fractionated irradiation of the major lymphoid tissues (*23*). It is possible that differences in the lymphoid tissues, techniques of irradiation, choice of organ allograft, or transplantation techniques could explain the different results to date in dogs and mice. Further animal experimentation will be required to determine whether TLI may be of use in clinical organ transplantation.

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Localization of Apolipoprotein B in Intestinal Epithelial Cells

Abstract. *Indirect immunofluorescence techniques were employed to determine the distribution within intestinal epithelial cells of apolipoprotein B, a protein essential for the normal transport of fat. Isolated intestinal cells were prepared from rats either during active lipid absorption or after biliary diversion. Specific immunofluorescence from an antiserum to apolipoprotein B was detected in the apical portion of epithelial cells from bile-diverted animals, demonstrating that a pool of apolipoprotein B is present in the nonabsorptive epithelial cell and may be a component of intestinal cell membranes. During lipid absorption in normal rats, an early and sustained increase in immunofluorescence was demonstrated, consistent with an increased synthesis of apolipoprotein B during lipid absorption. This study demonstrates the presence of apolipoprotein B within intestinal epithelium and provides evidence for the participation of this apoprotein in intestinal lipid transport.*

The intestine has recently been recognized as an active site of synthesis of lipoproteins that transport endogenous as well as exogenous lipid (*1*). Long chain fatty acids and monoglycerides, the products of luminal lipolysis of triglyceride, cross the microvillus membrane and are resynthesized to triglyceride in the endoplasmic reticulum of the epithelial cell. Endogenous fatty acids may be derived from biliary lipid and are similarly resynthesized to triglyceride. The newly resynthesized triglyceride is then subjected to a series of synthetic steps with the addition of phospholipid, specific apoproteins, and carbohydrate to form a completed lipoprotein particle (chylomicron or very low density lipoprotein). This completed particle is then ready for egress from the intestinal cell to enter the lymph and systemic circulation.

While there is information on the individual synthetic events in lipoprotein formation, little is known concerning the sequence of additions or the steps that are critical to the process. Although quantitatively small, the protein moiety of the $d < 1.006$ lipoproteins, especially apolipoprotein B (apo LDL), is thought to be of fundamental importance in the process of intestinal triglyceride transport. Indirect evidence for the importance of apolipoprotein B in intestinal lipid transport is provided by the disease abetalipoproteinemia where the presumed inability to synthesize this apoprotein is associated with a failure of intestinal lipoprotein formation (*2*). While apolipoprotein B is known to be synthesized by the intestinal epithelial cell during chylomicron formation (*3*, *4*), the precise subcellular distribution of this apoprotein and whether a pool of apolipoprotein B is present in the nonabsorptive state is unknown. Using fluorescent antibody techniques we have demonstrated the presence of apolipoprotein B in intestinal epithelial cells in the fasting and absorptive state.

Antiserum to apolipoprotein B was prepared in rabbits immunized with rat low density lipoprotein (LDL) isolated from fresh rat plasma between densities 1.025 and 1.050 (*5*). Rabbits were immunized with purified LDL in complete Freund's adjuvant in multiple intracutaneous sites along the back; booster injections were given 2 weeks later, and blood was withdrawn 1 to 2 weeks subsequently. Antiserums were characterized by double diffusion in agarose (Fig. 1) and by immunoelectrophoresis against whole rat serum and purified LDL, and they gave a single precipitin line which stained with oil red O. Additional antiserums to apolipoprotein B were prepared by injecting intact chylomicrons isolated from rat mesenteric lymph and purified on 2 percent agarose columns (*4*). Immunization and bleedings were carried out as described above. Early bleedings yielded an antiserum that was monospecific for apolipoprotein B (Fig. 1) when tested against rat plasma or delipidated chylomicrons.

Isolated rat intestinal epithelial cells were prepared from fasting animals, bile-diverted animals, or from animals during active lipid absorption. Isolated intestinal cells were prepared by the method of Weiser by incubating jejunal segments with phosphate-buffered saline (*p*H 7.4) containing EDTA and dithiothreitol (*6*). Isolated rat colonic cells were similarly prepared. To produce active lipid absorption, corn oil emulsion in 10 m*M* taurocholate was instilled into isolated jejunal segments in situ for varying periods of time as in-

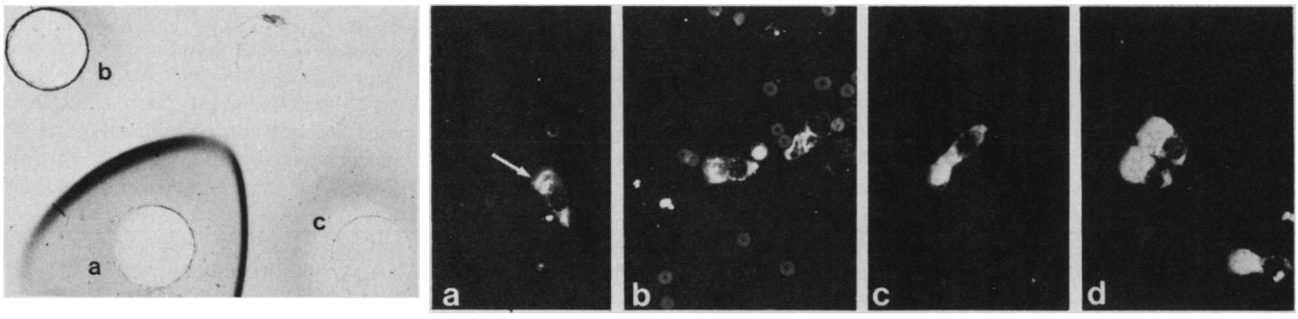

Fig. 1 (left). Immunodiffusion of antiserums to LDL and chylomicrons. Center well a, rat serum; well b, antiserum to LDL; well c, antiserum to chylomicrons. A reaction of identity is shown between the two antiserums. Stain: oil red O. No additional precipitin lines were visible after staining for protein. Fig. 2 (right). Immunofluorescence of apolipoprotein B within intestinal epithelial cells. (a) Intestinal cell from bile-diverted, fasted animal. Note the immunofluorescence in the supranuclear portion of the cell beneath the microvillus membrane (arrow). (b to d) The effect of lipid administration on intestinal apolipoprotein B. Isolated intestinal cells were prepared at varying intervals after the instillation of lipid into isolated intestinal segments; (b) 1 minute; (c) 5 minutes; and (d) 10 minutes. A progressive increase in the apolipoprotein B fluorescence, which gradually fills the entire apex of the cell, is evident.

dicated below. In some animals biliary diversion was performed 3 days previously, and the animals were allowed to drink only 5 percent dextrose in 0.9 percent saline after the operation. Immediately after preparation, isolated cells were smeared as a thin suspension on glass slides and dried in air. The slides were immersed in methanol for 4 minutes at −29°C, then acetone for 2 minutes, and again they were dried in air. The slides were incubated with antiserum to apolipoprotein B diluted with phosphate-buffered saline (1 : 8 by volume) for 30 minutes in a moist chamber at 37°C. Unreacted antiserum was then removed by sequential washing in three or four changes of phosphate-buffered saline at 37°C with mild agitation. Fluorescein-labeled antiserum to rabbit gamma globulin (the ratio of fluorescein to protein being 7) was then applied for 30 minutes and the washing procedure repeated. Nonimmune rabbit serum or antiserum to apolipoprotein B which had been subjected to prior absorption with immunochemically pure LDL were tested simultaneously. Slides were examined under a Zeiss fluorescent microscope and were photographed (at ×40) with a high-speed Ektachrome film.

Figure 2a shows that intestinal epithelial cells from fasting, bile-diverted animals contain significant quantities of apolipoprotein B. The distribution of fluorescence in the apical portion of the cell and the supranuclear region suggests that a pool of apoprotein is present in the endoplasmic reticulum and Golgi regions, consistent with the known ultrastructural localization of these organelles in intestinal epithelium (*7*). Since it is known that fasting and bile diversion for as little as 8 hours is sufficient to deplete the intestinal epithelial cell of all lipoproteins (*8*), it is unlikely that the apolipoprotein B that is visible in these cells is associated with lipoprotein particles. Furthermore, the known hydrophobicity of apolipoprotein B (*9*) would make it unlikely that this apoprotein exists unassociated with lipid in a soluble form within the cell. The present findings suggest that apolipoprotein B exists within nonabsorptive intestinal epithelium, perhaps as a constituent of cellular membranes. While ultrastructural studies are required to determine the precise site of apolipoprotein B and triglyceride association, the distribution of this apoprotein in the apical portion of the cell beneath the microvillus membrane suggests that an early association of apolipoprotein B and resynthesized triglyceride may occur during intestinal lipoprotein formation.

Supporting an early association of apolipoprotein B with triglyceride are ultrastructural observations in abetalipoproteinemia (*10*) where triglyceride accumulation is proximal to the Golgi region and suggests that apolipoprotein B deficiency interfered with chylomicron formation at an early step. Our findings, which demonstrate apolipoprotein B in the apical portion of the intestinal epithelial cell, are consistent with an early participation of this apoprotein in lipoprotein formation. In addition, with active lipid absorption (Fig. 2, b to d), there is a progressive increase in apolipoprotein B fluorescence consistent with the known increase in synthesis of this apoprotein during chylomicron formation (*11*). Within minutes after the intestinal mucosa is exposed to lipid, increased apolipoprotein B fluorescence becomes more intense in the apical portion of the cell (Fig. 2b) and progressively fills the entire apex of the cell from the nucleus to the microvillus membrane (Fig. 2, c and d). While the present studies do not define the precise stimulus for increased apolipoprotein B synthesis, the apparent rapidity with which this occurs and the finding of increased fluorescence progressing from beneath the microvillus membrane toward the supranuclear area suggests that this stimulus occurs early in the process of chylomicron formation.

All immunofluorescence was blocked by prior absorption of the antiserums with immunochemically pure LDL. In addition, isolated colon cells contained no apolipoprotein B fluorescence.

The ability to localize apolipoprotein B within intestinal epithelial cells should be of great value in the study of this important apoprotein in intestinal lipid transport in health and disease.

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