Intestinal lipids and lipoproteins in the human fetus: modulation by epidermal growth factor

Emile Levy,[1] Louise Thibault, and Daniel Ménard

Departments of Nutrition, Centre de Recherche, Hôpital Ste-Justine, Université de Montréal, and Groupe de recherche en biologie de développement, Département d'anatomie et de biologie cellulaire, Faculté de Médecine, Université de Sherbrooke, Québec, Canada

Abstract The aim of the present investigation was first, to examine the ability of human fetal intestine (17–20 wk) to incorporate fatty acid into esterified lipids; and second, to study in vitro lipoprotein synthesis and secretion by fetal explants, as well as the effect of epidermal growth factor (EGF) on these processes. Cultured fetal jejunal explants were incubated in Leibovitz medium for 42 h with [^{14}C]oleate. Both triglycerides (TG) and phospholipids (PL) were the major labeled products. Whereas TG were predominant (80%) in the culture medium, PL accounted for more than 50% of total tissue lipids. More than 60% of the radioactivity in PL was associated with phosphatidylcholine. Some labeling (<5%) was also recovered in the cholesteryl ester fraction. Active exocytosis was demonstrated by the accumulation of newly synthesized esterified lipids in the medium and the presence of lipoproteins in the basolateral membrane region and intercellular spaces. Most of the newly synthesized lipids were found in lipoproteins of d < 0.97 g/ml (51.2%) and d < 1.21 g/ml (39.3%), whereas the rest were recovered in d < 1.006 g/ml (9.8%) and 1.063 g/ml (5.6%). A similar trend characterized the lipoprotein secretion. The synthesis of the d < 0.97 g/ml fraction (30,653 ± 4,122 dpm/mg protein) was significantly greater than the 1.006 g/ml fraction (5,897 ± 1,734), $P < 0.005$. The secretion of d < 0.97 g/ml particles into the medium was also five fold higher than that of the d < 1.006 g/ml fraction ($P < 0.01$). The addition of EGF to the culture medium (25, 50, and 100 ng/ml) significantly enhanced the d < 0.97 g/ml lipoprotein secretion (25–40%) and decreased the d 1.006 g/ml and 1.063 g/ml fraction output. The lipid composition of these lipoprotein fractions was never altered by the presence of EGF, suggesting that the number of lipoprotein particles, rather than size, was modified by the growth factor. The present findings provide the first evidence that the human fetal intestine has the capacity to elaborate lipoprotein fractions for the transport of newly synthesized lipids. Furthermore, our data suggest that EGF, present in significant quantity in saliva, amniotic fluid, and bile, can modulate the release of TG-rich lipoproteins by fetal intestinal explants. **—Levy, E., L. Thibault, and D. Ménard.** Intestinal lipids and lipoproteins in the human fetus: modulation by epidermal growth factor. *J. Lipid Res.* 1992. **33:** 1607–1617.

Supplementary key words jejunal explants in culture • lipid esterification • lipoprotein secretion

Dietary lipids represent a major portion of the total daily calory intake of humans, and play an important role in the nutritional and physiological processes of the body. The intestinal absorption of long-chain fatty acid triglycerides (TG) is assured by two sequential processes: intraluminal and intracellular phases leading to the formation of chylomicrons (CM) (1, 2). When the rate of TG transport is low, as in the fasting state, the small gut becomes a considerable source of very low density lipoproteins (VLDL) (3).

In addition to the production of TG-rich lipoproteins, the enterocyte secretes nascent high density lipoproteins (HDL), consisting of spherical as well as disc-shaped particles (4, 5).

If this lipoprotein-lipid transport system, providing efficient mechanisms for moving large amounts of luminal fatty acids into the blood circulation, is well documented in the adult, it still remains largely unknown in the fetus and in the newborn. With a morphological approach, VLDL particles were visible within the endoplasmic reticulum, the Golgi apparatus of the absorptive cell as well as the intercellular spaces, the lamina propria, and the lumen of lymphatic lacteals (6). Mak and Trier (6) postulated that the VLDL particles might serve as precursors for CM formation during absorption of dietary fat upon initiation of suckling. However, available data in suckling rats emphasized that during infusion of lipid load, luminal fatty acids were incorporated into TG carried by VLDL alone, implying that CM are not involved as carriers of lipids from intestinal absorption (7). The conclusions that can be drawn at this time are that the intracellular metabolic mechanisms required for VLDL production are already present in rat jejunal epithelial cells at birth, but these are largely based on morphologic evidence. It re-

Abbreviations: CE, cholesteryl esters; CM, chylomicrons; DG, diglyceride; HDL, high density lipoproteins; EGF, epidermal growth factor; MG, monoglyceride; M/T, medium/tissue ratio; PL, phospholipid; TG, triglyceride; TLC, thin-layer chromatography.

[1]To whom correspondence should be addressed at: GI-Nutrition Unit, Ste-Justine Hospital, 3175 Ste-Catherine Road, Montreal, Québec, Canada, H3T 1C5.

mains to determine whether the developing intestine is able to serve as an important source of CM and HDL as in adults.

The paucity of information regarding both the developmental aspect of intestinal lipoproteins and their regulation is partly due to the complexity of the processes involved and the lack of appropriate experimental models. Furthermore, there are no studies on human material. The only available date concerning these processes during perinatal development pertain to animals.

Contrary to our meager knowledge of developmental lipoproteins, several reports have firmly stressed the intestinal synthesis of human apolipoproteins in human fetuses (8, 9) and suckling animals (10). It is therefore possible that the presence of the latter may reflect an active synthesis and secretion of lipoproteins. Another unanswered question relates to the physiologic regulation of lipoprotein synthesis and secretion. On the basis of available information, hormones appear to play a key role in the modulation of apolipoprotein synthesis in adults (11, 12). Surprisingly, many of the factors that serve a regulatory role in control of fetal enterocyte maturation and differentiation (13) have not been investigated. Epidermal growth factor (EGF) is one of the best characterized growth factors that produces a variety of biologic responses, most of which involve enhanced proliferation or differentiation, or both, of epidermal as well as epithelial tissues (14). Its presence in many biologic fluids such as amniotic fluids, milk, and saliva has led us to postulate a role for EGF either in the development of the gastrointestinal tract or in the functional maintenance of the adult gastrointestinal tract tissues (15, 16). Over recent years, the developing human gut has been shown to possess specific EGF receptors (17, 18). Furthermore, the successful morphologic and physiologic maintenance of human intestinal tissue in serum-free organ culture up to 5 days (19) permitted the study of the biological effects of EGF in human fetal jejunum (20). The presence of EGF receptors together with the known biological effects of EGF in both fetal small intestine and colon suggest an important role for EGF in human prenatal gastrointestinal maturation. In this report, we have studied the synthesis and secretion of the typical lipoprotein classes associated with the human fetal intestine. Using the organ culture technique it has been possible to demonstrate direct EGF regulation of the intracellular events governing the intestinal formation and release of lipoproteins.

MATERIALS AND METHODS

Specimens

Tissues from six fetuses ranging from 17 to 20 weeks in age were obtained after legal abortion by suction curettage, for medical-social indications. Studies were approved by the appropriate Human Subject Review Committee. The entire small intestine was immersed in Leibovitz-L15 medium (room temperature) containing garamycin (40 μg/ml) and mycostatin (40 μg/ml). The proximal half of the intestine excluding the first 3 cm was used and defined as jejunum. The time required to bring the tissue to the culture room after abortion and to place it in culture never exceeded 30 min.

Organ culture

The jejunum was cleansed of mesentery, split longitudinally, washed in culture medium, and cut into explants (3 × 7 mm). Five to seven explants were randomly transferred onto lens paper with the mucosal side facing up in each organ culture dish (Falcon Plastics, Los Angeles, CA). Six dishes were used for each experimental condition. An amount of medium (0.8 ml) sufficient to dampen the lens paper was added. Explants were cultured in serum-free Leibovitz L-15 medium according to the technique described previously (19, 20). After a 3-h stabilization period, the medium was changed with a fresh one containing a final amount of 0.13 μmol/ml of nonlabeled oleic acid with 0.3 μCi of [^{14}C]oleic acid attached to albumin (sp act 53.9 mCi/mmol, Amersham, Montreal, Quebec, Canada). The preparation of oleate/albumin complex was prepared according to the method of Fielding et al. (21). EGF (Collaborative Research, Waltham, MA) dissolved in redistilled water was added at concentrations of 25, 50, and 100 ng/ml. Intestinal explants were cultured for 42 h.

Light and electron microscopy

For morphological studies, explants freshly prepared from human fetal intestine, after a 42-h incubation period, were fixed in ice-cold 2.8% glutaraldehyde buffered with 0.1 M sodium cacodylate for 60 min. After being rinsed with cacodylate buffer, the tissues were postfixed in 2% osmium tetroxide/0.1 M cacodylate for 30 min, dehydrated, and embedded in Epon. One-micrometer sections were stained with a mixture of 1% methylene blue, 1% azure II, and 1% borax for high resolution light microscopy. Ultra-thin sections were stained with uranyl acetate followed by lead citrate and examined under a Philips EM-300 electron microscope.

Lipid carrier

Blood was drawn 3 h after the oral intake of 50 g of fat per 1.72 m^2 (flavored commercial cream) and postprandial plasma was prepared in order to serve as a carrier for the lipoproteins synthesized by the organ culture. The CM-enriched plasma was obtained after an oral fat loading as described previously (22), and was incubated at 56°C for 1 h to inactivate enzymatic activity.

Isolation of lipoproteins

After incubation, the explants were lightly homogenized (3 strokes) in a solution of saline containing antibacterial and antiprotease agents (sodium azide 0.01%, EDTA 0.1%, and Trasylol 10.000 Ik/ml). The medium and the homogenate were mixed with the plasma lipid carrier (2:1, v/v), and the lipoproteins were isolated by sequential ultracentrifugation using the newly available TL-100 ultracentrifuge (Beckman Instruments, Inc., Montreal, Quebec, Canada). We adapted the ultracentrifugation method of the Lipid Research Clinics Program for the separation of lipid subfractions (VLDL, LDL, and HDL) to this tabletop ultracentrifuge which provides a fast and convenient method for specimens with small volume. Excellent results were obtained for triglycerides and cholesterol contained in CM, VLDL, LDL, and HDL when compared with those from a L5-65 ultracentrifuge (Beckman Instruments). Furthermore, no contamination was detected in lipoprotein fractions when labeled lipoproteins were centrifuged and examined by agarose gel electrophoresis. Briefly, after the removal of 0.97 g/ml fraction by short centrifugation in a TLS 55 rotor (20,000 rpm for 20 min), lipoproteins of $d < 1.006$ g/ml and $d < 1.063$ g/ml were separated by spinning at 100,000 *g* for 2.26 h with TL 100.3 at 5°C. The high density fraction was obtained by adjusting the 1.063 g/ml infranatant to density 1.21 g/ml and by centrifuging for 6.30 h at 100,000 rpm. Each lipoprotein fraction was exhaustively dialyzed against 0.15 M NaCl, 0.001 M EDTA, pH 7.0, at 4°C for 24 h.

Agarose column chromatography

[^{14}C]oleate and [^{3}H]leucine were added to the culture medium of jejunal explants which were incubated for 42 h. The conditioned media and homogenates were adjusted to density 1.21 g/ml with KBr and ultracentrifuged at 4°C as detailed above. The top fraction of $d < 1.21$ g/ml was concentrated, dialyzed, and applied to a 1.0 × 70 cm column of Bio-Gel A-5m (Bio-Rad) previously calibrated with small amounts of human lipoprotein fractions (CM, $d < 0.96$ g/ml; VLDL, $d < 1.006$ g/ml; LDL, $d < 1.063$ g/ml; HDL, $d < 1.21$ g/ml) and eluted with 150 mM NaCl, 10 mM Tris-HCl, pH 8.0. Fractions of 1.0 ml were collected.

Analyses

Aliquots of explant homogenates and their respective incubation media were lipid-extracted with chloroform–methanol 2:1 (v/v) (23). Small amounts of lipid standards were added to the samples before separation of individual lipid classes by one-dimensional thin-layer chromatography (TLC) (silica gel, Eastman Kodak Rochester, N.Y.) as described previously (24). The nonpolar solvent system was hexane–diethylether–glacial acetic acid 80:20:3 (v/v/v) and the polar solvent was chloroform–methanol–water–acetic acid 65:25:4:1 (v/v/v/v). The radioactivity of the separated fractions was measured in a Beckman liquid scintillation spectrometer. Quenching was corrected using computerized curves generated with external standards. An aliquot of the tissue homogenate was used for protein determinations (25). The concentration of total lipid was determined by a Boehringer commercial kit (#124 303).

RESULTS

Morphological study

Fig. 1 (a–f) illustrates the general morphology both at light and electron microscopic levels of human fetal jejunal explants at the beginning and after 42 h of culture. The addition of oleic acid during 42 h of culture did not modify the general morphology of the jejunal explants (Fig. 1, a, c). Indeed, the jejunal mucosa exhibited well-developed villi and forming crypts. The epithelial cells lining the villi were tall and contained glycogen. The epithelium of the developing crypts was well preserved and mitotic figures were seen. The mucosal epithelium was always intact and no damaged cells were present. The addition of different concentrations of EGF did not modify the overall intestinal morphology (not shown). Therefore, the morphological aspect of the cultured intestinal explants with oleic acid and/or EGF was quite similar to that observed at the beginning of the culture and is in agreement with previously described results (19, 20). At the ultrastructural level, the morphological integrity of the epithelial cells was always noted after 42 h of culture (Fig. 1, d, e, f) and was quite comparable to that of uncultured explant (Fig. 1, b). The villus absorptive cells were tall with well-defined brush border. No modification of the apical tubular system, endoplasmic reticulum, mitochondria, lysosomes, and Golgi apparatus was recorded. Lipid droplets were observed in the smooth endoplasmic reticulum (Fig. 1, d). In the Golgi area, lipoprotein particles were visible within the cisternae (Fig. 1, e). Golgi-derived vesicles containing lipoproteins were present near the basolateral membrane (Fig. 1, e) and numerous lipoprotein particles were observed in the intercellular spaces (Fig. 1, e and f). Therefore, after the addition of oleic acid to the medium, the intestinal cells synthesized lipoproteins and secreted them into intercellular spaces. The excellent viability of the tissue was also reflected by the significant ($P < 0.05$) increased brush border sucrase activity during the course of the culture (56 ± 7 u) as compared with the uncultured tissue (40 ± 4 u), $P < 0.05$.

Incorporation of [^{14}C]oleic acid and secretion of ^{14}C-labeled lipids

To determine whether fetal intestinal tissue could synthesize and secrete newly formed lipids, jejunal explants

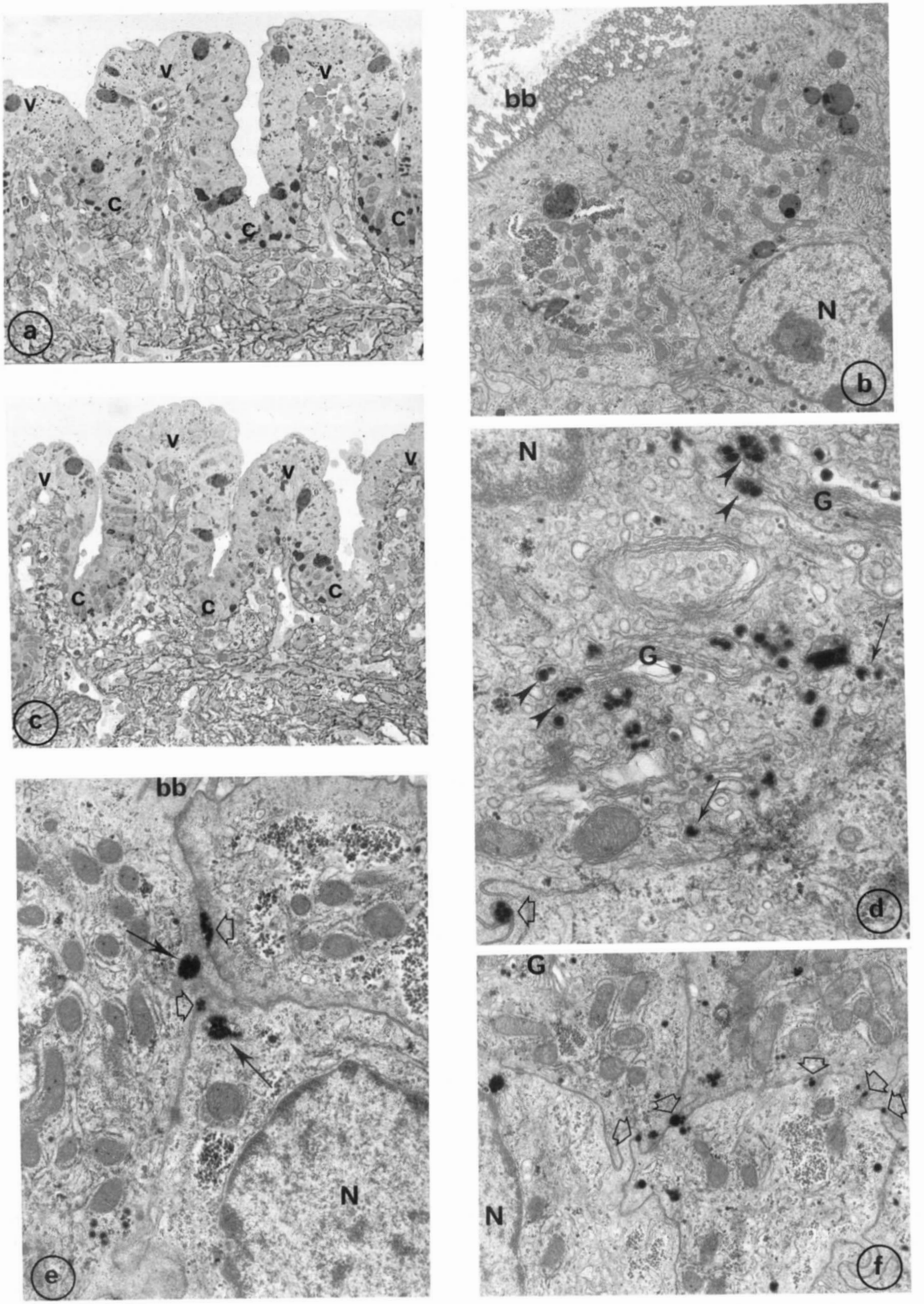

Fig. 1. Morphology of human fetal intestinal explants obtained from the same jejunum (18 wk) before (a, b) and after (c–f) 42 h of culture in presence of [^{14}C]oleic acid. a: Light micrograph of uncultured explant illustrating villi (V) and crypts (c) × 100; b: Electron micrograph of midvillous absorptive cells of uncultured jejunum × 7300; c: Light micrograph showing the good preservation of villi (V) and crypts (C) × 100; d: Electron micrograph of the Golgi area (G) of midvillous absorptive cells. Lipid droplets in smooth endoplasmic reticulum (arrows), lipoprotein particles within the Golgi cisternae (arrow heads), and lipoproteins in the intercellular space (open arrow), n: nucleus × 30,800; e: Electron micrograph of midvillous absorptive cells. Golgi-derived vesicles containing lipoproteins are present near the basolateral membranes (arrows) and lipoprotein particles secreted into intercellular spaces (open arrows), bb: brush border × 18,800; f: Transversal section of many villous absorptive cells. Lipoproteins are present in the intercellular spaces (arrows) × 15,400. (Figures reduced 20% in reproduction.)

were incubated with [^{14}C]oleic acid substrate. The incorporation of [^{14}C]oleic acid into the intestinal tissue was linear (results not shown). In all experiments, the total amount of radiolabeled lipids was higher in the medium than in the tissue, which indicates that an active secretion takes place in intestinal organ culture (**Fig. 2**). Although both triglycerides (TG) and phospholipids (PL) were the major products of [^{14}C]oleic acid incorporation, PL accounted for more than 50% in the explants, while TG were consistently predominant (80%) in culture media. Lesser amounts incorporated into cholesterol esters (CE).

As illustrated in **Fig. 3,** individual PL classes were higher in tissue than in media. However, no differences were noted in the general PL profile. Phosphatidylcholine was the predominant form of ^{14}C-labeled lipids. It was preferentially synthesized by the tissue and secreted into the medium.

Synthesis and secretion of lipoproteins

The separation of lipoproteins from fetal explants is shown in **Table 1.** Lipoproteins of d < 0.97 g/ml were the predominant particles to carry the bulk of [^{14}C]oleic acid. Fractions of d 1.006 g/ml and 1.063 g/ml were less enriched with labeled fatty acid than the 0.97 g/ml fraction and even less than the 1.21 g/ml fraction. A similar pattern characterized the different lipoproteins isolated from the medium. The jejunal explant's distinct ability to secrete lipoproteins into the medium is well expressed by the M/T ratio. Particles of d 0.97 g/ml were evidently the major secreted lipoproteins, followed by d 1.006, 1.21, and 1.063 g/ml particles in terms of decreasing ratio criteria. Although the d 1.21 g/ml fraction was predominant in the tissue homogenate, the d 0.97 g/ml fraction was the most abundant in the medium.

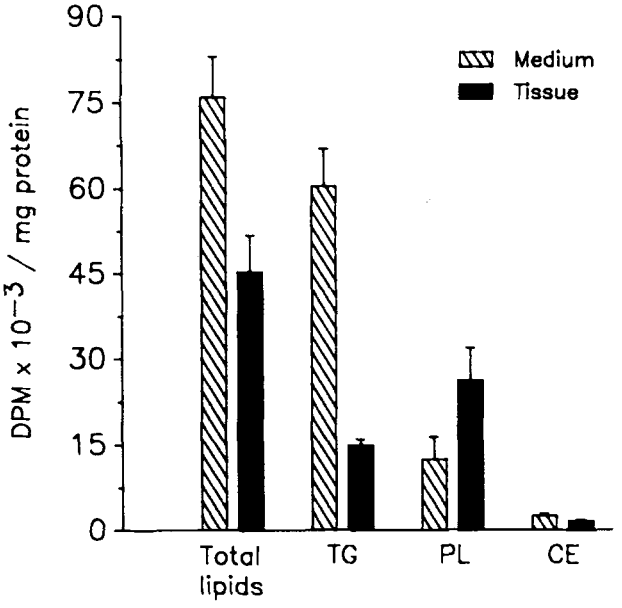

Fig. 2. Radiolabeled lipid content in tissue and medium of intestinal organ culture. Jejunal fetal explants were incubated with [^{14}C]oleic acid substrate for 42 h. Lipids of tissue homogenate and media were then extracted with chloroform–methanol 2:1, isolated by TLC, and quantitated as described in Methods. Values represent means ± SEM of six experiments; TG, triglycerides; PL, phospholipids; CE, cholesteryl esters.

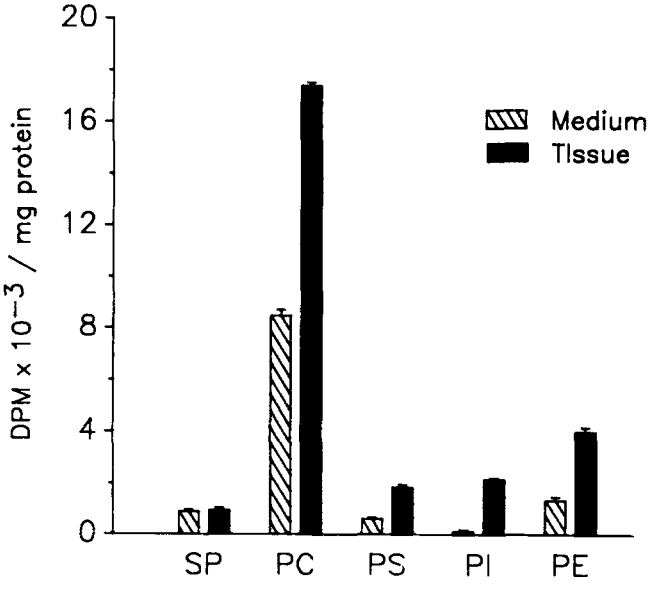

Fig. 3. Incorporation of [^{14}C]oleic acid substrate into phospholipid classes. Lipids from jejunal explants and from the medium were extracted, isolated by TLC, and quantitated; SP, sphingomyelin; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; PE, phosphatidylethanolamine. Values represent means ± SEM of six experiments.

TABLE 1. Synthesis and secretion of lipoproteins

Lipoprotein Fraction	Tissue (T)	Medium (M)	Tissue + Medium (T + M)	M/T
		dpm/mg protein		
0.97 g/ml	2184 ± 477	28469 ± 3879	30653 ± 4122	14.73 ± 2.78
1.006 g/ml	1240 ± 165	4655 ± 1999	5895 ± 1734	4.12 ± 1.43
1.063 g/ml	2046 ± 119	1309 ± 354	3355 ± 393	0.78 ± 0.13
1.21 g/ml	8184 ± 320	11777 ± 2388	19961 ± 1874	1.43 ± 0.29

Intestinal explants were cultured in the presence of [^{14}C]oleic acid. After 42 h incubation, the explants were gently homogenized, and lipoproteins from tissue homogenate (T) and medium (M) were isolated by ultracentrifugation as described in Methods. The sum of radioactivity incorporated into both tissue and medium (T + M) represents the total synthetic activity or production. Values represent means ± SEM of six experiments.

The elution profile of the d < 1.21 g/ml fraction of both [^{14}C]oleate- and [^{3}H]leucine-labeled media is depicted in **Fig. 4.** Peaks of particles, containing labeled protein and lipids corresponding in size to human plasma CM, VLDL, LDL, and HDL were observed. While the majority of the labeled lipids was distributed in the region of chylomicrons + VLDL, most of the labeled protein radioactivity eluted at the HDL region.

When the chromatographic peaks for these lipoproteins were analyzed for their total lipid and protein content, the highest proportion of lipids was found in the d 0.97 g/ml and 1.006 g/ml fractions as expected, whereas the highest proportion of proteins was associated with the d 1.21 g/ml fraction (Fig. 4).

Effects of EGF on synthesis and secretion of lipoproteins

Organ culture enabled us to evaluate the direct effect of EGF on lipoprotein formation and release in the fetal period. All the lipoprotein classes were affected by the presence of EGF (**Fig. 5**). While production of the d 0.97 g/ml fraction was substantially increased by rising concentrations of EGF, its secretion in particular was stimulated. The discrepancy between d 0.97 g/ml fraction synthesis by explants and the ability of the latter to secrete the d 0.97 g/ml fraction becomes even more striking when the relationship between the two is expressed as a ratio. At an intermediate concentration of 50 ng/ml, an increase in secretion of approximately 30% was visible. In contrast to this d 0.97 g/ml fraction stimulation, the addition of EGF to the medium led to a consistent reduction in both the synthesis and secretion of labeled d 1.006 g/ml fraction. As the secretion was more affected than the synthesis, the M/T ratio was markedly lower than that for controls. Jejunal explants cultured with [^{14}C]oleic acid showed only a moderate effect on the d 1.063 g/ml and 1.21 g/ml fractions. There was a trend towards a d 1.21 g/ml secretion decrease at 50 ng/ml without any substantial effect on the M/T ratio. As illustrated by this ratio, the secretion of d 1.063 g/ml particles was markedly diminished by EGF at 100 μg/ml.

Effect of EGF on intestinal lipid and lipoprotein characterization

To determine whether the increasing concentrations of EGF could have altered the lipid and lipoprotein composition, the distribution of the lipid content was examined. While important variations were noted between the lipid composition of tissue and medium in absence of EGF, the addition of this factor, however, had no substantial effect on total lipid distribution (**Table 2**). Similarly, phospholipid composition was not affected by EGF availability under our experimental conditions (**Table 3**).

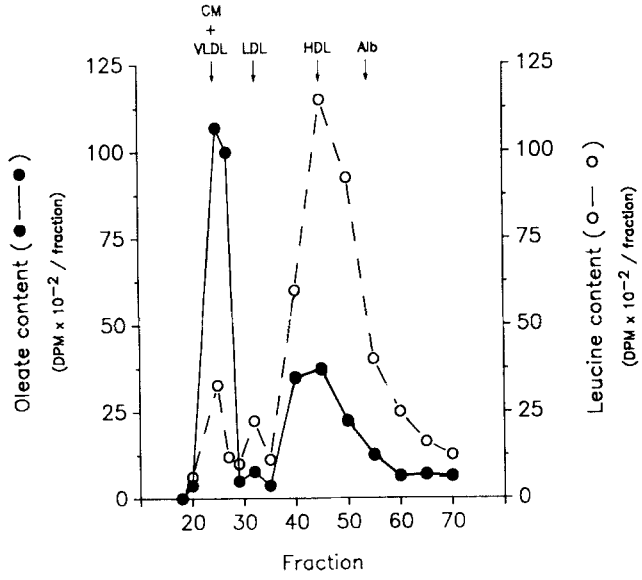

Fig. 4. Profile of newly synthesized lipoproteins secreted by cultured jejunal explants. Intestinal explants were incubated for 42 h in the presence of [^{14}C]oleic acid (closed circles) or [^{3}H]leucine (open circles). The medium was concentrated and applied on agarose column chromatography as described in Materials and Methods. Plasma carrier was omitted in this experiment. For calibration, human lipoprotein fractions isolated by sequential ultracentrifugation were run under identical conditions. When lipoproteins from pooled media of two organ-cultures were isolated by agarose column chromatography, the percent total lipid (95.8, 86.1, 74.7, and 49.1%) and protein (4.2, 13.9, 25.3, and 50.9%) was found in d 0.97, 1.006, 1.063, and 1.21 g/ml fractions, respectively.

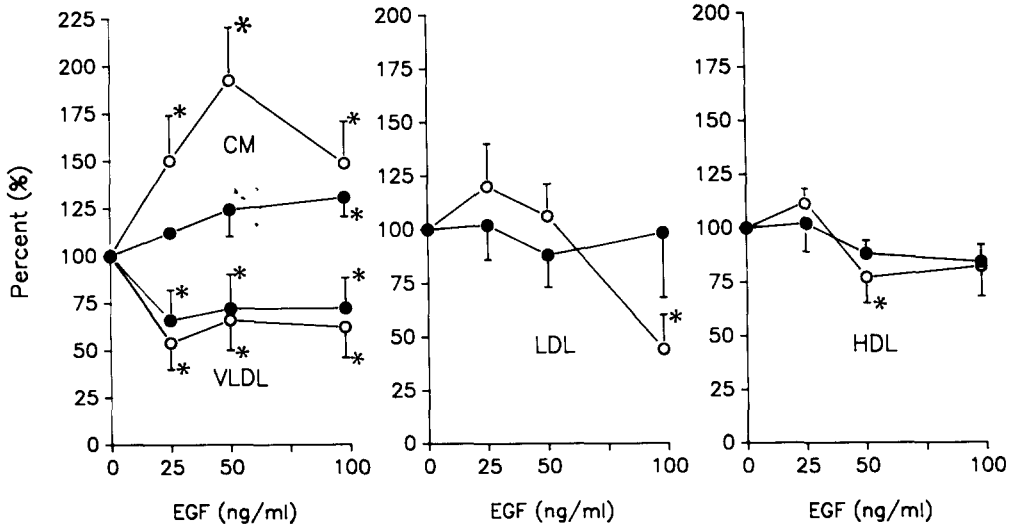

Fig. 5. Effects of EGF on synthesis and secretion of lipoproteins during 42 h of culture. Data were expressed as the percentage of increase or decrease relative to control values (culture without EGF). The closed circles represent the lipoproteins in the medium and the open circles illustrate the medium/tissue ratio in the presence of 25, 50, and 100 ng EGF/ml of medium.

Marked basal differences between tissue and medium lipoprotein lipid constituents were evident (**Table 4**). By comparison with tissue lipid distribution, TG enrichment and PL + MG depletion characterized the particles secreted to the medium. EGF at different concentrations had no particular effect on the whole lipid composition of the four lipoprotein fractions isolated from jejunal explant tissue. Only a slight trend toward TG depletion and PL + MG enrichment was noted in the d 0.97 g/ml lipoproteins isolated from the medium.

DISCUSSION

The first purpose of this work was to investigate the types and properties of lipoprotein particles that are synthesized and secreted by human fetal intestine. The second goal was to examine the effect of EGF on the formation and release processes of lipoproteins. The results reported herein show that the human fetal intestine possesses the capacity to elaborate and secrete four lipoprotein classes, in order to transport newly synthesized lipids. Furthermore, these experiments reveal that EGF causes antagonist modulation in secretion, increasing the d 0.97 g/ml fraction and decreasing the d 1.006 g/ml fraction.

It is obvious that several studies have largely advanced our knowledge of intestinal lipoprotein formation by using mesenteric lymph cannulation (26, 27). Despite the efficacy of this technique, intestinal lymph represents a combination of plasma filtrate from capillaries and material released by the enterocytes. Thus, apolipoproteins

TABLE 2. Lipid composition of jejunal explants and their incubation medium

Sample	EGF Concentration	PL + MG	FC	DG	TG	CE
	ng/ml			*% composition*		
Tissue	0	53.0 ± 4.6	2.4 ± 0.3	1.7 ± 0.3	37.5 ± 3.9	2.7 ± 0.2
	25	60.1 ± 3.3	1.8 ± 0.2	1.7 ± 0.2	33.8 ± 3.3	2.3 ± 0.2
	50	55.8 ± 3.1	2.0 ± 0.3	1.7 ± 0.4	35.5 ± 3.1	2.7 ± 0.2
	100	52.4 ± 4.5	2.0 ± 0.4	1.7 ± 0.2	38.8 ± 4.1	2.6 ± 0.2
Medium	0	17.6 ± 3.3	1.4 ± 0.5	0.9 ± 0.3	80.0 ± 3.9	2.4 ± 0.3
	25	14.8 ± 4.2	1.4 ± 0.4	0.9 ± 0.4	86.6 ± 4.4	2.2 ± 0.3
	50	15.9 ± 3.9	1.4 ± 0.5	0.7 ± 0.3	79.9 ± 4.2	2.3 ± 0.3
	100	14.1 ± 0.3	1.7 ± 0.6	0.8 ± 0.3	81.1 ± 3.8	2.1 ± 0.3

Jejunal explants were incubated with [^{14}C]oleic acid substrate for 42 h in absence or presence of increasing amounts of EGF. Lipids of tissue homogenates and media were then extracted with chloroform–methanol 2:1, isolated by TLC, and quantitated as described in Methods.

TABLE 3. Phospholipid composition of jejunal explants and their incubation medium

Sample	EGF Concentration	Sphingomyelin	Phosphatidylcholine	Phosphatidylserine	Phosphatidylinositol	Phosphatidylethanolamine
	mg/ml			*% composition*		
Homogenate	0	3.2 ± 0.2	66.0 ± 0.4	7.2 ± 0.5	8.2 ± 0.2	15.4 ± 0.4
	25	3.0 ± 0.6	66.0 ± 1.4	6.3 ± 0.6	8.0 ± 0.1	16.7 ± 0.8
	50	4.5 ± 0.5	67.5 ± 1.2	6.5 ± 0.5	7.5 ± 0.5	14.0 ± 1.0
	100	3.1 ± 0.1	67.1 ± 0.1	7.0 ± 0.6	8.1 ± 0.1	14.7 ± 0.3
Medium	0	7.8 ± 0.2	66.4 ± 1.3	4.5 ± 0.6	9.4 ± 0.6	11.9 ± 0.8
	25	7.6 ± 0.9	66.4 ± 0.3	4.5 ± 0.3	9.1 ± 0.9	12.3 ± 0.9
	50	10.5 ± 1.5	64.5 ± 2.5	4.0 ± 1.0	10.0 ± 1.0	11.0 ± 1.0
	100	9.9 ± 2.0	64.0 ± 2.5	4.9 ± 0.6	9.3 ± 0.9	11.9 ± 0.6

Jejunal explants were cultured under experimental conditions described in the legend of Table 4. Individual phospholipids were separated by thin-layer chromatography as described in Methods and counted in order to determine [^{14}C]oleate incorporation.

and denser lipoprotein classes can potentially be derived from circulating plasma through filtration. Moreover, this technique cannot be used either in human studies or, especially, in experiments with fetuses. In the present work, we utilized an organ culture technique that is known to isolate the intestine from various influences. Previously, this approach was also successfully used to clarify the mechanism of intracellular lipoprotein alterations in human disorders (22, 24). In this investigation, we took advantage of newly developed organ culture conditions, i.e., the incubation of the human fetal intestine in a serum-free medium, to avoid the effect of several hormones and growth factors that affect the morphological and functional development of the mucosa (28, 29). Another important aspect of organ culture involves the use of whole tissue instead of individual cells. This manipulation maintains the structural relationship among epithelial cells, basal lamina, and underlying mesenchymal elements.

Little attention has been paid to the lipid composition of human fetal explants in culture. Analysis of [^{14}C]oleic acid incorporation indicated that intracellular products were predominantly PL followed by TG, with a low level of CE content. The medium, however, was enriched with TG. On the other hand, the PL level was only 20% that of TG. It is of interest to notice that CE was barely detectable, which suggests a limited ability of fetal explants to elaborate this lipid fraction, similar to that of Caco-2 cells (30) and liver-derived cell line HepG2 (31, 32). Presumably this finding may reflect the low activity of acyl-CoA:cholesterol acyltransferase (ACAT), whereas the massive [^{14}C]oleic acid-labeled triglyceride in medium is indicative of increased TG-rich lipoprotein export.

Large amounts of lipoproteins were measurable in media and evidenced an active process of secretion. The data fit conveniently with the morphological study (Fig. 1) illustrating the presence of lipoproteins in the basolateral membrane region and in the intercellular spaces. In this context, the mucosal epithelium was always intact and no damaged cells were observed. Moreover, DNA in the medium was undetectable. Furthermore, the fact that there is a differential secretion rate of the four lipoprotein classes and that EGF affects the secretion of very low density particles in opposite ways make a leakage explanation quite unlikely.

It should be pointed out that lipoproteins accumulated in the medium during a 42-h period. However, we can exclude the possibility that these particles underwent modification by lipases, lipid exchange proteins, or ACAT, as the activity of such factors was undetected (results not shown). These particles can therefore be referred to as *nascent lipoproteins*.

The lipoprotein fractions found in the fetal organ culture and isolated at densities of 0.97, 1.006, 1.063, and 1.21 g/ml may correspond to their plasma counterparts, i.e., CM, VLDL, LDL, and HDL, respectively. This was compatible with the elution profile generated by agarose column chromatography and the resulting lipoprotein composition. However, the specific apolipoprotein constituents that conventionally characterize the different lipoprotein classes have not been examined in the present study. Thus, one should be very careful in hastily defining the nascent particles of the fetal intestine by the commonly used terms. Further studies are evidently needed to determine the major apolipoproteins associated with the lipoproteins particles.

In these experiments we were able to demonstrate that fetuses have in place the biochemical machinery necessary to assemble lipoproteins. Earlier, Mak and Trier (6) described the appearance and distribution of particles with morphological features of VLDL in the intestinal mucosa of rat fetuses in concert with the morphogenesis of villi. The size of the lipid particles in the jejunal mucosa of fetal rats was limited only to the range of VLDL particles. These workers postulated that the appearance of VLDL in the absorptive cells of fetal rats prior to birth may be important in neonatal and postnatal nutrition. In this

TABLE 4. Composition of lipoproteins isolated from jejunal explants and organotypic medium

Lipoproteins	EGF Concentration	PL + MG	FC	DG	TG	CE
		Lipoproteins Isolated from Jejunal Explants				
	ng/ml	*% composition*				
CM	0	39.7 ± 5.4	6.6 ± 1.4	6.3 ± 3.4	40.7 ± 5.3	6.7 ± 1.6
	25	48.4 ± 0.9	5.0 ± 1.4	5.0 ± 1.4	36.5 ± 4.7	5.0 ± 0.1
	50	45.4 ± 2.6	5.2 ± 1.4	5.2 ± 1.4	38.0 ± 4.7	6.2 ± 0.1
	100	39.2 ± 1.7	3.4 ± 0.1	3.7 ± 0.1	47.4 ± 0.1	6.3 ± 0.1
VLDL	0	32.7 ± 2.3	5.9 ± 2.1	6.0 ± 3.1	50.1 ± 2.9	5.3 ± 1.1
	25	33.5 ± 6.6	8.0 ± 3.7	3.8 ± 0.6	48.1 ± 9.3	5.6 ± 1.0
	50	36.2 ± 2.9	4.3 ± 0.1	4.3 ± 0.1	50.0 ± 2.2	5.4 ± 0.1
	100	32.6 ± 3.3	3.3 ± 0.1	3.3 ± 0.1	54.8 ± 2.7	5.9 ± 0.1
LDL	0	57.6 ± 0.3	5.7 ± 0.5	4.7 ± 0.5	26.7 ± 0.7	5.2 ± 0.1
	25	51.8 ± 5.5	5.7 ± 0.5	5.2 ± 2.1	30.9 ± 1.7	6.3 ± 1.1
	50	50.7 ± 0.2	5.2 ± 0.1	5.7 ± 0.4	31.4 ± 0.2	6.8 ± 0.6
	100	45.2 ± 2.1	6.4 ± 1.1	5.8 ± 0.5	37.1 ± 3.8	5.8 ± 0.5
HDL	0	72.8 ± 1.6	2.7 ± 0.4	2.4 ± 0.2	16.2 ± 1.8	2.9 ± 0.3
	25	75.1 ± 2.1	2.8 ± 0.2	2.6 ± 0.2	16.2 ± 1.7	3.1 ± 0.4
	50	75.7 ± 1.8	2.6 ± 0.2	2.6 ± 0.2	16.2 ± 1.6	2.8 ± 0.4
	100	74.4 ± 2.3	2.6 ± 0.3	2.7 ± 0.4	17.2 ± 1.6	2.9 ± 0.4
		Lipoproteins Isolated from Organotypic Medium				
CM	0	11.7 ± 2.8	3.1 ± 1.2	2.5 ± 0.6	81.1 ± 4.2	1.65 ± 0.5
	25	16.5 ± 4.5	7.5 ± 1.5	4.5 ± 0.5	68.0 ± 7.0	3.5 ± 0.5
	50	15.0 ± 7.1	7.5 ± 2.5	4.5 ± 1.5	68.5 ± 10.5	4.5 ± 1.0
	100	21.0 ± 8.9	8.0 ± 1.0	4.0 ± 0.1	64.0 ± 9.5	3.0 ± 1.0
VLDL	0	4.0 ± 0.6	1.3 ± 0.3	1.2 ± 0.4	91.8 ± 1.2	1.7 ± 0.7
	25	4.5 ± 0.5	1.5 ± 0.5	1.0 ± 0.1	92.5 ± 1.5	4.5 ± 2.5
	50	12.0 ± 8.9	3.0 ± 1.9	1.1 ± 0.5	83.0 ± 11.9	1.0 ± 0.2
	100	3.5 ± 0.5	1.5 ± 0.5	0.1 ± 0.5	93.0 ± 1.9	1.0 ± 0.3
LDL	0	5.5 ± 0.5	1.0 ± 0.8	ND	90.5 ± 0.5	3.0 ± 0.9
	25	5.5 ± 1.5	1.1 ± 0.9	ND	91.0 ± 1.0	2.5 ± 0.1
	50	4.0 ± 0.1	0.5 ± 0.5	ND	94.0 ± 1.3	1.5 ± 0.5
	100	5.5 ± 0.5	1.5 ± 1.5	ND	92.0 ± 0.9	1.0 ± 0.5
HDL	0	19.6 ± 3.0	1.0 ± 0.4	1.9 ± 0.7	75.3 ± 3.1	2.2 ± 0.6
	25	20.8 ± 3.5	1.2 ± 0.5	2.2 ± 0.9	74.2 ± 3.9	1.5 ± 0.5
	50	19.4 ± 4.3	1.3 ± 0.5	2.5 ± 0.9	75.7 ± 4.8	1.4 ± 0.6
	100	20.4 ± 4.8	1.7 ± 0.6	2.8 ± 1.0	73.3 ± 5.1	1.8 ± 0.4

After incubation of jejunal explants with [^{14}C]oleic acid, the tissue homogenates and media were submitted to sequential ultracentrifugation to separate the four types of lipoproteins. Lipids were extracted from the lipoproteins, analyzed by TLC, and counted; ND, not detectable.

way, VLDL particles may be used as precursors for CM formation during the absorption of dietary lipids upon initiation of suckling immediately after birth. The suggestion was in fact based on the assumption of Tytgat, Rubin, and Saunders (33) that intestinal VLDL, elaborated by endogenous lipids, expand to form CM in adult rats by incorporating dietary TG into the VLDL particles. Nevertheless, the patterns of lipoprotein distribution using human fetuses have revealed that a large proportion of lipoproteins in response to exogenous [^{14}C]oleic acid substrate refers to d 0.97 g/ml and 1.21 g/ml fractions that might be associated with CM and HDL.

It has been proposed that EGF plays a trophic role in the regulation of developmental processes in various tissues and organs (13, 15). The presence of EGF in the amniotic fluid (34) and milk (16), its synthesis by the small intestine among numerous organs (14, 15), and localization of its receptors on the cell membranes of developing human gut tissues (18) have indicated that this factor may be important for the development of GI tract. Not only does EGF play a role in organismic development, affecting both growth and differentiation, but it also triggers a number of biological responses. Using the organ culture of human fetal jejunum, Ménard, Arsenault, and Pothier (20) have demonstrated that EGF (25–100 ng/ml) induced an increase in lactase activity and a repression of increases in sucrase, trehalase, and glucoamylase activity that normally occur during the culture period. Similarly, our study demonstrates that during incubation with jejunal explants, purified EGF has a differential effect particularly on d 0.97 mg/ml and 1.006 g/ml fractions. Although the accurate mechanism of EGF action remains

unclear, taken together, all these observations support a direct EGF regulation of the synthesis and secretion of cellular macromolecules.

In conclusion, the culture of the human fetal jejunum is a reliable and useful model to study the ontogeny of lipid transport and metabolism. Our data have clearly demonstrated that fetal explants (17–20 weeks of gestation) are able to synthesize and secrete all four lipoprotein density fractions. Furthermore, our results establish for the first time the implication of EGF in the modulation of the intracellular events governing the formation and release of lipoproteins during intestinal ontogeny in human. The characterization of intestinal apolipoprotein formation and release during development as well as related physiologic regulation will be the focus of future studies.

This work was presented in part at the Annual Meeting of the American Gastroenterological Association, New Orleans, May 18–23, 1991, and published in abstract form in *Gastroenterology* **100:** A530, 1991. This study was supported by grants MT-10583 (EL) and MT-5969 (DM) from the Medical Research Council of Canada and constituted part of a M.Sc. thesis of LT. The authors thank Lina Corriveau for technical assistance. They also thank Drs. A. Bilodeau, C. Poulin, M. Morin, F. Jacot, and L. Lemieux of Département de la santé communautaire for their excellent cooperation in providing tissue specimens for this study. The authors thank Danielle St-Cyr Huot for typing the manuscript.

Manuscript received 12 December 1991 and in revised form 19 March 1992.

REFERENCES

1. Glickman, R. M., J. L. Perrotto, and K. Kirsch. 1976. Intestinal lipoprotein formation: effect of colchicine. *Gastroenterology.* **70:** 347–352.
2. Dietschy, J. M., H. S. Salomon, and D. Siperstein. 1966. Bile acid metabolism. I. Studies on the mechanisms of intestinal transport. *J. Clin. Invest.* **45:** 832–846.
3. Risser, T. R., G. M. Reaven, and E. P. Reaven. 1985. Intestinal contribution to secretion of very-low density lipoproteins into plasma. *Am. J. Physiol.* **234:** E277–E281.
4. Alpers, D. H., D. R. Lock, N. Lancaster, K. Poksay, and G. Schonfeld. 1985. Distribution of apolipoproteins A-I and B among intestinal lipoproteins. *J. Lipid Res.* **26:** 1–10.
5. Magun, A. M., T. A. Brasitus, and R. M. Glickman. 1985. Isolation of high density lipoproteins from rat intestinal epithelial cells. *J. Clin. Invest.* **75:** 209–218.
6. Mak, K. M., and J. S. Trier. 1975. Lipoprotein particles in the jejunal mucosa of fetal rats. *Dev. Biol.* **43:** 204–211.
7. Frost, S. C., W. A. Clark, and M. A. Wells. 1983. Studies on fat digestion, absorption, and transport in the suckling rat. IV. In vivo rates of triacylglycerol secretion by intestine and liver. *J. Lipid Res.* **24:** 899–903.
8. Glickman, R. M., M. Rogers, and J. N. Glickman. 1986. Apolipoprotein B synthesis by human liver and intestine in vitro. *Proc. Natl. Acad. Sci. USA.* **83:** 5296–5300.
9. Zannis, V. I., J. L. Breslow, and A. J. Katz. 1980. Isoproteins of human apolipoprotein A-I demonstrated in plasma and intestinal organ culture. *J. Biol. Chem.* **255:** 8612–8617.
10. Black, D. B., and N. O. Davidson. 1989. Intestinal apolipoprotein synthesis and secretion in the suckling pig. *J. Lipid Res.* **30:** 207–218.
11. Davidson, N. O., and R. M. Glickman. 1985. Apolipoprotein A-I synthesis in rat small intestine: regulation by dietary triglyceride and biliary lipid. *J. Lipid Res.* **26:** 368–379.
12. Rachmilewitz, D., P. Sharon, and S. Eisenberg. 1980. Lipoprotein synthesis and secretion by cultured human intestinal mucosa. *Eur. J. Clin. Invest.* **10:** 125–131.
13. Malo, C., and D. Ménard. 1983. Synergistic effects of insulin and thyroxine on the differentiation and proliferation of epithelial cells of suckling mouse small intestine. *Biol. Neonate.* **44:** 177–184.
14. Malo, C., and D. Ménard. 1982. Influence of epidermal growth factor on the development of suckling mouse intestinal mucosa. *Gastroenterology.* **83:** 28–35.
15. Dembinsky, A., H. Gregory, S. J. Konturek, and M. Polanski. 1982. Trophic action of epidermal growth factor on the pancreas and gastroduodenal mucosa in rats. *J. Physiol.* **325:** 35–42.
16. Hirata, Y., and D. N. Orth. 1979. Concentration of epidermal growth factor, nerve growth factor, and submandibular gland renin in male and female mouse tissue and fluids. *Endocrinology.* **105:** 1382–1387.
17. Ménard, D., and P. Pothier. 1991. Radioautographic localization of epidermal growth factor receptors in human fetal gut. *Gastroenterology.* **101:** 640–649.
18. Pothier, P., and D. Ménard. 1988. Presence and characteristics of epidermal growth factor receptors in human fetal small intestine and colon. *FEBS Lett.* **228:** 113–117.
19. Ménard, D., and P. Arsenault. 1985. Explant culture of human fetal small intestine. *Gastroenterology.* **88:** 691–700.
20. Ménard, D., P. Arsenault, and P. Pothier. 1988. Biological effects of epidermal growth factor in human fetal jejunum. *Gastroenterology.* **53:** 319–326.
21. Fielding, P. E., I. Vlodavsky, D. Gospodarowica, and C. J. Fielding. 1979. Effect of contact inhibition on the regulation of cholesterol metabolism in cultured vascular endothelial cells. *J. Biol. Chem.* **254:** 749–755.
22. Levy, E., Y. Marcel, R. J. Deckelbaum, R. Milne, G. Lepage, E. Seidman, M. Bendayan, and C. C. Roy. 1987. Intestinal apoB synthesis, lipids, and lipoproteins in chylomicron retention disease. *J. Lipid Res.* **28:** 1263–1274.
23. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226:** 497–509.
24. Levy, E., Y. Marcel, R. Milne, V. Grey, and C. C. Roy. 1987. Absence of intestinal synthesis of apolipoprotein B-48 in 2 cases of abetalipoproteinemia. *Gastroenterology.* **73:** 1119–1126.
25. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
26. Davidson, N. O., R. C. Carlos, M. J. Drewek, and T. G. Parmer. 1988. Apolipoprotein gene expression in the rat is regulated in a tissue-specific manner by thyroid hormone. *J. Lipid Res.* **29:** 1511–1522.
27. Tso, P., and S. R. Gollamudi. 1984. Pluronic L-81: a potent inhibitor of the transport of intestinal chylomicrons. *Am. J. Physiol.* **247:** G32–G36.
28. Arsenault, P., and D. Ménard. 1985. Explant culture of human fetal esophagus, stomach and colon. *Pediatr. Res.* **19:** 149A. Abstract.
29. Malo, C., P. Arsenault, and D. Ménard. 1983. Organ cul-

ture of the small intestine of the suckling mouse in a serum-free medium. *Cell Tissue Res.* **228:** 75–84.
30. Traber, M. G., H. J. Kayden, and M. J. Rindler. 1987. Polarized secretion of newly synthesized lipoproteins by the Caco-2 human intestinal cell line. *J. Lipid Res.* **28:** 1350–1363.
31. Ellsworth, J. L., S. K. Erickson, and A. D. Cooper. 1986. Very low and low density lipoprotein synthesis and secretion by the human hepatoma cell line Hep G2: effects of free fatty acid. *J. Lipid Res.* **27:** 858–874.
32. Thrift, R. N., T. M. Forte, B. E. Cahoon, and V. G. Shore. 1986. Characterization of lipoproteins produced by the human liver cell line, Hep G2, under defined conditions. *J. Lipid Res.* **27:** 236–250.
33. Tytgat, G. N., C. E. Rubin, and D. R. Saunders. 1971. Synthesis and transport of lipoprotein particles by intestinal absorption cells in man. *J. Clin. Invest.* **50:** 2065–2078.
34. Weaver, L. T., E. Freiberg, E. J. Israel, and W. A. Walker. 1988. Epidermal growth factor in human amniotic fluid. *Gastroenterology.* **95:** 1436.