Chylomicron apoprotein localization within rat intestinal epithelium: studies of normal and impaired lipid absorption[1]

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Abstract Monospecific antisera were produced to two chylomicron apoproteins (apoB, apoA-I) and utilized for indirect immunofluorescent localization of these apoproteins within rat intestinal epithelium during normal and impaired lipid absorption. Isolated intestinal epithelial cells prepared after different periods of lipid absorption from in situ intestinal segments revealed a rapid increase in fluorescence for both apoproteins that filled the entire apical portion of the cell. Prolonged lipid absorption for as long as 5 hr demonstrated sustained immunofluorescence and gave no indication of a depletion of the intestinal mucosa for either apoprotein during normal lipid absorption. [^{3}H]Leucine incorporation into mesenteric lymph chylomicron apoproteins showed a linear decrease in specific activity of total chylomicron protein as well as apoB over 4 hr of a continuous lipid infusion indicating sustained active apoprotein synthesis during prolonged lipid absorption. Acetoxycycloheximide, a potent inhibitor of protein synthesis, was employed to determine the dynamics of chylomicron apoproteins during an experimental condition of impaired lipid absorption. In animals with inhibited protein synthesis, fluorescence for both apoproteins was present early in the course of lipid absorption; however, at 60 min after the onset of lipid absorption, fluorescence for both apoproteins was absent. Fluorescence for both apoproteins returned during the recovery of protein synthesis. The present studies have confirmed previous results that localized two chylomicron apoproteins within intestinal epithelial cells. The present studies extend these observations and disclose a rapid and sustained synthesis of these apoproteins during prolonged chylomicron formation. During an experimental condition of impaired protein synthesis there was a marked reduction in the mucosal content of both apoA-I and apoB. These results are the first demonstration of impaired mucosal apoprotein synthesis during an experimental model of impaired lipid absorption.

Supplementary key words immunofluorescence · ApoB · ApoA-I · acetoxycycloheximide · inhibition of protein synthesis

The process of intestinal lipoprotein formation involves a complex sequence of biosynthetic events within the intestinal epithelium that combines specific apoproteins (1), phospholipid, cholesterol, and carbohydrate (2) with resynthesized triglyceride to transform it to a completed lipoprotein. Although quantitatively small, the protein moieties associated with the intestinal lipoproteins are thought to be of fundamental importance in the process of intestinal lipid transport. In this regard, apoprotein B is thought to be of particular importance, as suggested by the genetic disease abetalipoproteinemia (3). In this disorder, the presumed inability to synthesize apoB is associated with total inability to form chylomicrons. Yet despite this presumptive evidence, few data are available concerning the participation of apoB in normal lipid absorption.

Previous studies have shown that intestinal microsomes can synthesize apoB (4), and that de novo synthesis of apoB, as well as of other lymph chylomicron apoproteins, occurs during active lipid absorption (5, 6). While these studies indicate that chylomicron apoproteins are actively synthesized during lipid absorption, little is known concerning the dynamics of apoprotein synthesis within the intestinal mucosa. Direct measurement of apoprotein content of the intestinal mucosa during different conditions of lipid absorption is particularly difficult in view of the small quantities of protein involved. In addition, the known hydrophobicity of apoB (7) makes measurement of the intracellular content of this particular apoprotein difficult. A recent study (8) of the apoB content of human intestinal mucosa, employing a radioimmunoassay, indicated that apoB was present in the nonabsorptive mucosa; unexpectedly, however, the content of this apoprotein decreased significantly during maximal

Abbreviations: SDS, sodium dodecylsulfate; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; TMU, tetramethylurea; PBS, phosphate-buffered saline; ACH, acetoxycycloheximide.

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lipid absorption. While it is possible that the immunoreactivity of apoB differs in the fasting compared to the fed state, these results also suggest that the apoB content of the mucosa had been depleted during the process of chylomicron formation and repletion of this apoprotein had not yet occurred.

These observations are difficult to reconcile with the considerable capacity of the upper small intestine to absorb lipid (9) and underscore the necessity for determining the dynamics of important chylomicron apoproteins during intestinal lipid absorption. We have recently developed fluorescence antibody techniques to permit the localization of chylomicron apoproteins within rat intestinal epithelial cells (10). These studies showed that a pool of apoB is present in the apical portion of intestinal epithelial cells prepared from fasting biliary-diverted animals, an experimental condition that depletes the intestinal mucosa of chylomicrons and very low density lipoproteins (11). Within minutes after the onset of lipid absorption, there was a marked increase in apoB immunofluorescence which filled the entire apex of the cell, consistent with an increased synthesis of apoB during chylomicron formation (10).

The present studies employ similar fluorescence antibody methods to localize chylomicron apoproteins during conditions of normal and impaired lipid absorption. [^{3}H]Leucine incorporation into chylomicron apoproteins during lipid absorption was also studied. The results of these studies indicate that a sustained synthesis of apoB occurs with prolonged lipid absorption and shows no evidence of apoB depletion within intestinal mucosa. During an experimental condition of impaired lipid absorption resulting from impaired protein synthesis, there is a striking reduction in apoprotein fluorescence within the intestinal mucosa, providing direct evidence for impaired apoprotein synthesis during this experimental condition of impaired lipid absorption.

METHODS

Production of antisera

Mesenteric lymph chylomicrons were obtained from rats with indwelling mesenteric lymph cannulas as previously described (6). Lymph was defibrinated and chylomicrons were separated by ultracentrifugation at 3×10^6 g-min in a SW 41 rotor. Purification of chylomicrons from contaminating serum proteins was accomplished by gel filtration through 2% agarose columns (Biogel A50 100–200 mesh) as described previously (6). Two passages were sufficient to achieve a purification yielding a consistent pattern of chylomicron apoproteins on SDS–acrylamide electrophoresis as validated earlier in this laboratory (6). Intact chylomicrons (100–200 μg of protein) were mixed with complete Freund's adjuvant and injected at multiple intracutaneous sites along the backs of New Zealand white rabbits. The animals were boosted 2 weeks later and bled 1–2 weeks subsequently. Characterization of this antiserum has been reported previously (10). The antiserum was characterized by double diffusion in agarose and by immunoelectrophoresis against whole rat serum and purified LDL. Early bleedings yielded an antiserum that gave a single arc, which was Oil Red O-staining when reacted against whole rat serum or purified LDL. In particular, no precipitin arcs were visible when this antiserum was tested against rat HDL or lipoprotein-depleted ($d > 1.21$ g/ml) serum, indicating that it did not contain the additional antibodies to chylomicron apoproteins that are common to these substances. Additional antisera to apoB were similarly prepared to rat LDL isolated from fresh rat plasma between densities 1.025 and 1.050 (12). When tested against rat serum and purified LDL, these antisera gave a single precipitin arc which stained with Oil Red O and exhibited a reaction of identity with the anti apoB antiserum prepared from chylomicrons (10). Both antisera reacted with apoB prepared from both chylomicrons and LDL by TMU delipidation, indicating that these antisera recognized apoB in its native and delipidated forms. As indicated below, both antisera gave similar results when used in fluorescence antibody studies and all fluorescence was specifically blocked by prior absorption of either antiserum with purified rat LDL.

Additional antisera were prepared to apoA-I, another chylomicron apoprotein which we have previously shown comprises approximately 50% of rat lymph chylomicron protein (6). This apoprotein has a molecular weight of ca. 25,000 and is immunologically identical to one of the rat high density apoproteins. Subsequent work in our laboratory has characterized and identified this apoprotein as apoA-I (13). Isolation of the apoprotein and characterization of the anti apoA-I antiserum have been reported previously (13). The resultant antisera reacted with rat serum HDL and gave no nonlipoprotein arcs when tested against whole rat serum. No reaction was present to rat LDL. As described below, these antisera were employed for immunofluorescence localization of chylomicron apoproteins within intestinal epithelium during various conditions of lipid absorption.

Immunofluorescence studies of lipid absorption

Lipid administration. In some experiments, lipid was administered intragastrically as corn oil, and intestinal

tissue was obtained at the indicated times (usually 90 min after lipid administration). In other experiments, lipid was instilled into in situ intestinal segments. Under light pentobarbital anesthesia, the abdomen was opened and ligatures were placed at the duodenum and below the ligament of Treitz, isolating a segment of duodenum and jejunum approximately 12–15 cm, in length. One to two ml of a sonicated corn oil emulsion (1 ml of corn oil, 2.5 ml of 40 mM taurocholate, 6.5 ml of PBS) was inserted into the loop and it filled the entire segment without producing distension. The abdomen was closed and the animals were allowed to recover from anesthesia (usually 15–20 min). At various time periods after lipid administration, the animals were killed by cervical dislocation. The intestinal segment was removed and flushed with phosphate-buffered saline. The intestinal segment was then longitudinally incised to expose the mucosa. Where indicated, aliquots of mucosa were frozen at this point for histologic demonstration of lipid (Oil Red O staining). The remainder of the mucosa was utilized to prepare isolated small intestinal epithelial cells for fluorescence antibody studies. Small intestinal epithelial cells were also prepared from rats that had been bile ligated and fasted for 12 hr previously to deplete the intestinal mucosa of lipoproteins (11). Bile-diverted animals were allowed free access to 0.9% saline, 5% dextrose.

Fluorescence studies. Isolated rat small intestinal epithelial cells were prepared according to the method of Weiser (14). Jejunal segments were incubated at room temperature for 1–3 min with a citrate buffer (KCl 1.5 mM; NaCl 96 mM; sodium citrate 27 mM; KH_2PO_4 8 mM; Na_2HPO_4 5.6 mM, pH 7.2). The tissue was then incubated with phosphate-buffered saline (no Ca^{2+} or Mg^{2+}) containing 1.5 mM EDTA and 0.5 mM dithiothreitol. Within minutes, isolated epithelial cells dissociated and were collected by centrifugation at 1000 *g*. The cell pellet was washed twice in PBS, pH 7.4, and immediately smeared as a thin suspension on glass slides and air dried. Slides were then immersed in cold methanol for 4 min, then immediately in acetone for 2 min and air dried (15). Isolated colonic epithelial cells were prepared in the same fashion. Immunofluorescence studies on intestinal epithelial cells were carried out as previously described (10). After fixation, isolated intestinal cells were overlaid with the indicated antiserum at an appropriate dilution (usually greater than 1:8) for 30 min at 37°C in a moist chamber. Unreacted antiserum was then removed by sequential washing in 3–4 changes of PBS at 37°C with mild agitation. Fluorescein-labeled anti rabbit gamma globulin (Behring Diagnostics, Somerville, NJ) (fluorescein:protein ratio 7:1) was then applied for 30 min and the washing procedure was repeated. Nonimmune rabbit serum or antiserum that had prior absorption with purified LDL or HDL was simultaneously tested. Slides were mounted in glycerol–PBS 9:1, pH 10, and viewed under a Zeiss fluorescence microscope equipped with an FITC specific filter. Slides were photographed with Agfachrome 64 film with a 40× oil immersion objective (total magnification ca. 400×). Five to ten slides for each experimental variable were examined and representative cells were photographed. Each experiment was repeated five times.

Studies of [^{3}H]leucine incorporation into chylomicron apoproteins

Cannulation of the main mesenteric lymphatic duct and duodenum and post operative maintenance of the animals have been described previously (16). All animals were studied 16–24 hr after surgery. Lipid was administered as a corn oil emulsion in 10 mM sodium taurocholate (described above) by constant intraduodenal infusion at a rate of 1 ml/hr. After 2 hr of perfusion, a single dose of 200 μCi [^{3}H]leucine was administered in saline intraduodenally. The lipid infusion was continued at the same rate and lymph was collected at hourly intervals for the duration of the experiment. Lymph flow was at least 1.5 ml per hr. Lymph was defibrinated and chylomicrons were isolated by ultracentrifugation. Isolated chylomicrons were purified of contaminating serum proteins by passage through 2% agarose columns as described above. Purified chylomicrons were then mixed with an equal volume of TMU (Burdick and Jackson Laboratories, Muskegon, MI) at 30°C as described by Kane (17) and allowed to stand overnight. The TMU-soluble material was removed from the TMU-insoluble residue by aspiration. The TMU-insoluble material was washed once with TMU–saline 1:1. The residue was hydrolyzed in 1 N NaOH at 80°C for 30 min, neutralized, and then extracted with ether. Protein determinations and radioactive counting were carried out directly. SDS gel electrophoresis of this material revealed only a single band identified as apoB, validating that TMU delipidation solubilized all chylomicron apoproteins with the exception of apoB.

The specific activity in purified chylomicrons was also determined by direct counting of intact chylomicrons that were directly solubilized in NCS. Protein determinations were carried out by the method of Lowry.

Inhibition of protein synthesis

Acetoxycycloheximide was administered intraperitoneally 1 hr prior to lipid administration in a 0.9% saline solution at a dose of 0.25–0.5 mg/kg. This dose level and schedule was previously shown by us to in-

hibit L-[^{14}C]leucine incorporation into intestinal mucosal protein by at least 80% for 3 hr (17) and to decrease the lymphatic absorption of [^{14}C]oleic acid mixed micelles by approximately 33% (18). One hour after ACH administration, a lipid emulsion was inserted into a jejunal loop as described above. Animals were killed at the indicated times and isolated cells were prepared.

Materials

Male Sprague-Dawley rats (200–250 g) were purchased from Charles River Breeding Co., Boston, MA. Taurocholate was obtained from Calbiochem and found to be chromatographically pure as described earlier (6). ACH was generously supplied by Chas. Pfizer and Co., Inc., New York. All other chemicals were obtained from standard sources and were the purest grades commercially available.

RESULTS

Immunofluorescence localization of chylomicron apoproteins

As indicated above, isolated jejunal epithelial cells were prepared from rats that had been given 2 ml of corn oil by stomach tube 90 min previously. **Fig. 1** (left) illustrates the fact that intense immunofluorescence is present in the supranuclear region filling the entire apex of the cell. Prior absorption of the anti apoB antiserum with an excess of purified rat serum LDL completely eliminates all fluorescence (Fig. 1, middle). Nonimmune rabbit serum (Fig. 1, right) shows no reaction with lipid-laden cells. No fluorescence was present in isolated colon cells.

Similar results were obtained when anti apoA-I antiserum was employed with fluorescence visible in the apex of the lipid-laden cells, confirming previous results from our laboratory (13). Again, prior absorption of the antiserum with rat serum HDL eliminated all fluorescence.

In order to determine the dynamics of apoB appearance within intestinal epithelial cells, a timed study from in situ intestinal loops was carried out. As described in Methods, isolated epithelial cells were prepared at various time intervals after lipid absorption from in situ jejunal segments. These experiments were carried out on animals that had been bile-duct ligated and fasted for 12 hr previously to deplete the intestinal epithelium of lipoproteins (11). Previous results from our laboratory (10) have shown that intestinal epithelial cells contain a pool of apoB localized in the apical portion of the cell. Within minutes after the onset of lipid absorption, there was a rapid increase in immunofluorescence that filled the entire apex of the cell. While these results indicated a rapid synthesis of apoB within minutes after the start of lipid absorption, they did not indicate whether a sustained synthesis of apoB occurred with continued maximal lipid absorption. As shown in **Fig. 2** (upper), a pool of apoB is present in nonabsorptive epithelium. Within 10 min after the instillation of a lipid emulsion into an in situ jejunal loop, there was a marked increase in apoB fluorescence filling the entire apical portion of the cell (Fig. 2, middle). Intense fluorescence persisted throughout the duration of lipid absorption. Fig. 2 (lower) shows the appearances of apoB fluorescence after 4 hr of lipid absorption. These results are consistent with a sustained increase in intracellular apoB during maximal lipid absorption. Similar results were obtained for the appearance of apoA-I during active lipid absorption, consistent with earlier results in mesenteric lymph (6) showing that both of these chylomicron apoproteins are actively synthesized during intestinal lipid absorption. In order to investigate further the effects of sustained lipid absorption on apoB synthesis, iso-

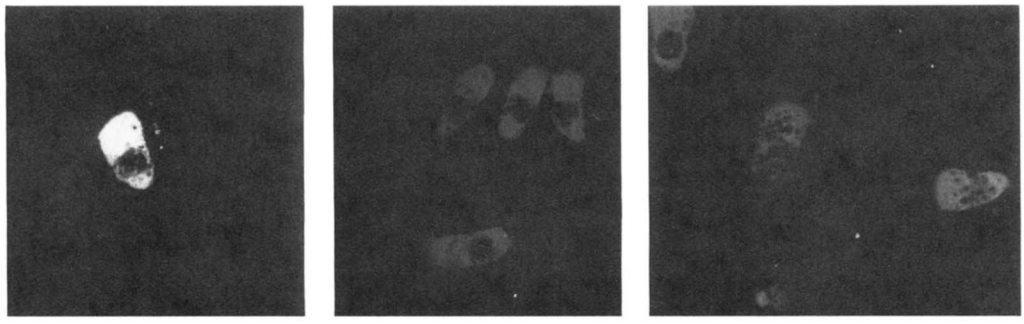

Fig. 1. ApoB immunofluorescence on isolated jejunal epithelial cells. Ninety min after the aministration of corn oil by gastric tube, isolated small intestine epithelial cells were prepared and examined for immunofluorescence (Methods). Left, anti apoB antiserum; middle, anti apoB + LDL; right, normal rabbit serum.

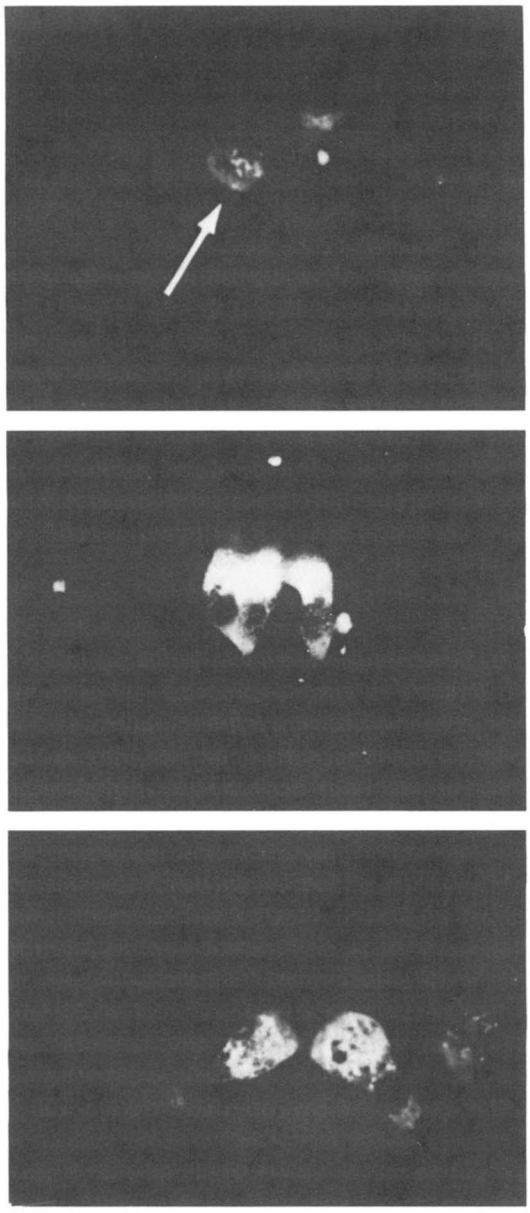

Fig. 2. ApoB immunofluorescence after biliary ligation and lipid feeding. Isolated intestinal cells were prepared from bile ligated animals (upper) and after various times after lipid is instilled into in situ jejunal loops (middle, lower). Middle, 10 min; lower, 4 hrs. The arrow indicates the microvillus membrane.

topic incorporation studies on lymph chylomicron apoB were carried out.

[^{3}H]Leucine incorporation into mesenteric lymph chylomicrons

After a constant intraduodenal infusion of a corn oil emulsion was established in lymph fistula rats, a single dose of 200 μCi of [^{3}H]leucine was administered intraduodenally. Chylomicrons were purified and the specific activity in chylomicron protein was determined. Specific radioactivity incorporated into apoB was also determined after TMU delipidation. As shown in **Fig. 3,** there was a linear decrease with time in the specific activity in chylomicron protein, with the highest specific activity recorded 1 hr after the administration of the isotope. Since a prolonged lipid infusion was continued throughout the entire experiment, the linear decrease in specific activity observed is compatible with a continued synthesis of chylomicron apoproteins. In view of the central role afforded to apoB in lipid absorption, it was of interest to determine apoB specific activity under the same experimental conditions (Fig. 3). Similarly there was a linear decrease in apoB specific activity with time, suggesting a continued active synthesis of this apoprotein during prolonged maximal lipid perfusion. SDS–acrylamide gels of delipidated chylomicron apoproteins after 1 and 4 hr of lipid perfusion showed no difference in apoprotein patterns or distribution as determined densitometrically from stained gels. In particular, apoB accounted for 10–12% of chylomicron protein and apoA-I for 38–42% of chylomicron protein at both times. In addition, fluorescence antibody studies carried out on jejunal cells isolated after 4 hr of lipid perfusion showed intense fluorescence for apoB as well as for apoA-I, again consistent with a sustained synthesis of these apoproteins during maximal lipid absorption.

Studies of protein synthesis inhibition

The above results reveal an early and sustained increase in the content of both apoB and apoA-I

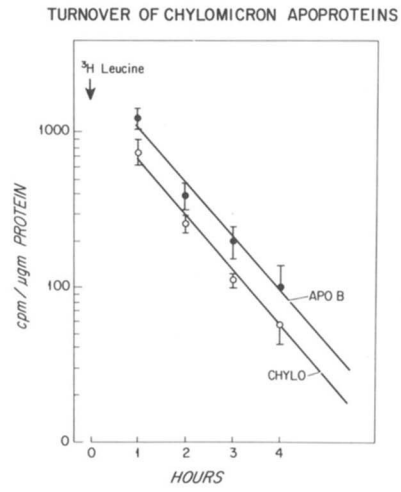

Fig. 3. [^{3}H]Leucine incorporation into mesenteric lymph chylomicron apoproteins. After a constant lipid infusion had been established (Methods), 200 μCi of [^{3}H]leucine was administered intraduodenally to mesenteric lymph fistula rats. The lipid infusion was continued and hourly lymph samples were collected. Chylomicrons were purified and the specific radioactivity was determined in chylomicron protein and chylomicron apoB (Methods). N = 5. Note the logarithmic scale.

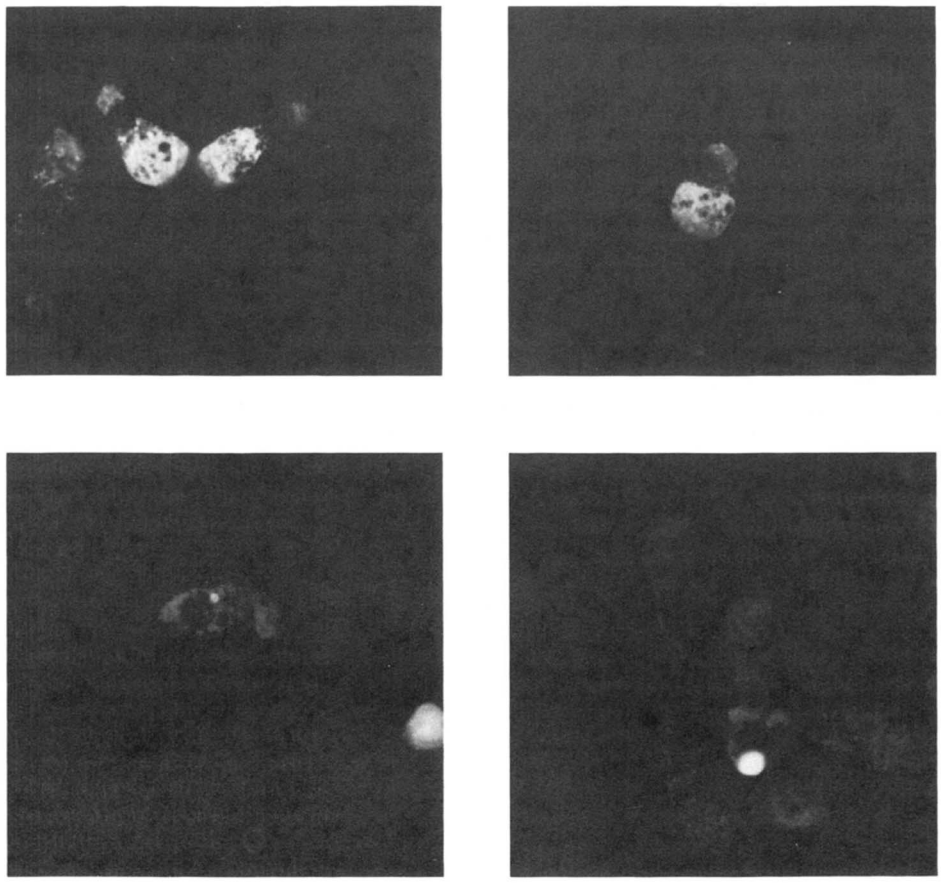

Fig. 4. Effect of protein synthesis inhibition (ACH) on chylomicron apoprotein immunofluorescence. Isolated intestinal cells were prepared from control and ACH-treated animals 1 hr after lipid had been instilled into isolated in situ intestinal segments (Methods). Upper left, control, anti apoB; upper right, control, anti apoA-I; lower left, ACH-treated, anti apoB; lower right, ACH-treated, anti apoA-I. A marked decrease in immunofluorescence for both apoproteins is evident in ACTH-treated animals. Note the large lipid spaces within the cytoplasm in ACH-treated animals.

within the intestinal epithelium during lipid absorption. In addition, a pool of formed apoB is present within the intestinal epithelium in the nonabsorptive state. In order to determine the extent of this preformed apoprotein pool, as well as the dynamics of apoprotein synthesis during an experimental condition of impaired lipid absorption, the effect of acetoxycycloheximide (ACH), a potent inhibitor of protein synthesis, was studied. ACH was administered to animals 1 hr prior to lipid administration into an isolated jejunal segment (Methods) in a dose sufficient to inhibit intestinal protein synthesis for 3–4 hr (17). Isolated cells were prepared at 5 and 60 min after the start of lipid absorption. Histologic stains for lipid (Oil Red O) at these two times confirmed that the inhibition of protein synthesis did not affect the uptake of lipid or its resynthesis within the intestinal mucosa (19) since large lipid droplets were seen within epithelial cells at both times. Isolated cells prepared from intestinal mucosa that had not been exposed to lipid still showed a fine pattern of apoB fluorescence in the apical portion of the cell beneath the microvillus membrane, indicating that protein synthesis inhibition has no effect on preformed apoB present within intestinal cells.

Fig. 4 shows that 60 min after the start of lipid absorption in control animals, the entire apical portion of the cell showed bright fluorescence for both apoB (Fig. 4, upper left) and apoA-I (Fig. 4, upper right). In contrast, in animals with impaired protein synthesis (Fig. 4, lower) no fluorescence was visible for either apoprotein despite the intestinal mucosa containing large amounts of triglyceride as shown by histological staining. Earlier time points (i.e., 5, 10 min after lipid administration) showed positive fluorescence for both apoproteins in ACH-treated animals. These results indicate that, under the stimulus of continued lipid uptake, impaired intestinal protein synthesis is associated with a depletion of two major chylomicron apoproteins within the intestinal

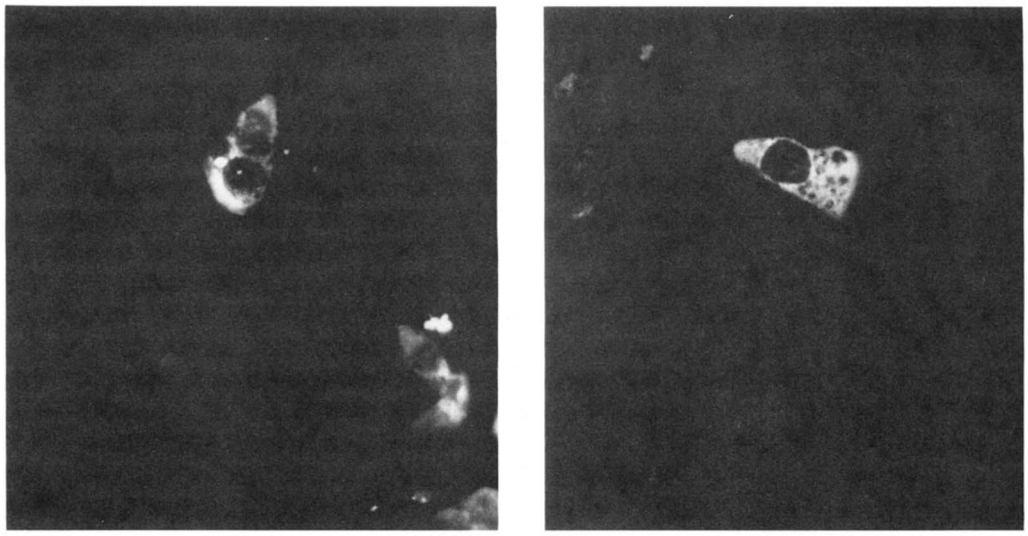

Fig. 5. ApoB immunofluorescence during the recovery of intestinal protein synthesis. Lipid was instilled into in situ intestinal loops in ACH-treated animals and isolated intestinal cells prepared after 6 hr (left) and 8 hr (right). Note the progressive reappearance of fluorescence in the apex of the epithelial cells.

mucosa. In order to determine whether the effect of impaired protein synthesis on chylomicron apoprotein synthesis is reversible, animals were studied at 6 and 8 hr after the administration of ACH, at a time when protein synthesis is returning to normal. As shown in **Fig. 5,** the intestinal cells were still engorged with large lipid droplets. It is of interest that at 6 hr (Fig. 5, left) the first appearance of apoB was beneath the microvillus membrane in close proximity to the large triglyceride droplets in the apex of the cell. Sufficient resolution was not possible, however, to determine whether this newly formed apoB had arranged itself on the surface of the lipid droplet. Two hours later (Fig. 5, right), lipid droplets were still visible; however, there was a marked increase in apoB fluorescence that filled the entire apex of the cell. In parallel histologic sections stained for lipid, a progressive clearing of triglyceride from the epithelium followed the return of apoprotein synthesis. Virtually identical results were obtained for apoA-I (data not shown).

DISCUSSION

While the process of intestinal lipoprotein formation involves many biosynthetic events within the intestinal epithelium that transform resynthesized triglyceride to a completed lipoprotein, few data are available concerning which steps are truly critical to the process. It is widely held that apoB, an apoprotein present in intestinal lipoproteins (VLDL, chylomicrons) is a major determinant for lipid transport through the intestine. The major basis for this belief is the disease abetalipoproteinemia where the presumed inability to synthesize apoB is associated with a total inability to form intestinal chylomicrons (3). Recent advances in our understanding of lipoprotein interactions (20) would now suggest that the hallmark of the disease, the absence in peripheral blood of any circulating lipoproteins containing apoB, could arise from any cellular defect interfering with the synthesis of the triglyceride-rich lipoproteins (VLDL, chylomicrons). It is therefore of interest to examine the physiological role of apoB in lipid absorption.

It has been shown previously that the intestinal mucosa can synthesize apoB as well as other chylomicron apoproteins during lipid absorption (1, 5, 6). In addition, nascent lipoproteins released from lipid-laden intestinal cells contain apoB (21). Yet, little is known concerning the dynamics of apoB synthesis within the intestinal mucosa. The present study has utilized fluorescence antibody techniques to localize apoB within the intestinal epithelium during conditions of normal and impaired lipid transport. Previous results from our laboratory (10) have shown that there is a preformed pool of apoB within rat intestinal epithelium scattered throughout the apical portion of the cell. Within minutes after the start of lipid absorption there was a progression of apoB fluorescence from beneath the microvillus membrane towards the supranuclear area, the known site of the Golgi apparatus in the intestine. This progression of apoB fluorescence parallels the known ultrastruc-

tural movement of triglyceride (22) during lipid absorption and suggested that apoB synthesis is an early event in chylomicron formation. The present studies have investigated the synthesis of chylomicron apoproteins during prolonged lipid infusion. The results of [^{3}H]leucine incorporation into lymph chylomicron apoB are consistent with a continued active synthesis of this apoprotein during a 4-hr period of lipid perfusion (Fig. 3). Supporting this interpretation are the fluorescence antibody studies that show a marked increase in immunofluorescence during all phases of lipid absorption (Fig. 2). The present study gives no indication of a mucosal depletion of apoB during normal prolonged lipid absorption by either of these techniques, and lymph chylomicron apoprotein composition as determined by SDS gel electrophoresis was unchanged after prolonged lipid infusion. A recent study in humans (8) suggested that, 45 min after the onset of lipid absorption, there was a depletion of small intestinal mucosal apoB as measured by radioimmunoassay. These results could be explained by either a depletion of preformed apoB or a decreased immunoreactivity of de novo synthesized apoB. While the present studies cannot exclude a transient decrease in the apoB content of small intestinal mucosa at a time period not studied, there was no indication of such a depletion. The marked increase in immunofluorescence for apoB seen at all points during lipid absorption could argue against a major decrease in immunoreactivity of newly synthesized apoB. The present results are in keeping with the capacity of the jejunum to absorb considerable quantities of lipid without apparent saturation (9) and suggest that continued chylomicron apoprotein synthesis accompanies this process. In addition, the present studies have confirmed previous reports that apoA-I is actively synthesized by rat intestinal mucosa during chylomicron formation (6, 13).

While the above results confirm a continued active synthesis of chylomicron apoproteins during intestinal lipid absorption, little is known concerning the intracellular metabolism of chylomicron apoproteins during conditions of impaired lipid absorption. One experimental model of impaired lipid absorption employs pharmacological inhibitors of protein synthesis and is associated with an accumulation of large lipid droplets distending the apical cytoplasm of intestinal epithelial cells (19, 23). Previous studies from our laboratory have shown that acetoxycycloheximide, a potent inhibitor of protein synthesis, was associated with marked changes in mesenteric lymph chylomicrons formed under these conditions. In addition to a decreased lipid absorption into lymph, chylomicrons were found to be considerably larger (18) and deficient in a major chylomicron protein subsequently identified as apoA-I (6, 13). Those chylomicrons, which appeared in lymph during the inhibition of protein synthesis, showed a relative preservation of apoB despite a marked decrease in other apoproteins, indirectly underscoring the importance of apoB in lipid transport. No direct conclusions could be made, however, on the apoprotein content of the intestinal mucosa.

The present study provides direct evidence that during protein synthesis inhibition there is a significant reduction in the mucosal content of two major chylomicron apoproteins, apoB and apoA-I (Fig. 4). The finding that prior inhibition of protein synthesis did not visibly decrease the preformed pool of apoproteins within the intestinal cell and that, at early times after the onset of lipid absorption, fluorescence for both apoproteins was present, indicates that there was no interference with the detection of intracellular apoproteins under these experimental conditions. It should be noted that the design of the present experiments utilized large amounts of lipid inserted into an isolated in situ intestinal segment, thus ensuring a maximal absorptive stress since the same segment of mucosa is continuously exposed to luminal lipid requiring chylomicron formation for transport. Under these conditions, it was possible to demonstrate a depletion of the pool of apoB from within the intestinal epithelium under conditions of impaired protein synthesis (Fig. 4).

These results also strongly argue against the theoretical possibility that the intracellular apoB visualized in the present study was transferred from plasma, since this should be unaffected by inhibiting de novo protein synthesis. While the present studies are not directly comparable to previous studies of lymph chylomicron formation during impaired protein synthesis, the results obtained do offer direct evidence that, during conditions of impaired protein synthesis, changes in mucosal apoprotein content also occur.

The results of the present study that confirm the presence of a preformed pool of apoB within the intestinal epithelium (8, 10) show that, under conditions where de novo apoprotein synthesis is limited, this pool can be mobilized for lipoprotein formation. The present study, as well as the study of isolated perfused liver by Bar-on et al. (24), indicate that this pool is of limited size. It is not known, however, whether during normal lipid absorption there is initial utilization of this pool followed by de novo apoprotein synthesis.

The immunofluorescence techniques utilized in the

present study, while qualitative, should be of value in the study of apoprotein metabolism within the intestinal epithelium during conditions of normal and impaired lipid absorption.

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