Isolated rabbit enterocytes as a model cell system for investigations of chylomicron assembly and secretion

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Abstract **A method is described for the isolation of viable enterocytes from rabbit small intestine. The procedure can also be used to isolate populations of epithelial cells from the crypt/villus gradient. The isolated enterocytes synthesized and secreted apoB-48 and triacylglycerol in particles of the density of chylomicrons. Secretion was stimulated by addition of bile salt/lipid micelles. Pulse-chase experiments demonstrated that newly synthesized apoB-48 is degraded intracellularly and that degradation is inhibited by provision of lipid micelles, suggesting that regulation of chylomicron assembly and secretion is broadly similar to that of very low density lipoprotein assembly in hepatocytes. This procedure for preparation of isolated enterocytes will provide a useful model system for investigation of the molecular details of chylomicron assembly.**—Cartwright, I. J., and J. A. Higgins. **Isolated rabbit enterocytes as a model cell system for investigations of chylomicron assembly and secretion.** ***J. Lipid Res.*** **1999.** 40: **1357–1365.**

Supplementary key words small intestine • fat absorption • crypt/villus gradient • thymidine kinase • alkaline phosphatase • apoB-48 • triacylglycerol • micelles • degradation

Absorption of dietary lipids involves a number of steps: *i*) intralumenal digestion, *ii*) transfer of the products of digestion across the brush border of the enterocytes, *iii*) resynthesis of lipids and assembly of these into chylomicrons, and (iv) release of chylomicrons at the lateral border of the enterocytes into the lamina propria, from where they move via the lymph into the circulation. Chylomicrons, consist of droplets of neutral lipid (predominantly triacylglycerol [TAG] with a varied amount of cholesteryl ester), stabilized by a shell of amphipathic lipid (phospholipid and cholesterol) and protein (1). Apolipoprotein B-48 (apoB-48) is the major apolipoprotein of chylomicrons and is essential for their assembly (2). Electron microscopy studies, including autoradiography and immunocytochemistry, have shown that chylomicron assembly and secretion follow the classical secretory pathway (3–5). ApoB-48 is synthesized by bound ribosomes in the rough endoplasmic reticulum (RER). Lipid droplets, presumed to be chylomicron precursors, are observed within the lumen of the RER and the smooth endoplasmic reticulum (SER) and mature chylomicrons accumulate in the lumen of the trans-Golgi, before release at the lateral border of the enterocyte.

In many ways assembly of chylomicrons in enterocytes resembles that of very low density lipoproteins (VLDL) in hepatocytes. However, chylomicrons contain more triacylglycerol and are larger and lighter than VLDL (1). The major protein component of VLDL is apoB-100, while that of chylomicrons is apoB-48, a truncated form of apoB-100. There has been considerable progress in the elucidation of the molecular events in assembly of VLDL in hepatic cells (6–8). This has been facilitated by the availability of the human hepatoma cell line, HepG2, and of methods for the isolation and maintenance or primary culture of adult hepatocytes. However, very little is known about the assembly of chylomicrons. This is partly due to the lack of an appropriate enterocyte-derived cell line and because methods for the isolation and maintenance of viable enterocytes have not been fully developed (1). Studies of lipid absorption have been carried out on lymph duct-cannulated animals and intestinal segments. However, these studies do not identify cellular or intracellular events. Mucosal scrapings have also been used. However, these preparations suffer from the limitation that they include the absorptive cells of the villi and the immature crypt cells, as well as cells of the lamina propria. In order to investigate cellular, intracellular, and molecular events in lipid absorption and chylomicron assembly, homogeneous preparations of viable enterocytes are necessary. For this reason, a number of investigators have used the CaCo-2 cell line (9–11). When cultured on filters, this colonic cell line differentiates into enterocyte-like cells that form monolayers and secrete lipoproteins of LDL/VLDL density, which contain both apoB-100 and apoB-48. However, CaCo-2 cells are not an ideal model for enterocytes and resemble fetal rather than adult enterocytes (1).

One of the aims of our laboratory is to investigate the ef-

Abbreviations: TAG, triacylglycerol; apoB, apolipoprotein B; RER, rough endoplasmic reticulum; SER, smooth endoplasmic reticulum, VLDL, very low density lipoproteins; DMEM, Dulbecco's modified Eagle's medium, HPTLC, high performance thin-layer chromatography.

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fect of factors such as diet on the regulation of lipid absorption. We consider that it is essential to use mature enterocytes for these studies. Here we report a method for the preparation of suspensions of viable adult rabbit enterocytes. We chose the rabbit because it is considered a good model for studies of lipoprotein metabolism and is sufficiently large to provide a good yield of enterocytes. The isolated enterocytes synthesize and secrete chylomicrons when provided with bile salt/lipid micelles, their physiological substrate, and should provide a useful model system for studies of chylomicron assembly and the regulation of lipid absorption.

MATERIALS AND METHODS

Materials

Dulbecco's modification of Eagle's medium without methionine (DMEM) was purchased from ICN (Thane, Oxfordshire, UK). Optiprep™ was from Lipotek Ltd (Peter Jost Enterprise Centre, Liverpool John Moores University, Liverpool L3 3AF, UK) [^{35}S]methionine, [^{3}H]thymidine, and [^{3}H]oleate were from Amersham, UK. High performance thin-layer chromatography (HPTLC) silica gel plates were from Camlab (Cambridge, UK). PHM-Liposorb and protease inhibitor cocktail (Complete™) were from Calbiochem (Nottingham, UK) and Boehringer Mannheim (East Sussex, UK), respectively. All other chemicals were from Sigma (Poole, Dorset, UK) or as described previously (12–16).

Animals

Dwarf lop rabbits (6 months old, 2.5–3.0 kg body weight) were used for these studies. They were bred and housed in the University Field Laboratories, maintained on a 12-h light/dark cycle, and were allowed free access to standard chow.

Isolation of viable enterocytes

Rabbits were killed by terminal anesthesia (Nembutal) and the small intestine was immediately removed for preparation of isolated enterocytes (**Fig. 1**). In some experiments, using 20-cm seg-

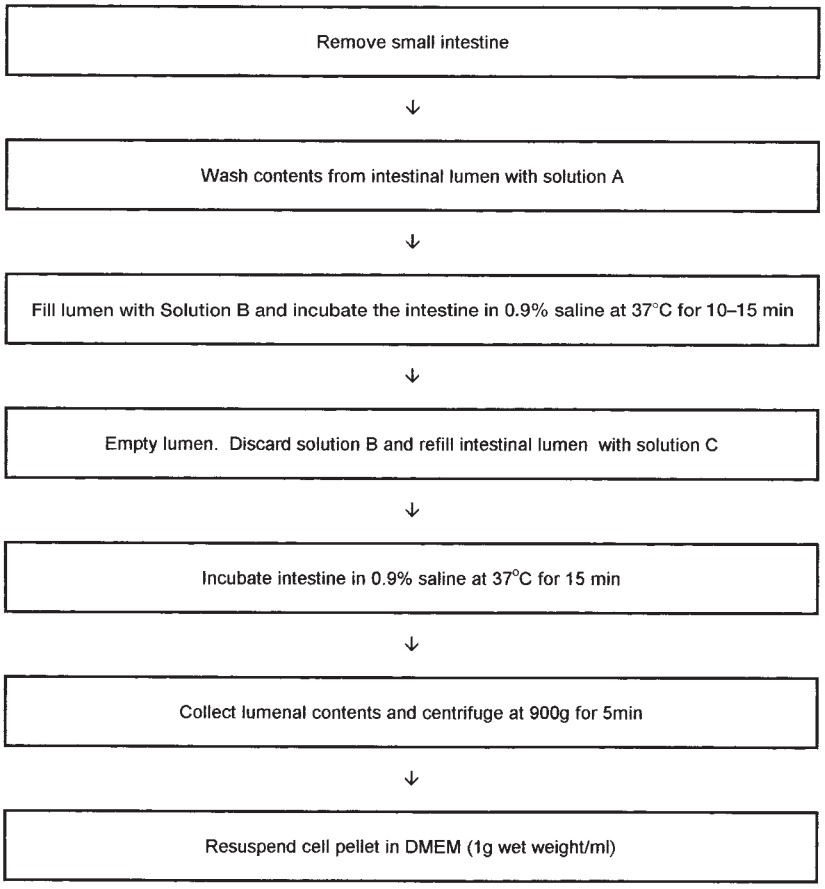

Fig. 1. Isolation of enterocytes from the small intestine.

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ments of small intestine, the incubation time in solution C varied between 5 and 30 min. The relative proportions of crypt to villus cells recovered from these segments were determined by alkaline phosphatase and thymidine kinase assays, as described below. All enterocytes used for studies of chylomicron secretion were isolated after 10 min incubation in solution C.

Preparation of micelles

Lipids were mixed and dissolved in chloroform–methanol 1:1 (v/v) and were dried under vacuum. The appropriate volume of DMEM containing bile salts was added. Micelles were then prepared by sonication using an MSE Soniprobe at maximum power for 3 min with cooling. Preliminary experiments showed that bile salts and phospholipids were essential for the secretion of radiolabeled TAG by isolated enterocytes, and the following final concentrations of the micelle components in the incubations were selected, based on the physiological composition of micelles in the lumen of the small intestine (17): sodium cholate (0.14 mm), sodium deoxycholate (0.15 mm), phosphatidylcholine (palmitoleoyl) (0.17 mm), oleic acid (0.22 mm), and monopalmitoylglycerol (0.19 mm). Micelles were prepared at 10 times these concentrations in DMEM and 0.4 ml was added to a 3.6-ml of enterocyte suspension. In some experiments [^{3}H] or [^{14}C]oleic acid was added to the initial lipid mixture prior to micelle preparation, in order to study enterocyte TAG synthesis and secretion.

Incubation of enterocytes

Enterocytes were suspended to 55.5% w/v in DMEM (pregassed with 95% O_2/5% CO_2 and adjusted to pH 7.4). Glutamine (4 mm final concentration) was added to 3.6 ml cell suspension immediately prior to incubation with [^{35}S]methionine (100 μCi), to radiolabel apoB-48, and/or micelles (0.4 ml) containing [^{3}H]oleic acid (20 μCi), to radiolabel TAG. Cell suspensions were incubated for up to 120 min in 50 ml siliconized flasks maintained at 37°C in a shaking incubator. The flasks were gassed (95% O_2/5% CO_2) at 15-min intervals. In some experiments, enterocytes were pulsed with [^{35}S]methionine (100 μCi) for 30 min, isolated by centrifugation (800 *g* for 2 min), and reincubated in DMEM containing unlabeled methionine (40 mm) to follow the fate of newly synthesized apoB-48 (14–16). At the end of all incubations, enterocytes were isolated by centrifugation at 800 *g* for 2 min. The cell pellet and supernatants were then processed for analysis of cellular and secreted apoB-48 and lipid.

Isolation of chylomicrons

Two different centrifugation techniques were used to isolate chylomicron-density particles from the incubation media of enterocytes. In the first method, chylomicrons were floated by centrifugation of the total medium at 13,000 *g* for 20 min at 16°C. In the second method, Optiprep™ (60% iodixanol) was added to the media to give a final concentration of 12.5% iodixanol (18). This was layered onto a cushion of 30% iodixanol (w/v) in HEPES-buffered saline (137 mm NaCl, 60 mm HEPES:K; pH 7.4), overlaid with Tris-buffered saline (150 mm NaCl: 10 mm Tris-HCl, pH 7.4) and centrifuged at 330,000 *g* at 16°C for 3 h in the Beckman VTi 65.1 rotor (18). The gradients were collected by tube puncture in five fractions (18).

Analysis of apoB-48

Secreted apoB-48 was recovered from the media by adsorption onto PHM-Liposorb, as previously described for apoB-100 (14–16), and from chylomicron-containing fractions using Vivaspin microconcentrator tubes (100 K mol/wt cut-off). Incorporation of [^{35}S]methionine into cellular and secreted apoB-48 was determined as described previously for apoB-100, with the modification that the radiolabel in the apoB-48-containing bands on SDS-PAGE gels was quantified using a Packard InstantImager (two dimensional counter) (14–16).

Analysis of triacylglycerol

Cellular and secreted lipids were extracted and separated by HPTLC, as described previously (15, 19). Quantitation of ^{3}H radioactivity in TAG was performed by scintillation counting. TAG mass was determined by laser densitometry as described previously (15, 19).

Immunoblotting of apoB

Cellular and secreted apoB-48 was detected by immunoblotting, as described previously for apoB-100, using an anti-rabbit monoclonal antibody (MAC31) that recognizes the N-terminal 48% of apoB-100 (12, 20).

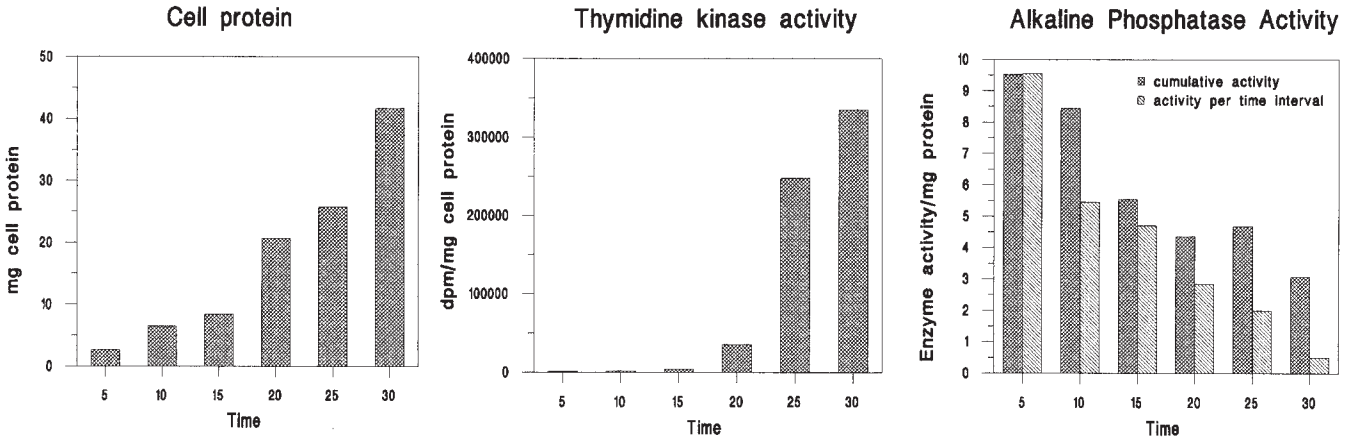

Fig. 2. Release of intestinal cells from villi and crypt. Segments of small intestine were treated as in Fig. 1. The times of incubation with solution C were 5, 10, 15, 20, 25, 30 min. The cells released after each time point were pelleted by centrifugation. Protein, alkaline phosphatase activity (phosphate released/min/mg cell protein), and thymidine kinase activity (dpm [^{3}H]thymidine incorporated/min/mg cell protein) were determined. The mg of cell protein and the specific activity of thymidine kinase are plotted against time of incubation. The specific activity of alkaline phosphatase in cells released at each time point (cumulative activity) and the calculated specific activity of cells released at each 5-min time interval are also plotted. Results plotted are from a typical experiment performed in duplicate.

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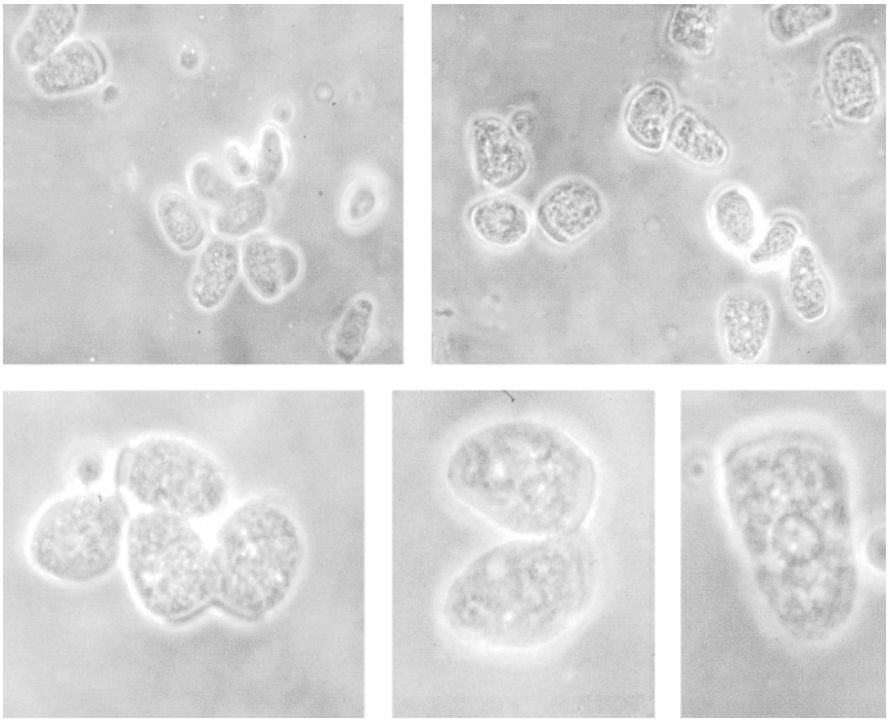

Fig. 3. Morphology of enterocytes isolated after 10 min incubation in solution C. Enterocytes were isolated as described in Methods and viewed using a Zeiss light microscope. A collage of images is presented: top, ×200; lower, ×300–×500.

Assay of thymidine kinase

The activity of thymidine kinase (a marker for crypt cells) was determined as follows. Aliquots (0.5 ml) of cells (0.5 mg of protein in 10 mm Tris-HCl, 150 mm NaCl, pH 7.4) were incubated for 30 min at 37°C in 0.5 ml of assay buffer containing 90 mm Tris-HCl (pH 7.5), 20 mm ATP, 20 mm phosphocreatine, 10 mm $MgCl_2$, 10 mm NaF, 2 mm dithiothreitol, 1 mm thymidine (containing 4 μCi of [^{3}H]thymidine) and 20 μg of creatine phosphokinase. After 30 min, 50-μl aliquots of the reaction mixture were removed and mixed with 1.0 ml of 100 mm $LaCl_3$/5 mm triethanolamine followed by incubation at 4°C for 30 min. Samples were then centrifuged at 15,000 *g* for 20 min at 20°C. Supernatants were removed and the pellets were washed by resuspension in 1 ml of 100 mm $LaCl_3$/5 mm triethanolamine followed by centrifugation as above. The pellets were resuspended in 0.5 ml of 0.1 m HCl/0.2 m KCl and mixed with 5 ml of scintillation fluid. [^{3}H]thymidine phosphate generated over 30 min was determined by counting.

Assay of alkaline phosphatase

The activity of alkaline phosphatase (a marker for differentiated enterocytes) was determined using a kit purchased from Sigma.

RESULTS

Isolation of viable enterocytes

The protocol developed for isolation of epithelial cells from rabbit small intestine was based on that described by Weiser (21) for preparation of enterocytes from rat intestine. In order to maintain the viability of the isolated rabbit enterocytes, we found that it was necessary to use oxygen-

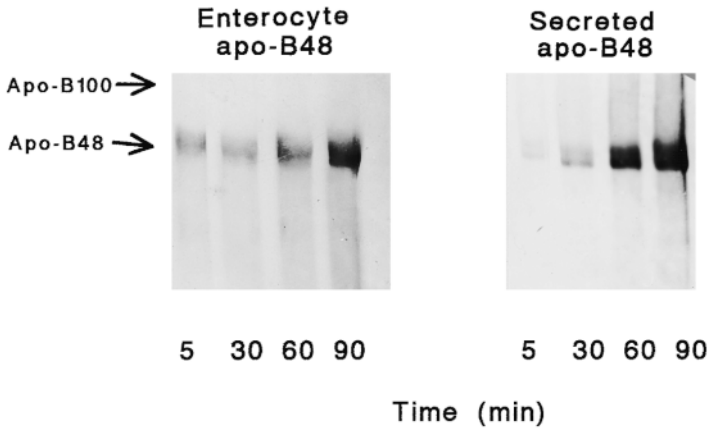

Fig. 4. ApoB-48 in isolated enterocytes detected by immunoblotting. Enterocytes were incubated with micelles for a range of times as described in Methods. The cells and media were separated by centrifugation. The media were concentrated, and both cells and media were dissolved in sample buffer and separated by SDS-PAGE as described in Methods. The proteins were transferred to nitrocellulose and apoB was detected by immunoblotting using a monoclonal antibody (MAC31) that recognizes the N-terminal of apoB (20). ApoB-100 and apoB-48 prepared from plasma as previously reported (13) were used as standards.

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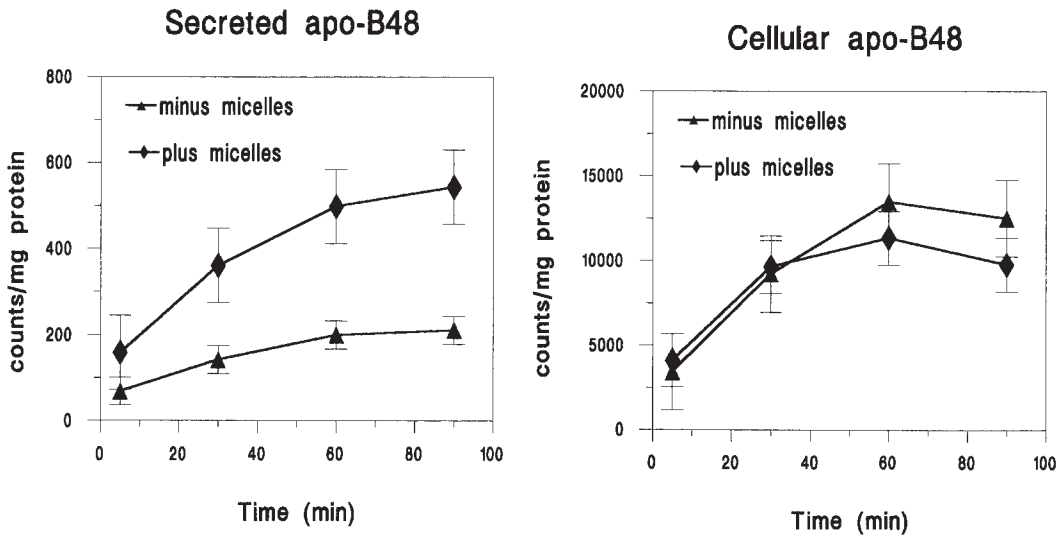

Fig. 5. Effect of addition of micelles on the synthesis and secretion of apoB-48 by isolated enterocytes. Isolated enterocytes were incubated with [^{35}S]methionine with and without addition of micelles. Enterocytes were isolated by centrifugation and the radiolabel in apoB-48 was determined as described in Methods. Results plotted are counts measured per mg of cell protein in three experiments ± SEM.

ated and osmotically balanced solutions containing glucose during the isolation procedure, and to resuspend the isolated cells in oxygenated DMEM. Using these modifications, the preparation of isolated villus enterocytes was homogeneous and the cells were viable as judged by trypan blue exclusion and the ability to incorporate [^{35}S]methionine into proteins during the experimental incubations.

Isolation of epithelial cells from villi and crypts

The number of cells released from the small intestine increased with the time of incubation with solution C (**Fig. 2**). After 30 min, approximately 45 mg of cell protein was released from each 20-cm intestinal segment. Twenty percent of the cells was released during the first 15-min incubation and this contained >80% of the alkaline phosphatase activity, which is characteristic of villus cells, but had an almost undetectable thymidine kinase activity, a marker for dividing crypt cells. In contrast, 75% of the recovered thymidine kinase was in cells released between 25 and 30 min incubation with solution C. These results suggest that the intestinal epithelial cells are released in a gradient from villus to crypt and that 10–15 min is the op-

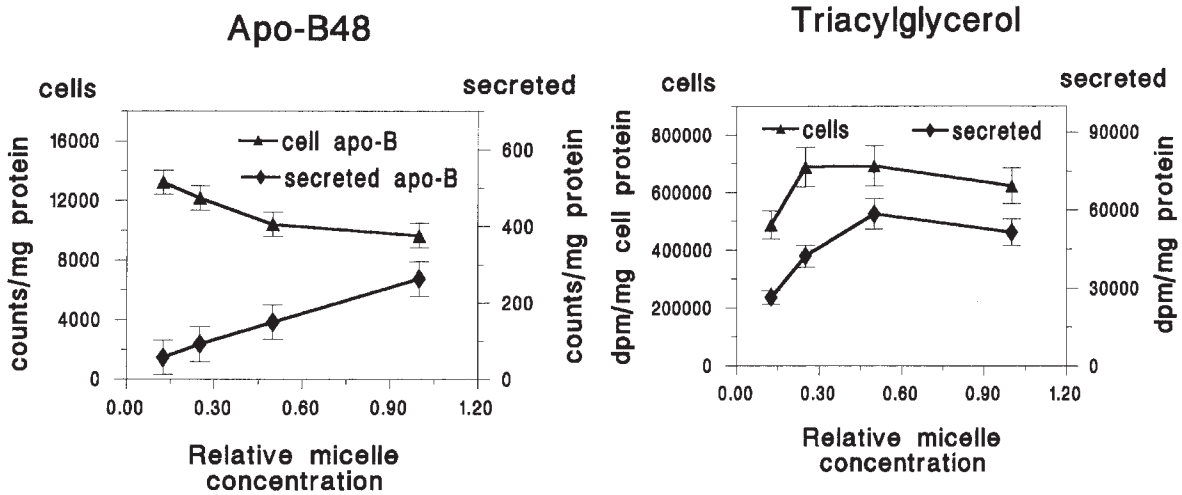

Fig. 6. Effect of micelle concentration on the synthesis and secretion of apoB-48 and TAG by isolated enterocytes. Isolated enterocytes were incubated for 30 min with a range of micelle concentrations and with [^{35}S]methionine or [^{3}H]oleic acid as described in Methods. The enterocytes were isolated by centrifugation and the media were concentrated. The incorporation of each radiolabel into apoB-48 and into TAG was determined as described in Methods. The highest concentration of micelles used is equivalent to 1.5-fold the standard concentration described in Methods. Results plotted are counts (for [^{35}S]methionine determined by two-dimensional counting) and dpm (for [^{3}H]oleic acid determined by scintillation counting) per mg of cell protein for three determinations ± SEM.

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timum time for preparation of villus enterocytes. This incubation time was used in all subsequent preparations.

The isolated enterocytes exhibited the typical morphology of absorptive cells (**Fig. 3**). The cells were columnar and showed well-defined brush borders. Overall, the preparation was uniform consisting of separate cells and clusters of two or more cells.

Isolated enterocytes synthesize and secrete apoB-48 and TAG in particles of the density of chylomicrons

Isolated enterocytes contained and secreted immunodetectable apoB-48, with no evidence of the larger isoform apoB-100 (**Fig. 4**). During the 90-min incubation period, the immunodetectable apoB-48 increased in the cells and at the same time appeared in the incubation medium (Fig. 4). Incorporation of [^{35}S]methionine into cellular apoB-48 was linear for about 60 min and then fell slightly. The rate of synthesis of apoB-48 was unaffected by addition of micelles; however, the final level of incorporation of [^{35}S]methionine into apoB-48 was decreased by approximately 20% (P = 0.05) (**Fig. 5** and **Fig. 6**). Secretion of radiolabeled apoB-48 was significantly increased by addition of micelles ($P <$ 0.001 after 120 min incubation) (Figs. 5 and 6). However, during the 120 min incubation period, only a small fraction (<5%) of the newly synthesized cellular apoB-48 was secreted, suggesting that transit of apoB-48 through the secretory pathway is slow and/or that there may be a pool of newly synthesized intracellular apoB-48 that is not secreted.

Incorporation of [^{3}H]oleate into cellular TAG was very rapid, reaching more than 60% of the final level in the few minutes needed to separate the cells and media after addition of micelles (**Fig. 7**, 0 min incubation), and was complete within 30 min (Fig. 7). Secretion of radiolabeled TAG exhibited a lag of about 30 min followed by an increase for the remaining incubation time (Fig. 7). Only a small fraction (<5%) of the total cellular radiolabeled TAG was secreted after 90 min incubation. As the radiolabelled oleate is incorporated into micelles, it was not possible to measure synthesis and secretion of TAG without added micelles. However, incorporation of [^{3}H]oleate into cell and secreted TAG was stimulated with increased micelle concentration, reaching a plateau with micelles at half the standard concentration (Fig. 6). The cellular mass of TAG remained relatively constant and the secreted mass of TAG increased after a 30-min lag to approximately 35% of that of the cells, indicating net synthesis (Fig. 7). The specific activity of the cellular TAG was more than 10-fold higher than that secreted, suggesting that newly synthesized TAG does not move immediately into the secretion pathway. From the specific activities of the cell and secreted TAG, it also appears that although there is a relatively large net synthesis of TAG during the incubation period, this is not all formed from the radiolabeled oleate added.

More than 90% of the radiolabeled apoB-48 and TAG secreted by enterocytes incubated with micelles floated to the top of iodixanol gradients to the density of chylomicrons and VLDL (**Fig. 8**). When the total medium was centrifuged at 12,000 *g* for 20 min, the conditions for isolation of chylomicrons from plasma, 90% of the radiolabeled apoB-48 and TAG was recovered in the top 1 ml. The small amount of apoB-48 secreted by enterocytes incubated without added micelles was also in particles of chylomicron density.

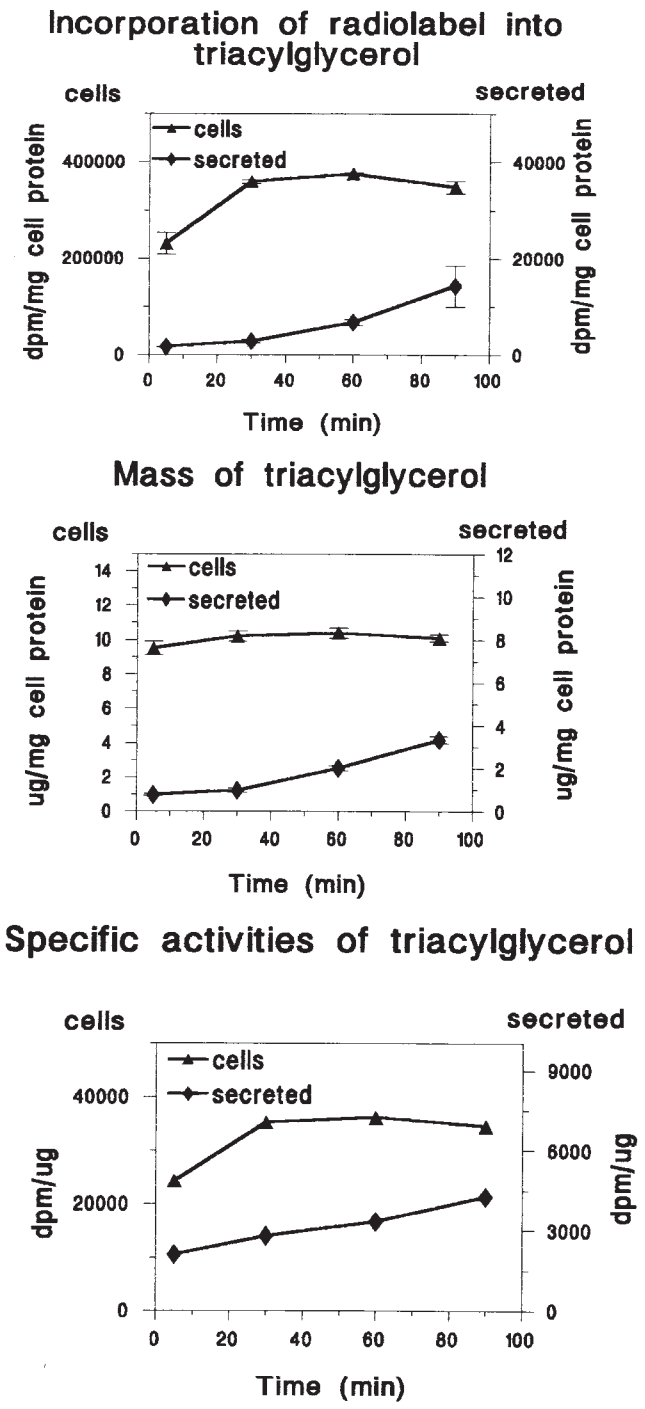

Fig. 7. Synthesis and secretion of TAG by isolated enterocytes. Isolated enterocytes were incubated for a range of times with micelles containing [^{3}H]oleic acid. The cells were isolated by centrifugation, and the lipids were extracted from both cells and media and separated by HPTLC. The incorporation of [^{3}H]oleic acid into cellular and secreted TAG, the mass of TAG, and the calculated specific activities of the TAG per mg of cell protein are plotted against incubation time.

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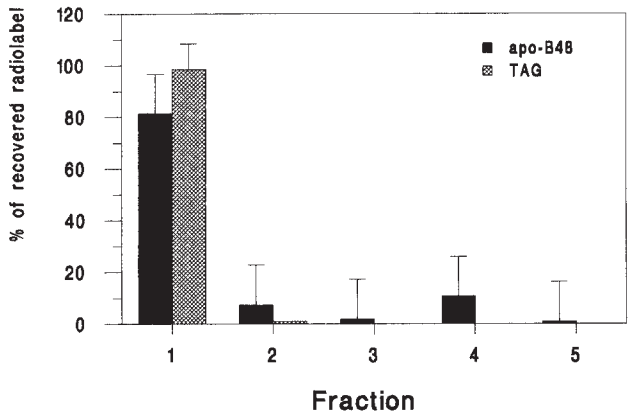

Fig. 8. Density of radiolabeled apoB and TAG secreted by isolated enterocytes. Isolated enterocytes were incubated with micelles containing [^{3}H]oleic acid with [^{35}S]methionine for 30 min. The media were collected, adjusted to 12.5% iodixanol, and centrifuged as described in Methods. Five fractions were collected from each gradient: 1 is the top fraction and 5 the bottom fraction. The radiolabeled apoB and the radiolabeled TAG in each fraction were determined. The results plotted are the % distribution of the recovered radiolabel in each fraction. The total counts in apoB-48 were 8,000–110,000 and in TAG were 100,000–140,000 dpm.

Newly synthesized intracellular apoB-48 is degraded and this is inhibited by addition of micelles

To follow the fate of newly synthesized intracellular apoB-48, enterocytes were pulse-labeled with [^{35}S]methionine and chased in the presence of a large excess of unlabeled methionine. Intracellular apoB-48 was lost at a rate that could not be accounted for by secretion, indicating that intracellular degradation of apoB took place (**Fig. 9**). Addition of micelles to the incubation medium significantly inhibited intracellular degradation and stimulated secretion of apoB-48 ($P < 0.001$) (Fig. 9).

DISCUSSION

The aim of the present study was to develop a protocol for the isolation and maintenance of viable enterocytes and to demonstrate that the cells secrete chylomicrons. The enterocytes isolated using the protocol described synthesized apoB-48, the essential structural apolipoprotein of chylomicrons, and TAG, the major lipid, and secreted these components in particles of the density of chylomicrons. The procedure also offers a method for isolation of cells derived from the villi/crypt gradient which will allow studies of the differentiation of enterocytes.

[^{3}H]oleic acid was rapidly esterified by isolated enterocytes. A similar finding was reported by Mansbach and Nevin (22), from in vivo studies of rats infused via a duodenum cannula with radiolabeled oleate or triolein. Thus, the enterocyte rapidly converts potentially damaging fatty acids to inert TAG. The newly synthesized TAG was secreted only slowly by isolated enterocytes with a lag of ~30 min. A similar delay occurs in vivo (22). During the 120 min incubation period, only about 5% of the newly synthesized apoB-48 and TAG was secreted by isolated enterocytes. However, this is not because the cells have a low secretory ability. The mass of TAG synthesized and secreted during the incubation period was ~4 μg/mg cell protein and equivalent to about 40% of the cellular TAG, which remained relatively constant. These observations demonstrate that there is more than one intracellular pool of TAG in enterocytes and the pools are not in equilibrium. Newly synthesized TAG is preferentially retained in the cells at least during the 120-min

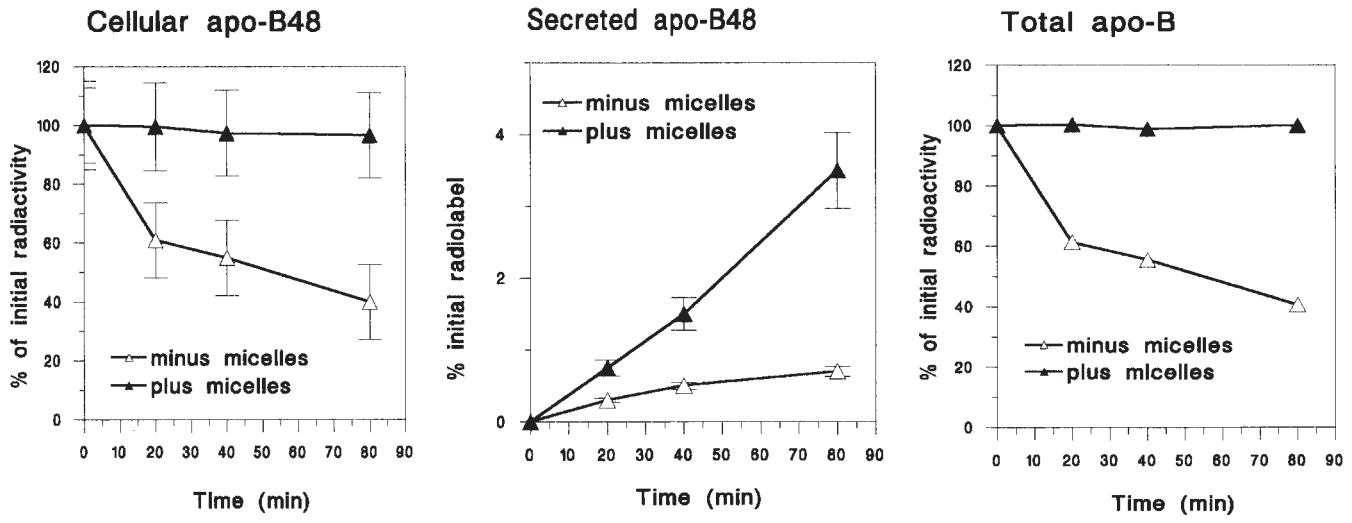

Fig. 9. Fate of radiolabeled apoB-48 in enterocytes. Isolated enterocytes were incubated with [^{35}S]methionine for 30 min without added micelles as described in Methods. The cells were isolated by centrifugation and reincubated with and without added micelles, with an excess of unlabeled methionine, as described in Methods. The cells were isolated by centrifugation and the radiolabeled cellular and secreted apoB-48 were determined as described in Methods. To allow comparison between different experiments, the results are plotted as % change in radiolabeled apoB with 100% being the initial counts, which were between 6,000 and 8,000. The dpm in cellular apoB, secreted apoB, and the sum of the two values are plotted.

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incubation period we used. These results are in contrast to the results reported by Mansbach and Nevin (22), who found that the specific activity of TAG in the ER isolated from rat intestinal mucosa, after labeling in vivo, was less than that of TAG in the Golgi, suggesting that newly synthesized ER-TAG is preferentially channeled into secretion. The most probable explanation for this discrepancy lies in the markedly different experimental protocols. Mansbach and Nevin (22) infused lipid in vivo into the duodenum of rats for 6 h followed by a bolus of radiolabeled triolein. They then scraped the mucosa and prepared subcellular fractions for analysis. Their ER and Golgi fractions were, therefore, from the total mucosal cell population. We prepared villus enterocytes from rabbits fed a low fat diet and these were incubated with micelles for 120 min. The total cellular TAG including cytosolic stores was extracted. The kinetics of intracellular TAG movement are undoubtedly complex and may also be affected by background diet and also differ between regions of the villi and crypt.

Studies of human lipid absorption have shown that about 4–6 h after feeding a high fat meal, chylomicron TAG and apoB-48 peak in the plasma and then fall to premeal levels (23, 24). However, if a further high fat meal is given within about 6 h, there is a small peak in the chylomicrons appearing in the plasma after about 1 h and before the main post-prandial peak. The chylomicron lipids in the initial rapidly appearing peak have the same fatty acid composition as the earlier meal (24) suggesting that TAG is retained in the lacteals or in the enterocytes, and that the second fat challenge moves this pool of lipid into the plasma chylomicrons. In the present study, our observation, that stimulation of enterocytes with micelles increases secretion of TAG of low specific activity compared with the cellular TAG, suggests that secreted TAG comes initially from a cellular pool and not directly from the micelles provided. The observation that isolated enterocytes secrete newly synthesized apoB-48 in particles of chylomicron density, albeit at a low rate, in the absence of added micelles also suggests that intracellular triacylglycerol is used for chylomicron assembly.

In hepatic cells, secretion of VLDL is driven by the availability of lipid. ApoB is synthesized constitutively and in excess, and the protein not assembled into VLDL is degraded intracellularly (6–8). Provision of lipid inhibits intracellular degradation and stimulates secretion of apoB; however, only a small fraction of newly synthesized apoB is secreted by hepatic cells. In the present investigation, we have shown that in enterocytes apoB-48 is synthesized in large excess of that secreted and is degraded intracellularly. Degradation is inhibited by provision of lipid in the form of micelles, which stimulate chylomicron secretion, but, as in the case of hepatic cells, only a small fraction of newly synthesized apoB is secreted. In hamster and human intestine, mRNA levels for apoB do not increase in response to a fat-enriched diet, suggesting that apoB is synthesized constitutively (25, 26). Thus, the basic events in chylomicron assembly appear to be similar to those in VLDL assembly. A mechanism in which apoB-48 is synthesized in excess and available for chylomicron assembly would enable the small intestinal enterocytes to respond to the large fluctuations in dietary lipid, which must be absorbed. However, chylomicrons are considerably larger than VLDL and contain apoB-48 exclusively. The molecular details of chylomicron assembly and their regulation undoubtedly differ from those of VLDL assembly. The availability of isolated adult rabbit enterocytes will provide a good model system for studies of chylomicron assembly and in particular will allow studies of the effect of diet on the process of fat absorption.

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