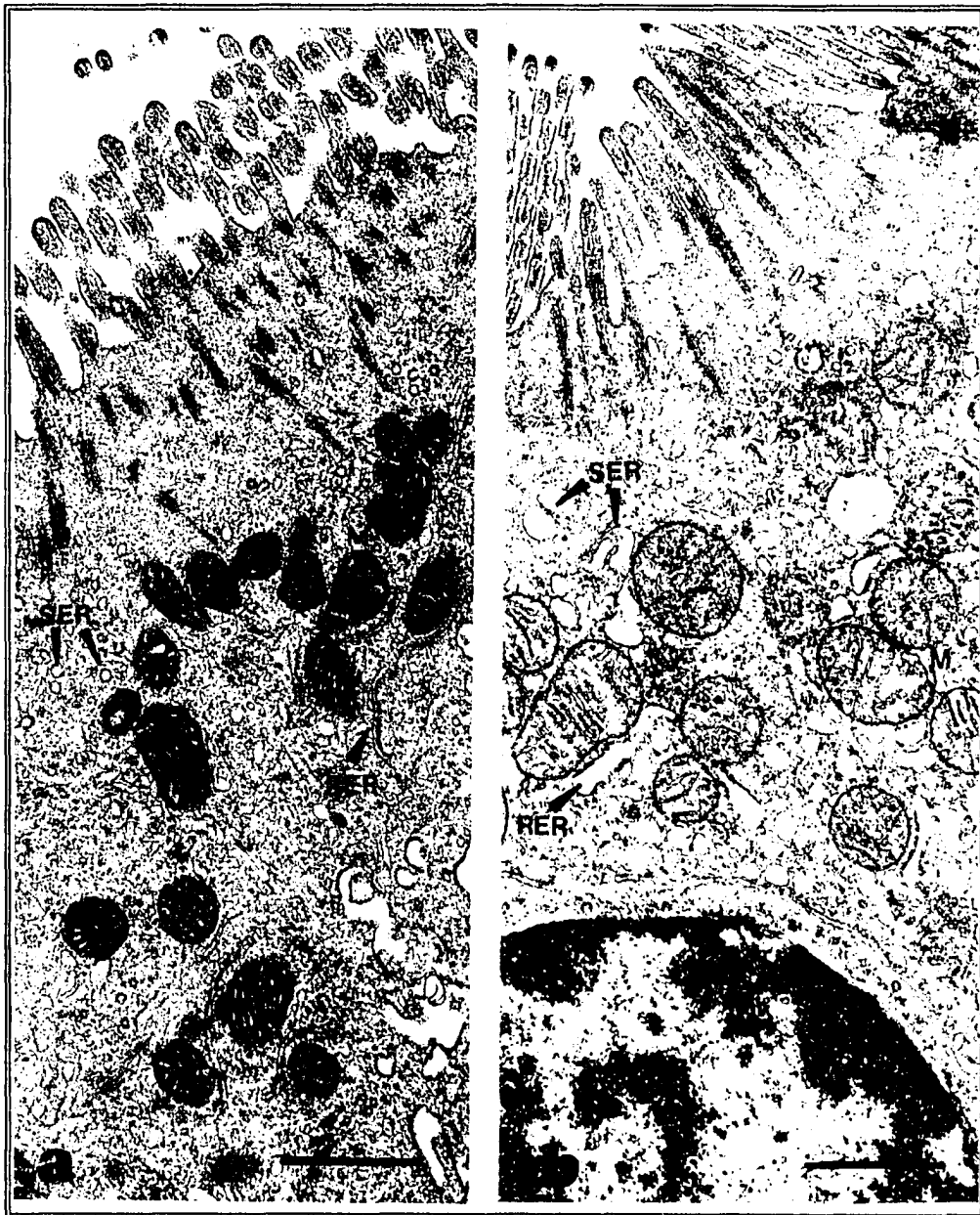


Figure 3. Ileal M cells demonstrating alterations in the endoplasmic reticulum between control (a) and ethanol-treated (b) animals. Note also the difference in mitochondrial appearance. SER, smooth endoplasmic reticulum; RER, rough endoplasmic reticulum; M, mitochondria. Bars: (a,b) 1 micron.



Figure 4. Ethanol-treated dome epithelium of the ileal Peyer's patch. Large vacuoles are present in the M cells' (arrows) cytoplasm. The vacuoles are membrane bound (see inset). V, vacuoles; LY, lymphocyte; BM, basement membrane. Bars: 1 micron; (inset) 1 micron.

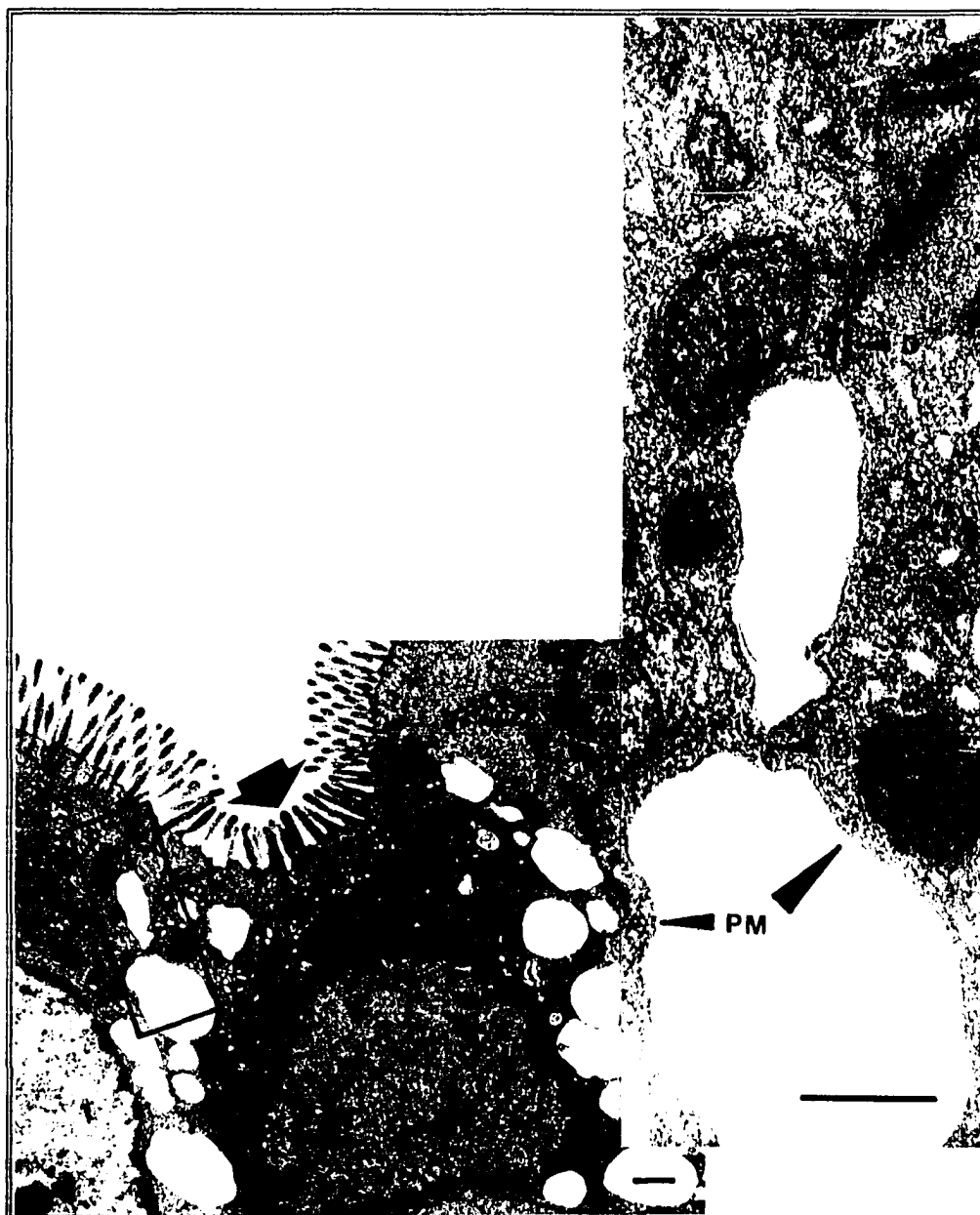


Figure 5. The lateral plasma membranes of this ethanol-treated M cell (arrow) show distinct separation from the adjacent absorptive cells. The membranes are completely separated between adherens junctions or desmosomes (see inset). PM, plasma membrane; D, desmosome. Bars: 1 micron; (inset) 0.5 micron.

controls. These intercellular vesicles were usually clear but sometimes contained granular or membrane-like components.

Ultrastructural analysis of the recovery group revealed no significant differences from controls (see Figure 6). Some irregular clear spaces were seen in the mitochondria of the recovery M cells but these spaces also appeared in controls. This was reported as a possible artifact in a similar study where glutaraldehyde was also the primary fixative (Rubin et al., 1972).

Ethanol Assay

Figure 7 gives the results of the ethanol determinations for serum and chyme samples. This data shows very similar ethanol levels for both types of samples in the experimental group. Only one animal showed chyme ethanol to exceed serum ethanol. It is of interest, that while the rat designated E8 showed little serum ethanol, the ultrastructural alterations were present in this animal. Observations during the dosing of these animals revealed cycles of fasting that may explain this discrepancy.



Figure 6. Ileal M cell (arrow) from the experimental group 15 days after the cessation of ethanol feeding. These recovery-group cells appeared like controls taken on days 45 and 60. N, nucleus; LY, lymphocyte; D, desmosome. Bar: 1 micron.

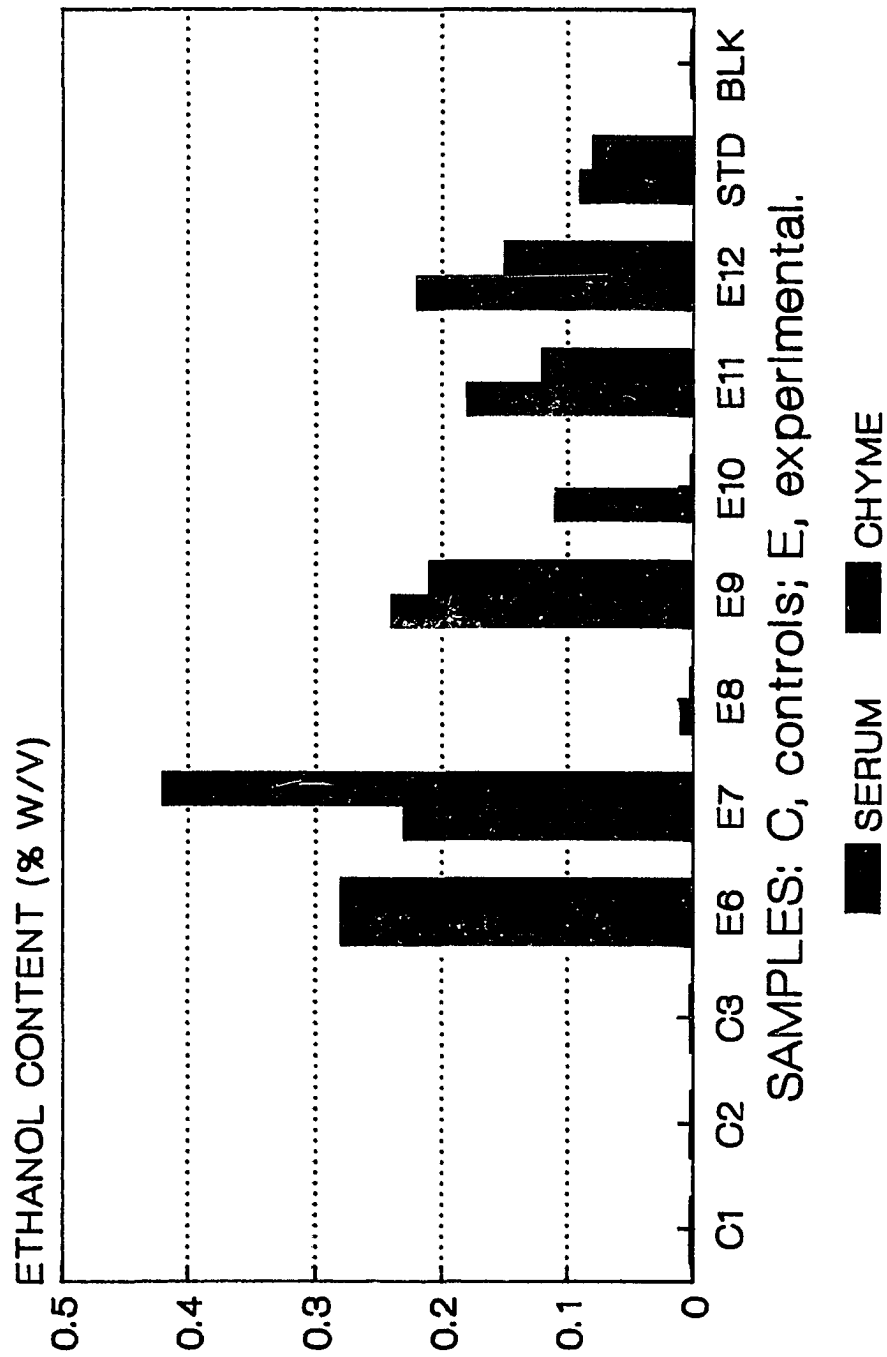


Figure 7. Ethanol Determinations.

DISCUSSION

Our findings on ethanol-induced changes in M cell ultrastructure, parallels similar research on many other cell types. The results of the ethanol assay indicates a systemic mode of action as opposed to contact toxicity. The lipid solubility and small size of the ethanol molecule allows it access to virtually every membrane component of the body (Hunt, 1985). The hepatocyte has been the object of intense study in regard to alterations in structure and function attributable to systemic ethanol. Rubin, et al.,(1972) state that, "The ultrastructural changes in epithelial cells of the intestine produced by chronic ethanol ingestion in this study bear a striking resemblance to those seen in the liver" (p. 807). This suggests similar pathogenic biochemical mechanisms operate to produce the pathogenesis among various cell types. Lieber (1988) proposed a complex mechanism for the oxidation of ethanol in the hepatocyte (see Figure 8). It seems likely that ileal M cells would respond as these cell populations in response to continued ethanol exposure.

The primary pathway for cellular ethanol oxidation involves ADH and NAD to produce acetaldehyde and NADH. These products may be involved in the mitochondrial changes observed in M cells. Chronic exposure to ethanol leads to

high systemic levels of acetaldehyde and increased cytosolic NADH. These excess reducing equivalents can not be totally compensated by the metabolic reactions that control cytosolic NADH. Consequently, the increased ratio of NADH/NAD affects metabolic pathways of the mitochondria. These include impaired oxidation of fatty acids and acetaldehyde, a reduction in cytochrome oxidase activity and oxidative phosphorylation. The excess NADH is not totally culpable. Exogenous acetaldehyde has been shown to mimic the alteration in oxidative phosphorylation produced by chronic ethanol exposure (Arai, Leo, Nakano, Gordon, and Lieber, 1984). Acetaldehyde has also been shown to depress mitochondrial glutathione content by binding with its cysteine moiety. The resulting reduced scavenging of toxic free radicals may well contribute to the structural damage observed in mitochondria (cited in Lieber, 1988).

Evidence of a microsomal ethanol-oxidizing system has been reported by Lieber & DeCarli (1968). An enzyme of the cytochrome P-450 system was shown to be induced by chronic ethanol exposure. Furthermore, acetaldehyde with its propensity for binding to sulfhydryl groups, forms a stable adduct with the enzyme (cited in Lieber, 1988). This may serve to enhance the induction and also help explain the dilation seen in the smooth endoplasmic reticulum.

The origin of the cytoplasmic vacuoles and the disruption of the lateral membrane junctions require

further investigation. The possibility of altered ion transport and osmotically retained water seems likely. A direct effect on membrane permeability may result from changes in lipid content due to chronic ethanol exposure. This result, while seen in some cells, is not universal (Hunt, 1985). Alternatively, the intercellular dilations may occur because of interrupted formation of the adherens-type junctions between desmosomes. These adherens-type junctions are linked to the actin filament system of the cell. Removal of extracellular calcium ions or blockage of actin polymerization will prevent junction formation (Volberg, Geiger, Kartenbeck, & Franke, 1986). Lieber (1988) proposes a similar theory of acetaldehyde blocked polymerization of tubulin, resulting in impaired protein secretion. Thus, the junctional alterations may precede, and contribute, to improper ion channeling and vacuole formation.

The pattern of renewal for follicular epithelium overlying the domes of ileal Peyer's patches clearly indicates a zone of cellular sloughing at the dome apex. M cells, being a rapidly renewing cell population, arise from the intestinal crypts and migrate to the dome apex in 3 days (Bye, Allen, & Trier, 1984). Considering the rapid turnover of M cells it is not suprising that the recovery group showed no ultrastructural alterations. Rubin et al., (1972) reported mitochondrial abnormalities in some

intestinal cell populations 23 days after cessation of ethanol. Although this is not consistent with our findings for M cells, it may apply to more stable populations like Paneth cells (cited in Bye et al., 1984).

The ultrastructural changes reported here lead us to the question of impaired antigen uptake of M cells exposed to ethanol. While no direct conclusions can be made at this stage; we do have evidence of distinct changes. Consideration must be given to the role other cells play in mucosa associated immunity. The M cell-associated lymphocytes and the cells of the underlying follicle cannot be exempt from scrutiny. Adjacent absorptive cells are certainly part of the interrupted cellular junctions with M cells. Indeed the structural integrity between these cells could play an important role in the development and maintenance of function of the M cell. The production of an energy deficit via the interruption of oxidative phosphorylation has merit as a causal factor in M cell pathology. How these various factors intertwine to produce and affect M cell function is the question. Further research on the M cell and ethanol-induced pathophysiology will provide some answers.

BIBLIOGRAPHY

- Arai, M., Leo, M., Nakano, M., Gordon, E., & Lieber, C. (1984). Biochemical and morphological alterations of baboon hepatic mitochondria after chronic ethanol consumption. Hepatology, 4, 165-174.
- Barona, E., Pirola, M., & Lieber, C. (1974). Small intestinal damage and changes in cell population produced by ethanol ingestion in the rat. Gastroenterology, 66, 226-234.
- Bjornboe, M., Prytz, H., & Orskov, F. (1972). Antibodies to intestinal microbes in serum of patients with cirrhosis of the liver. Lancet, i, 58-60.
- Bye, W., Allen, C., & Trier, J. (1984). Structure, distribution, and origin of M cells in Peyer's patches of mouse ileum. Gastroenterology, 86, 789-801.
- Dawes, C. (1988). Introduction to biological electron microscopy: theory and techniques. Burlington, VT: Ladd Research Industries.
- DeCarli, L., & Lieber, C. (1967). Fatty liver in the rat after prolonged intake of ethanol with a nutritionally adequate new liquid diet. Journal of Nutrition, 91, 331-336.
- Drenckhahn, D., & Franz, H. (1986). Identification of actin-, α -actinin-, and vinculin-containing plaques at the lateral membrane of epithelial cells. Journal of Cell Biology, 102, 1843-1852.
- Hall, P. (1985). Alcoholic liver disease: pathology, epidemiology, and clinical aspects. New York: Wiley-Medical.
- Havens, W., Dickensheets, J., Biely, J., & Eberhard, T. (1954). The half life of I^{131} labelled normal gamma globulin in patients with hepatic cirrhosis. Journal of Immunology, 73, 256-258.
- Hunt, W. (1985). Alcohol and biological membranes. New York: Guilford Press.

- Iturriaga, H., Pereda, T., Esterez, A., & Ugarte, G. (1977). Serum immunoglobulin A changes in alcoholic patients. Annals of Clinical Research, 9, 39-43.
- Lieber, C. (1988). Biochemical and molecular basis of alcohol-induced injury to liver and other tissues. New England Journal of Medicine, 319, 1639-1650.
- Lieber, C., & DeCarli, L. (1968). Ethanol oxidation by hepatic microsomes: adaptive increase after ethanol feeding. Science, 162, 917-918.
- Lieber, C., & DeCarli, L. (1976). Animal models of ethanol dependence and liver injury in rats and baboons. Federal Proceedings, 35, 1232-1236.
- Owen, R. (1977). Sequential uptake of horseradish peroxidase by lymphoid follicle epithelium of Peyer's patches in normal unobstructed mouse intestine: an ultrastructural study. Gastroenterology, 72, 440-451.
- Rubin, E., Rybak, B., Lindenbaum, J., Gerson, c., Walker, G., & Lieber, C. (1972). Ultrastructural changes in the small intestine induced by ethanol. Gastroenterology, 63, 801-814.
- Sneller, M., & Strober, W. (1986). M cells and host defense. Journal of Infectious Disease, 154, 737-741.
- Triger, D., Alp, M., & Wright, R. (1972). Bacterial and dietary antibodies in liver disease. Lancet, i, 60-63.
- Volberg, T., Geiger, B., Kartenbeck, J., & Franke, W. (1986). Changes in membrane-microfilament interaction in intercellular adherens junctions upon removal of extracellular calcium ions. Journal of Cell Biology, 102, 1832-1842.
- Zubay, G. (1988). Biochemistry. New York: Collier Macmillan.