Intestinal lipoprotein synthesis. Comparison of nascent Golgi lipoproteins from chow-fed and hypercholesterolemic rats[1]

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Abstract Hypercholesterolemia, induced by a cholesterol-enriched diet, is associated with distinctive modifications in the serum lipoproteins of a variety of species. Present in the serum of these animals are several classes of lipoproteins enriched in cholesteryl esters and apolipoprotein E. To investigate the role of intestinal lipoprotein synthesis in diet-induced hypercholesterolemia, we characterized nascent lipoproteins retrieved from Golgi apparatus-rich fractions of intestinal epithelial cells from chow-fed control and hypercholesterolemic rats. To eliminate chylomicrons from the preparations, rats were fasted overnight prior to the experiments. Golgi very low density lipoproteins (d < 1.006 g/ml) from control rats were triglyceride-rich lipoproteins that migrated slightly slower than pre-beta migrating serum very low density lipoproteins. These particles contained apoproteins B-240, A-IV, and A-I. Golgi very low density lipoproteins from hypercholesterolemic rats were likewise triglyceride-rich lipoproteins migrating electrophoretically like control Golgi very low density lipoproteins and they contained apoproteins B-240, A-IV, and A-I. However, these latter particles contained less triglyceride and more cholesterol compared to control Golgi very low density lipoproteins. In addition, by radioisotope incorporation studies, Golgi very low density lipoproteins from hypercholesterolemic rats contained relatively more apoprotein A-IV (21.6 vs. 11.0%) and less apoprotein B-240 (17.0 vs. 27.0%) than found in control Golgi very low density lipoproteins. Approximately 60% of the total apoprotein radioactivity was found in apoprotein A-I in both preparations. We conclude that intestinal lipoprotein synthesis is modified by diet-induced hypercholesterolemia. The significance of these modifications with respect to the marked hypercholesterolemia observed in these animals remains to be determined.—**Swift, L. L., P. D. Soulé, M. E. Gray, and V. S. LeQuire.** Intestinal lipoprotein synthesis. Comparison of nascent Golgi lipoproteins from chow-fed and hypercholesterolemic rats. *J. Lipid Res.* 1984. **25:** 1–13.

Supplementary key words Golgi apparatus • apoprotein B-240 • radioisotope incorporation • very low density lipoproteins

Previous studies in our laboratory have shown that hepatic lipoprotein synthesis is altered in hypercholesterolemic (HC) rats (1). By characterizing nascent hepatic Golgi lipoproteins we demonstrated that the livers of HC rats synthesize a cholesteryl ester-enriched VLDL similar to plasma β-VLDL found in these animals. Furthermore we found that these HC rat livers produce a cholesteryl ester-rich low density lipoprotein that is secreted into the plasma compartment in the d 1.006–1.019 g/ml range (2). We concluded that abnormal hepatic lipoprotein synthesis and secretion contribute to alterations in serum lipoproteins produced by diet-induced hypercholesterolemia in the rat.

It was the aim of these studies to investigate intestinal lipoprotein synthesis in control and HC rats. Previous studies on intestinal lipoprotein formation have for the most part been carried out on lymph lipoproteins collected from intact animals or the isolated perfused intestine (3–11). However, newly synthesized intestinal lipoproteins undoubtedly undergo rapid modifications after secretion into the lymph. Furthermore, the presence of plasma components in the lymph may serve to further disguise these nascent particles. Because of these problems lymph lipoproteins may be quite different from the lipoproteins actually synthesized by the intestine.

We have studied intestinal lipoprotein synthesis in control and HC rats by characterizing nascent lipoproteins retrieved from Golgi apparatus-rich fractions of intestinal epithelial cells. This model allows characterization of newly synthesized intestinal lipoproteins prior to any post-secretory modifications. In this study we report that the intestine of the fasting control rat synthesizes triglyceride-rich VLDL (Golgi VLDL) which displays an electrophoretic mobility on agarose slightly slower than pre-beta migrating serum VLDL, and which contains only apoproteins B-240, A-IV, and A-I. We also show that the intestine of the HC rat synthesizes a cholesteryl ester-enriched VLDL with an electrophoretic mobility similar

Abbreviations: VLDL, very low density lipoproteins of d < 1.006 g/ml; β-VLDL, beta-migrating very low density lipoproteins; apo, apoprotein; B-335, apoprotein B of higher molecular weight; B-240, apoprotein B of lower molecular weight; HC, hypercholesterolemic; EDTA, ethylenediamine tetraacetic acid; SDS, sodium dodecyl sulfate.

[1] Part of this work was presented at the 55th Scientific Session of the American Heart Association, Dallas, Texas, November 1982, and published in abstract form in *Circulation.* **66(4):** II-101, 1982.

to Golgi VLDL from control rats. In addition, Golgi VLDL from HC rats contains apoproteins B-240, A-IV, and A-I. However, by radioisotope incorporation studies, HC Golgi VLDL contains relatively more apoA-IV and less apoB-240 than Golgi VLDL from control rats.

We conclude that diet-induced hypercholesterolemia results in altered intestinal lipoprotein synthesis in the rat. However, the qualitative and quantitative significance of these alterations with regard to the marked hypercholesterolemia observed in these animals remains to be determined.

METHODS

Animals and diet

Male Sprague-Dawley albino rats (Harlan Sprague-Dawley, Indianapolis, IN and Sasco Inc., St. Louis, MO) were used for all experiments. Rats to be made hypercholesterolemic weighed 80–100 g upon initiation of feeding. They were fed a diet of laboratory chow (Wayne Lab-Blox, Allied Mills, Inc., Chicago, IL) supplemented with 5% lard, 0.1% 6-N-propyl-2-thiouracil (Sigma Chemical Co., St. Louis, MO), 0.3% sodium taurocholate, and 1% cholesterol (ICN Nutritional Biochemicals, Cleveland, OH) for 21 days. At the end of this period they weighed approximately 160 g (162.8 ± 14.4, n = 188). Control rats were fed standard laboratory chow and weighed approximately 170 g (169.3 ± 17.4, n = 187) at the time of the experiments. Weight-matched animals rather than age-matched animals were chosen for controls to provide similar amounts of tissue per animal as well as similar body masses between groups when doing radioisotope studies. The difference in ages of groups of animals could range from 0 to 14 days. The animals were housed under constant temperature and humidity conditions, and were permitted access to food and water ad libitum. To eliminate chylomicrons from the preparations, all rats were fasted for 16 hr prior to the experiments, and the experiments were begun between 8:00 and 9:00 AM.

Isolation of serum lipoproteins and intestinal Golgi lipoproteins

Under light ether anesthesia, rats were exsanguinated from the distal abdominal aorta, and the blood was permitted to coagulate at 4°C. Serum lipids were determined on pooled or individual serum samples. Lipoproteins were isolated from pooled serum. Usually 24 animals were used for each preparation.

Immediately after exsanguination the small intestine was removed and intestinal epithelial cells were isolated as described previously (12). Mucus was removed by passing the suspension through the filter of a blood administration set (Travenol Laboratories, Inc., Deerfield, IL), and the cells were pelleted (500 *g*, 10 min).

The method for isolating a Golgi apparatus-rich fraction was a modification of previously described procedures (1, 12). All solutions were kept at 0°C, and all centrifugations were carried out at 4°C. The cells were resuspended in 0.1 M phosphate buffer, pH 7.3, containing 0.25 M sucrose, 1% dextran, and 10 mM $MgCl_2$, by three strokes of a Potter-Elvejhem homogenizer with a motor-driven Teflon pestle. Total disruption of the cells was affected with a Parr bomb (Parr Instrument Co., Moline, IL). The cell suspension was placed in the bomb at 4°C, pressurized to 1000 *psi* for 60 min, and the contents were slowly released. More than 90% of the cells was disrupted by this procedure as judged by phase contrast microscopy.

The entire disrupted cell suspension was layered on an unbuffered aqueous 1.25 M sucrose pad and centrifuged in a Beckman SW 27 rotor (Beckman Instruments, Inc., Palo Alto, CA) in the following manner: 5000 rpm, 10 min; 10,000 rpm, 10 min; and 25,000 rpm, 45 min. The centrifuge was not stopped between the different speeds. The Golgi apparatus-rich fraction appeared as a white band at the interface and was removed with a Pasteur pipette. This fraction was diluted with saline and pelleted by centrifugation at 20,000 rpm for 30 min in the SW 27 rotor.

To release nascent lipoproteins, the Golgi-rich pellet was suspended in 0.04 M sodium barbital buffer, pH 8.5, and passed twice through a French pressure cell (American Instrument Co., Travenol Laboratories, Inc., Silver Spring, MD) under 4000 *psi*. The density of the suspension was adjusted to 1.006 g/ml with 0.52 M NaCl, and centrifuged in a Beckman 40.3 rotor at 38,000 rpm for 18 hr. The floating lipoproteins (Golgi VLDL) were removed by tube slicing in a volume of approximately 0.5 ml, and washed one time under the same conditions.

Serum lipoproteins were isolated by the methods of Havel, Eder, and Bragdon (13) using the 40.3 rotor. Serum VLDL was isolated in 18 hr at 38,000 rpm, removed by tube slicing, and washed under the same conditions.

All fractions of Golgi or serum lipoproteins were dialyzed at 4°C for 72 hr against three changes of 500 vol of 0.01% EDTA, pH 7.2, and a final change of distilled H_2O, pH 7.0. Aliquots were retained for electron microscopy, lipoprotein electrophoresis, and protein assay, and the remainder was lyophilized.

Analytical methods

Total serum cholesterol was determined on ethanol–ethyl acetate extracts as described by Babson, Shapiro, and Phillips (14). Serum triglycerides were determined on isopropanol extracts by the method of Van Handel

and Zilversmit (15). Serum and Golgi lipoprotein protein were estimated by the Coomassie microprotein assay (16) using bovine serum albumin as standard. Lipoproteins were delipidated and individual lipid classes were isolated on silica gel 60 thin-layer plates (EM Laboratories, Elmsford, NY) developed in petroleum ether–ethyl ether–acetic acid 80:20:1. For lipid quantitation, the individual lipid classes were visualized with iodine vapors, scraped from the plates, and eluted from the gel (1). Cholesterol (free and esterified) was analyzed by the method of Babson et al. (14), triglyceride according to Van Handel and Zilversmit (15), and phospholipid according to Bartlett (17). For fatty acid analysis of triglycerides, the lipid spots were visualized with rhodamine 6G, scraped from the plates, methylated, and analyzed by gas–liquid chromatography (1).

Agarose gel electrophoresis

Electrophoretic mobility of lipoproteins was determined by a modification of the method of Noble (18) using 0.8% agarose (Bio-Rad Laboratories, Richmond, CA) in 50 mM barbital buffer, pH 8.6, containing 0.2% bovine serum albumin (Sigma Chemical Co., St. Louis, MO).

Electron microscopic techniques

Pieces of jejunem were pre-fixed in 2% glutaraldehyde, 50 mM lysine, in phosphate buffer (pH 7.2) for 30 min, followed by 2% glutaraldehyde in phosphate buffer (pH 7.2) overnight (19). The tissue was then rinsed in 7.5% phosphate-buffered sucrose, and post-fixed in Millonig's osmium tetroxide for electron microscopy (20). Dehydration and embedding were by Luft's procedure (21). One-micron sections were mounted on glass slides and stained with 1% toluidine blue. Thin sections were mounted on bare copper grids and stained with uranyl acetate and lead citrate (22).

Small portions of Golgi-rich fractions were negatively stained on carbon stabilized Formvar-coated nickel grids using 2% aqueous phosphotungstic acid, pH 6.5.

Grids were viewed in a Philips 300 electron microscope (Philips Electronic Instruments, Inc., Mahwah, NJ) and various fields were selected and photographed. Measurements of lipoprotein diameters were made from electron micrographs at magnifications between 62,750 and 79,750 (primary magnifications 25,100–39,900).

Polyacrylamide gel electrophoresis

The apolipoprotein composition of serum and Golgi lipoproteins from HC and control rats was analyzed on SDS slab gels by a modification of the method of Laemmli (23). The running gel was a linear 3 to 20% acrylamide gradient in a 0.375 M Tris-HCl buffer, pH 8.8, containing 0.1% SDS. The stacking gel was 2.0% acrylamide in 0.125 M Tris-HCl, pH 6.8, containing 0.1% SDS. The running buffer was 0.05 M Tris, pH 8.3, containing 0.38 M glycine and 0.1% SDS. Apoproteins were solubilized in 0.06 M Tris-HCl, pH 6.8, containing 2% SDS, and approximately 30 μg of protein was applied to each lane of the slab. Proteins were electrophoresed at 10 mA/slab until the dye marker (Bromphenol Blue) entered the resolving gel and at 20 mA/slab until the dye came to the end of the gel. Gels were stained with 0.01% Coomassie Brilliant Blue in methanol–water–acetic acid 10:10:1 (v/v) and destained.

The apoproteins were identified on the basis of molecular weights, as determined by reference to purified protein standards, and by comparison to published SDS gel electrophoretograms of rat apolipoproteins.

Radioisotope incorporation studies

To quantitate the relative apoprotein distribution in intestinal Golgi lipoproteins and to confirm that the apoproteins observed on intestinal Golgi lipoproteins were indeed being synthesized by the gut, radioisotope incorporation studies were carried out. In these experiments, rats were anesthetized with ether and a small midline incision starting at the sternum was made. The duodenum was isolated, and 200–250 μCi of L-[4,5-^{3}H]leucine (130–190 Ci/mmol, Amersham, Arlington Heights, IL) in 0.4 ml of 0.02 M phosphate buffered saline, pH 7.4, was injected into the lumen. Fifteen minutes after injection the rats were killed, and the intestines were removed and flushed with 150–180 ml of ice-cold saline. Golgi lipoproteins were isolated as described above.

The radioactive Golgi apolipoproteins were separated on SDS slab gels as described above. Gel lanes were sliced in approximately 2-mm slices and prepared for liquid scintillation counting (24). Gel slice samples were counted in a Beckman LS 233 scintillation counter. Quenching in each sample was approximately the same and therefore no quench correction was necessary. Greater than 85% of the total counts applied to the gel were recovered and greater than 87% of the radioactivity in the gels was located in apoproteins B-240, A-IV, and A-I. The remainder of the activity was not localized in any identifiable bands.

Statistical analysis

Significance of the data was evaluated by Student's *t*-test. Values are expressed as mean ± SD.

RESULTS

Serum lipid and lipoprotein concentrations

As has been reported previously, the feeding of cholesterol-saturated fat diets to rats resulted in marked ele-

TABLE 1. Serum lipid concentrations

	Control	Hypercholesterolemic
	mg/dl	
Cholesterol	65.5 ± 8.3 (3)	504.9 ± 248.7 (8)
Triglycerides	84.5 ± 16.2 (3)	59.7 ± 12.9 (5)

Values represent mean ± SD from pooled serum from (n) groups of rats. Each group contained 20–24 rats.

vations of serum cholesterol (505 ± 249 mg/dl vs. 66 ± 8 mg/dl) (**Table 1**). Serum triglycerides were slightly decreased in hypercholesterolemic (HC) rats (60 ± 13 vs. 85 ± 16 mg/dl).

Morphology studies

In the columnar absorptive cells of the rat jejunem, the Golgi apparatus was identified in a relatively fixed position beween the lateral cell membranes and the upper pole of the nucleus. In each cell it appeared as a group of one to three dictyosomes, each containing four to six parallel flattened cisternae surrounded by numerous smooth surfaced vesicles.

In intestinal lining cells from control rats (**Fig. 1**), the flattened cisternae appeared to terminate in large dilated saccules. The location of the Golgi apparatus was so precise and the dilated saccules were so numerous that the Golgi apparatus appeared as a zone in the epithelium easily identified at low magnification (×4,000). At higher magnification (×20,000) many of the dilated saccules were found to contain electron-dense lipoprotein particles 250–800 Å in diameter. The particles were not packed in the saccules but were scattered loosely in an amorphous electron-translucent material. Myelin figures were found associated with the Golgi apparatus in rare instances.

In the HC rats (**Fig. 2**) the Golgi zone was difficult to identify at low magnification due to the fact there were few, if any, of the dilated saccules at the periphery of the cisternae. In the HC rats the Golgi apparatus also contained four to six parallel flattened cisternae, but the cisternae were markedly elongated when compared to those of control rats. Lipoprotein particles were not as numerous, and they were less electron-dense, making them more difficult to identify. However, enough particles were found to measure, and they ranged from 200–500 Å in diameter. The tendency toward formation of myelin figures was exaggerated markedly in HC animals. The myelin figures appeared more often at the ends of the cisternae but were also found within cisternae and in intercellular spaces.

By negative stain electron microscopy the isolated Golgi apparatus exhibited the characteristic features described for hepatic Golgi fractions (1). However, rat intestinal Golgi fractions appeared to be more fragmented than rat hepatic Golgi fractions. There were no notable differences in the morphologic features of Golgi fractions from HC or control rats. Identifiable in both fractions were flattened central plates or fenestrated plates as seen in **Fig. 3.** In places the plates were seen to be continuous with an anastomosing tubular network which terminated in lipoprotein-filled secretory vesicles. The lipoproteins in vesicles from control rats ranged from 300 to 750 Å in diameter, while particles in vesicles from HC rats ranged from 250 to 750 Å in diameter (**Fig. 4**).

Agarose electrophoresis

Agarose electrophoretograms of serum, and Golgi and serum VLDL from HC and control rats are shown in **Fig. 5.** Compared with control, HC rat serum contained a marked increase in lipoproteins migrating in the beta-pre-beta region, due to the presence of β-VLDL, and increasing concentrations of IDL and LDL in HC rat serum. Intestinal Golgi VLDL from control rats migrated in a somewhat diffuse band slightly slower than pre-beta migrating serum VLDL. Golgi VLDL from HC rats migrated in a sharper band with a mobility similar to that seen with control Golgi VLDL.

Lipoprotein compositional studies

The protein and lipid composition of intestinal Golgi VLDL from HC and control animals is shown in **Table 2.** Also shown is the composition of serum VLDL from HC and control animals. Golgi VLDL from control rats were triglyceride-rich lipoproteins similar in composition to serum VLDL from the same animals. Intestinal Golgi VLDL from HC animals were also triglyceride-rich lipoproteins but were enriched fourfold in cholesteryl esters and sevenfold in unesterified cholesterol compared to control Golgi VLDL. Unlike serum and Golgi VLDL from control rats, Golgi and serum VLDL from HC rats were quite different in composition.

Fatty acid composition

The fatty acid composition of triglycerides of Golgi and serum VLDL from control and HC rats is shown in **Table 3.** In both control and HC rats, 16:0, 18:0, 18:1, 18:2, and 20:4 were the major fatty acids of Golgi and serum VLDL triglycerides. Golgi VLDL triglycerides from control rats contained markedly more 18:0 and 20:4 and less 18:1 and 18:2 than serum VLDL triglycerides from control rats. Golgi VLDL triglycerides from HC rats contained more 18:2 and 20:4 and less 18:1 than serum VLDL triglycerides from the same animals.

The fatty acid compositions of Golgi VLDL triglycerides from control and HC rats were remarkably similar with the exception that the HC samples contained more 18:1 and less 20:4 than control samples. These changes, however, resulted in relatively less polyunsaturated fatty

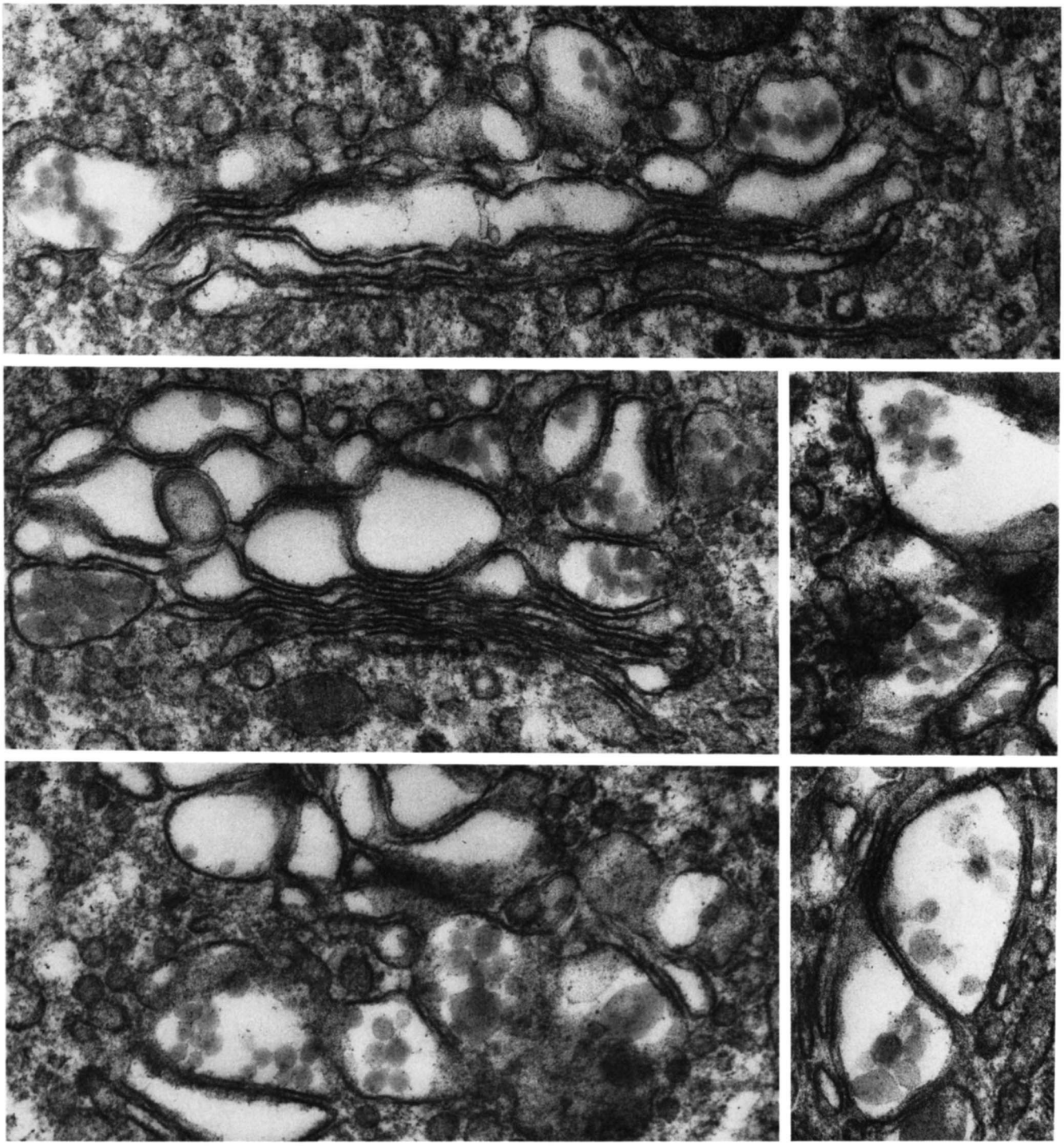


Fig. 1. Electron micrographs of Golgi apparatus of intestinal epithelial cells from control rats fasted 16 hr prior to death. The vesicles at ends of the cisternae and on the maturing face were dilated markedly and many appeared empty. Others were incompletely filled with electron dense lipoprotein particles ranging in diameter from 250–800 Å (average 440 ± 115 Å, n = 337). Uranyl acetate and lead citrate. ×79,750.

acids in HC Golgi VLDL triglycerides compared with control. The ratio of polyunsaturated/saturated and monounsaturated fatty acids for HC Golgi VLDL triglycerides was 0.71 compared with 0.96 for control samples.

Apolipoprotein composition

The apolipoprotein composition of serum and Golgi lipoproteins from HC and control rats was analyzed by SDS polyacrylamide gels on 3–20% acrylamide gradients.

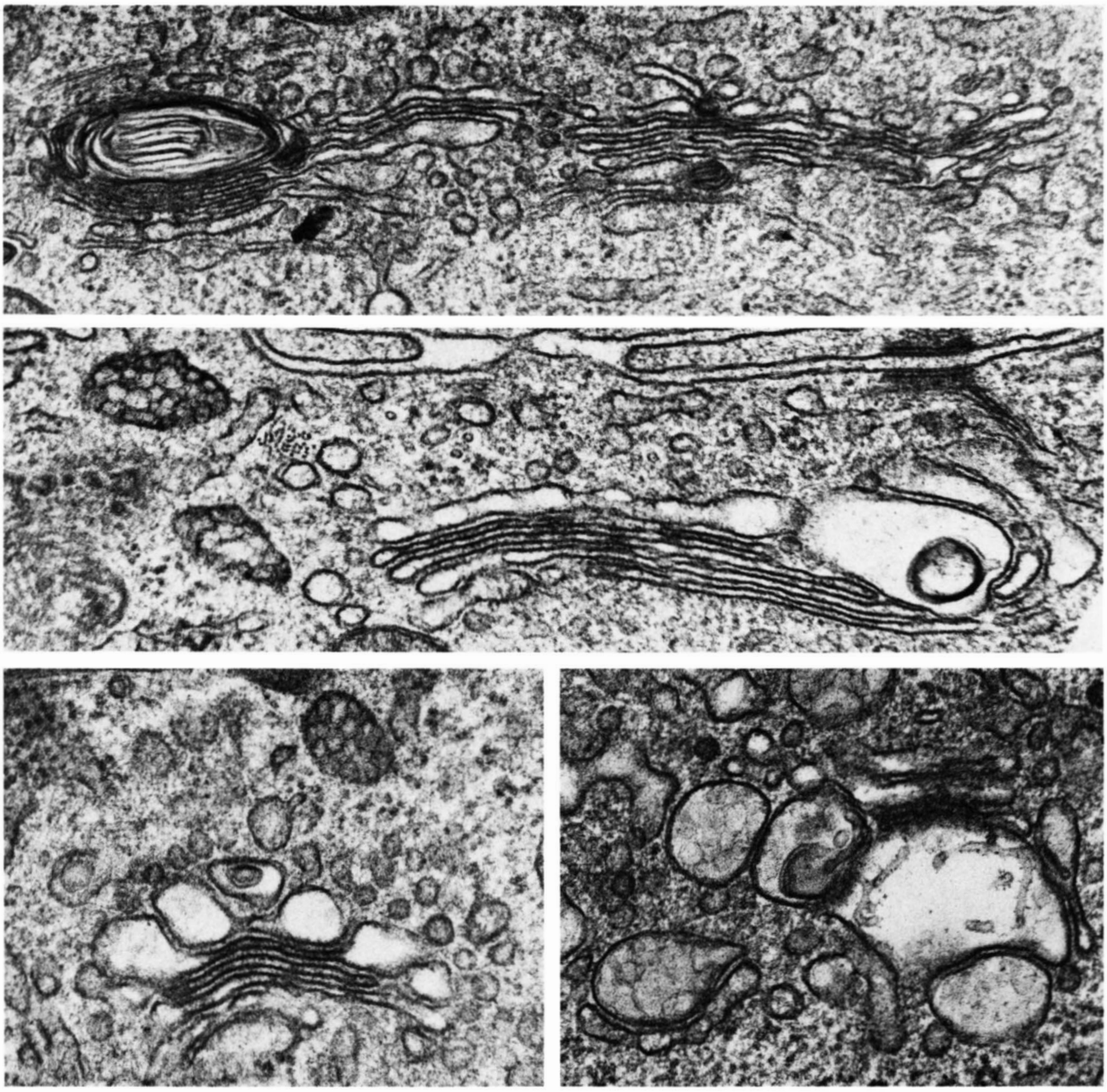


Fig. 2. Electron micrographs of Golgi apparatus of intestinal epithelial cells from HC rats fasted 16 hr prior to death. Dilated, electron-translucent vesicles were less apparent in the Golgi apparatus of these animals. Myelin figures were prevalent at ends of cisternae, within cisternae, and in intercellular spaces. Lipoprotein particles were less electron-dense than in control samples and were generally found packed into vesicles. Diameters of measurable particles ranged from 200–500 Å (average 330 ± 79 Å, m = 89). Uranyl acetate and lead citrate. ×79,750.

Intestinal Golgi VLDL (**Fig. 6**) contained apoproteins B-240, A-IV, A-I, and proteins migrating in the C region of the gel. No staining was observed in the apoB-335 or apoE region for any control Golgi VLDL samples. Golgi VLDL from HC rats contained the same apoprotein pattern. In addition to the known apoproteins, a protein of molecular weight of approximately 60,000 was observed in inconsistent amounts in Golgi VLDL samples from control and HC rats.

Intestinal VLDL apoprotein synthesis was studied by measuring the incorporation of intraduodenally administered [^{3}H]leucine into the apoproteins of Golgi lipoproteins from HC and control rats. Radioactivity profiles of SDS polyacrylamide gels of HC and control Golgi VLDL are shown in **Fig. 7.** Greater than 87% of the total radioactivity in the gels was recovered in apoproteins B-240, A-IV, and A-I. The relative amounts of activity incorporated into these apoproteins is shown in **Table 4.**

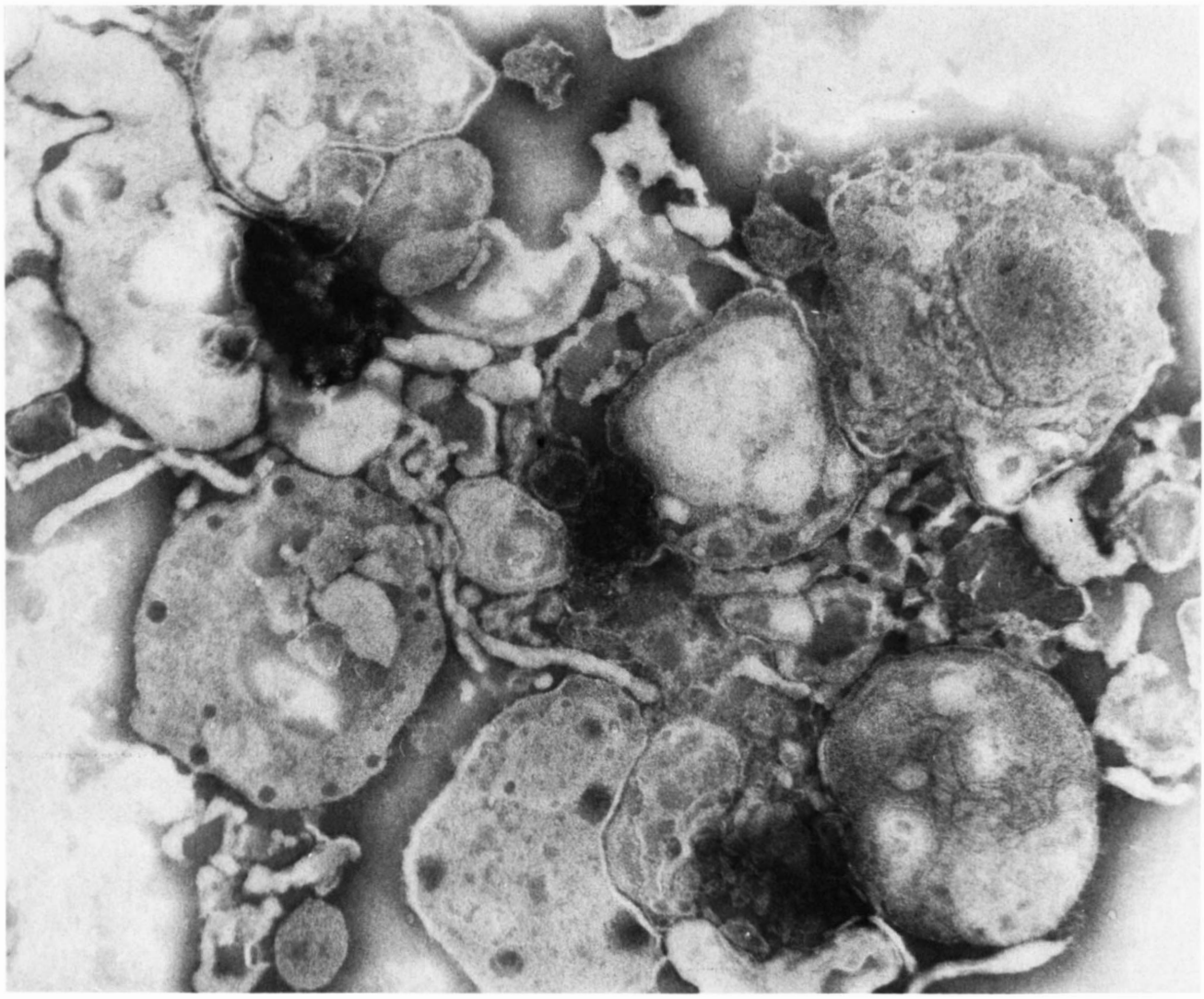
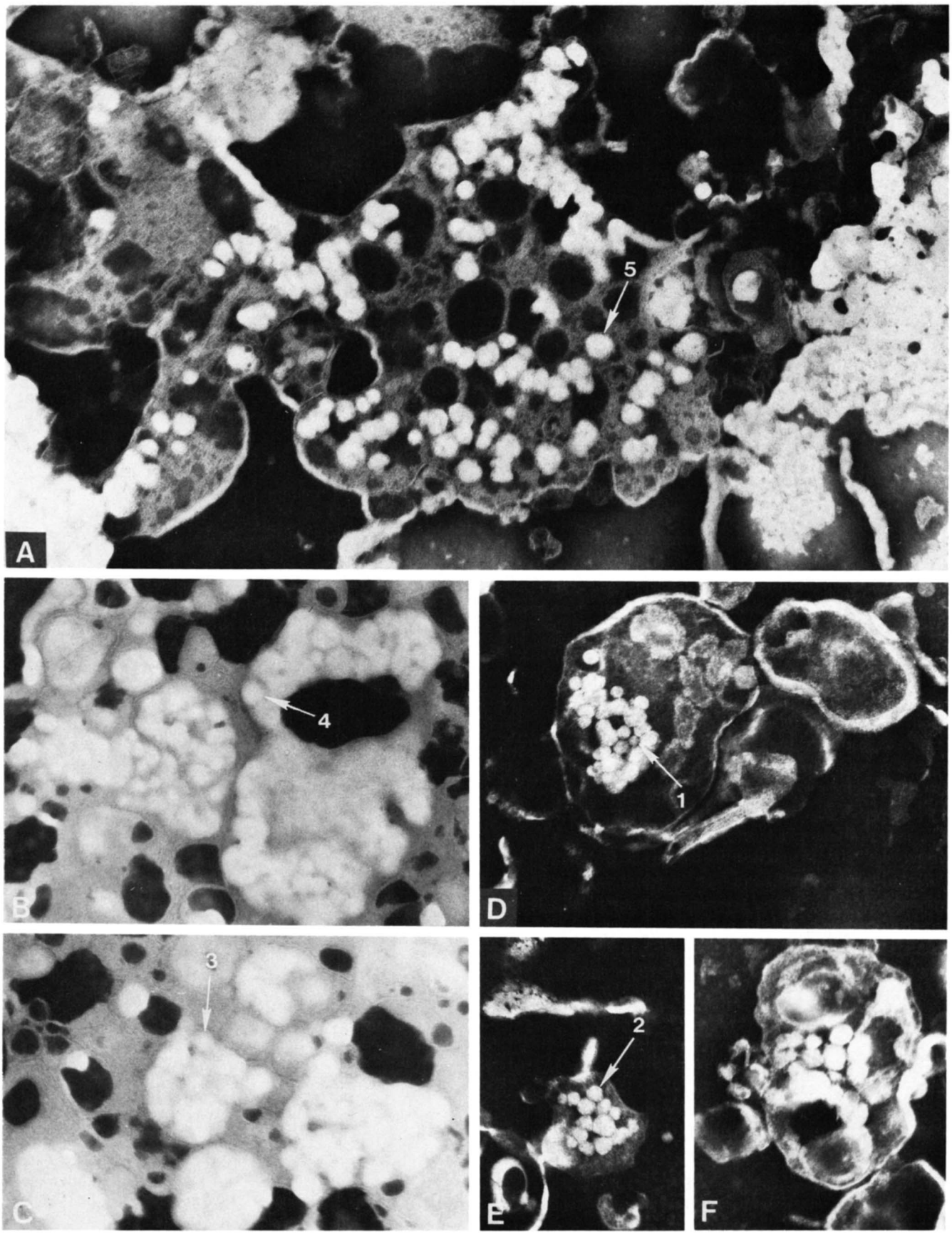


Fig. 3. Electron micrograph of negatively stained Golgi apparatus-rich fraction from HC rat. Several central plates, which correspond to the cisternae in thin sections, are present along with anastomotic tubules. Varicosities seen along the length of some tubules are probably caused by the presence of lipoprotein particles. 2% phosphotungstic acid. ×67,000.

Approximately 60% of the total apoprotein radioactivity in Golgi VLDL from both control and HC rats was found in apoA-I. ApoB-240 contained approximately 27% of the radioactivity in control Golgi VLDL compared with 17% in HC samples. Eleven percent of the radioactivity was found in apoA-IV in control samples compared with 21.6% in HC samples.

DISCUSSION

The small intestine has been recognized as an important source of plasma lipoproteins and lipoprotein components (for review, see ref. 25). While the major function of the intestine in lipoprotein metabolism may be the absorption of dietary fat and its packaging and secretion as chylomicrons, it is also an important site of VLDL production even in the absence of dietary fat. It has been reported that the intestine contributes from 10 to 40% of the total plasma VLDL-triglyceride pool in the fasting state (3, 9, 26). Risser, Reaven, and Reaven (27) have shown that intestinal triglyceride production is increased by 50% in nonfasted rats, but the overall contribution of the intestine represents 14–17% of the total body secretion in nonfasted rats compared with 11% in the fasting state. From their studies Risser et al. (27) concluded that the intestine does not play a major role in endogenous triglyceride production in normolipidemic rats. However, little is known concerning the role of the intestine in lipoprotein production in hyperlipidemic states. Furthermore, little is known concerning the types and characteristics of lipoproteins produced by the intestine in hyperlipidemic



states. In light of our present knowledge on atherogenesis, the latter information may be more important than the former.

Previous studies on intestinal lipoprotein synthesis have utilized lymph lipoproteins (3–11). However, because of the inherent dynamic nature of the lipoproteins, lipoproteins isolated from lymph may be quite different from the nascent intestinal lipoproteins. Our studies on uncirculated Golgi lipoproteins allow characterization of newly synthesized intestinal lipoproteins prior to post-secretory modifications which occur in lymph.

The animal model used for our studies is the hypercholesterolemic rat. The diet used to induce hypercholesterolemia contains propylthiouracil which has been reported to have an independent effect on lipoprotein metabolism (28, 29). We have shown previously that the livers of rats fed the complete diet (cholesterol, lard, taurocholate, and propylthiouracil) synthesize and secrete a cholesteryl ester-enriched VLDL and a cholesterol ester-rich LDL, while livers of rats fed diets containing lard, taurocholate, and propylthiouracil do not (1, 2). We concluded that the effects of propylthiouracil on hepatic lipoprotein synthesis were small in comparison with the effects of the complete diet. For our studies on lipoprotein synthesis by the small intestine, we have not made an effort to separate the individual effects of the components of the diet. However, it should be kept in mind that certain differences between control and HC rats might be attributable to propylthiouracil.

Our electron microscopic studies demonstrated the presence of VLDL-sized lipoprotein particles within the vesicles and saccules associated with the Golgi apparatus in intestinal epithelial cells of control rats (Fig. 1). In HC rat intestine, lipoprotein particles were more difficult to identify because they were less electron-dense and appeared to be packed tightly in the vesicles and saccules of the Golgi apparatus (Fig. 2).

While the Golgi-rich fractions were not completely characterized, the predominant organelle as determined by negative staining electron microscopy was the Golgi apparatus. Furthermore the characteristic features of the Golgi apparatus, including plates, anastomotic tubular network, and lipoprotein-filled vesicles, were evident (Figs. 3 and 4).

Our study on nascent uncirculated Golgi lipoproteins has shown that the intestine of the fasted control rat synthesizes a triglyceride-rich VLDL that migrates slightly slower than the pre-beta migrating serum VLDL from the same animals. While the compositions of Golgi and serum VLDL from control rats were found to be similar,

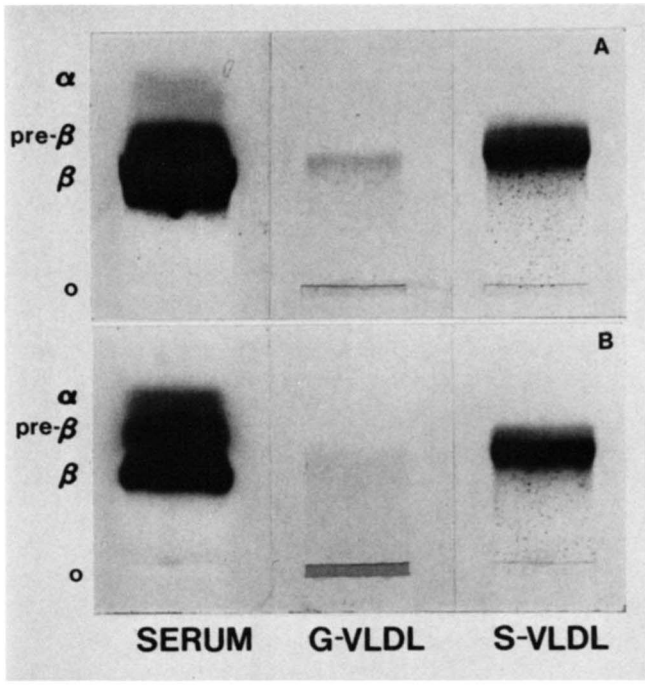


Fig. 5. Agarose gel electrophoretograms of serum and intestinal Golgi lipoproteins from HC (A) and control rats (B). Samples were electrophoresed in 0.8% agarose and stained with oil red O. G-VLDL, Golgi VLDL; S-VLDL, serum VLDL.

striking differences were observed in the spectrum of apoproteins associated with these lipoprotein classes. Golgi VLDL contained only apoproteins B-240, A-IV, and A-I in contrast to serum VLDL which contained apoproteins B-335, B-240, E, and C. The decreased electrophoretic mobility of Golgi VLDL compared with serum VLDL is probably due to the absence of apoC from these particles. By Coomassie staining we were unable to detect any protein in the apoB-335 or apoE regions of gels containing Golgi VLDL. Our finding of apoB-240 as the only B protein in intestinal Golgi lipoproteins confirms earlier reports suggesting that the intestine of control rats synthesizes only the lower molecular weight B protein (11, 30, 31). ApoA-IV and apoA-I have also been shown to be synthesized by the small intestine (10).

The marked difference in apoprotein composition of intestinal Golgi and serum VLDL indicates that VLDL synthesized by the intestine must undergo dramatic alterations in apoprotein content after secretion. Some of these alterations undoubtedly begin in the lymph. Lymph VLDL contains small amounts of apoproteins E and C in addition to apoproteins B-240, A-IV, and A-I (10). Furthermore, lymph VLDL has more phospholipid and less cholesteryl ester and protein than Golgi VLDL (3).

Fig. 4. Electron micrographs of negatively stained Golgi apparatus-rich fractions from HC and control rats. Lipoprotein particles are visible in plates and within secretory vesicles. A, D, E, and F, HC rats; B and C, control rats. Arrows indicate particles of various diameters: 1, 285 Å; 2, 450 Å; 3, 540 Å; 4, 600 Å; 5, 750 Å. 2% phosphotungstic acid. ×67,000.

TABLE 2. Percent composition of serum VLDL and intestinal Golgi VLDL[a]

	Control		HC	
	Serum (4)[b]	Golgi (3)	Serum (4)	Golgi (4)
Cholesteryl ester	8.1 ± 2.5	5.2 ± 2.6	56.6 ± 7.3	21.4 ± 4.5[c]
Triglyceride	61.6 ± 6.5	72.2 ± 2.3	9.9 ± 3.9	49.5 ± 3.2[c]
Unesterified cholesterol	5.1 ± 1.5	1.1 ± 0.9	12.4 ± 6.0	8.0 ± 3.4[d]
Phospholipid	11.8 ± 2.7	8.7 ± 3.3	7.8 ± 2.0	8.9 ± 4.3
Protein	13.4 ± 3.2	12.8 ± 3.6	13.3 ± 2.2	12.2 ± 4.7

[a] Values represent mean ± SD.
[b] Numbers of preparations analyzed.
[c] Significantly different from control Golgi VLDL at $P < 0.01$.
[d] Significantly different from control Golgi VLDL at $P < 0.05$.

Thus alterations in the nascent Golgi lipoproteins apparently begin immediately after secretion into the lymph and probably continue in the plasma compartment.

The fatty acid composition of Golgi VLDL triglycerides from control rats is similar to that reported previously for lymph VLDL triglycerides from fasted control rats (3). Ockner, Hughes, and Isselbacher (3) suggested that the relative abundance of 18:0 and 20:4 in intestinal VLDL triglycerides from these animals reflects the importance of biliary phospholipids as precursors. As would be predicted, the fatty acid pattern of triglycerides of intestinal Golgi lipoproteins from nonfasted rats reflects dietary triglycerides as major fatty acid precursors (data not shown).

VLDL synthesized by the intestine of HC rats differed from control Golgi VLDL in that it was enriched in cholesteryl esters and unesterified cholesterol. In addition, Golgi VLDL triglycerides from HC rats had more 18:1 and less 20:4 than Golgi VLDL triglycerides from control rats. These changes resulted in a relative decrease in the polyunsaturated fatty acids in HC Golgi VLDL triglycerides. This decrease in polyunsaturated fatty acids may explain the decreased osmiophilia of lipoprotein particles in the electron microscopic thin sections of HC rat intestine.

Our finding of differences in the nascent Golgi VLDL from HC and control rats differs from the results reported by Riley et al. (32). These workers studied mesenteric lymph lipoproteins in rats made hypercholesterolemic by feeding diets containing 1% cholesterol and 10% olive oil. They found no differences in the lipid composition

TABLE 3. Fatty acid composition of intestinal Golgi and serum VLDL triglycerides from control and HC rats

	Control		HC	
	Serum VLDL	Golgi VLDL	Serum VLDL	Golgi VLDL
16:0	23.2 ± 1.0	25.3 ± 0.8	23.1 ± 0.5	22.5 ± 3.6
16:1	0.5 ± 0.3	0.4 ± 0.4		0.4 ± 0.5
18:0	3.8 ± 1.1	11.1 ± 0.7	12.5 ± 0.2	13.0 ± 1.0
18:1	16.0 ± 0.5	13.5 ± 0.1	35.3 ± 0.3	21.1 ± 3.1[a]
18:2	42.9 ± 1.2	33.5 ± 0.8	24.0 ± 2.2	34.1 ± 1.7
18:3	0.3 ± 0.3	0.9 ± 0.1		0.7 ± 0.4
20:4	9.5 ± 1.2	14.2 ± 1.3	2.9 ± 0.2	6.2 ± 2.1[b]
20:5	0.5 ± 0.6		2.1 ± 1.2	0.4 ± 0.8
22:4	2.0 ± 1.1	0.7 ± 0.6		0.9 ± 0.8
22:5	1.5 ± 1.3	0.4 ± 0.8		0.3 ± 0.3

Fatty acids are denoted by ratio of chain length to number of double bonds. Values are mean ± SD for three experiments.
[a] Significantly different from control Golgi VLDL at $P < 0.05$.
[b] Significantly different from control Golgi VLDL at $P < 0.01$.

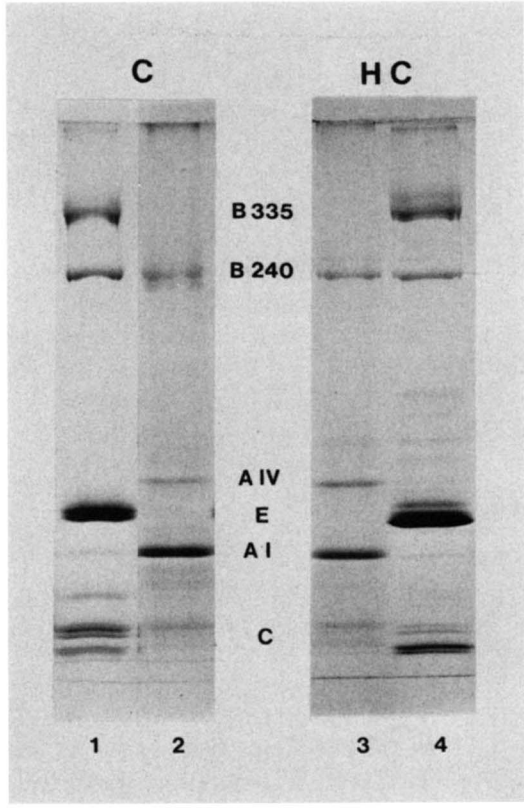


Fig. 6. SDS polyacrylamide gel electrophoretograms of intestinal Golgi VLDL and serum VLDL from HC and control rats. Approximately 30 μg of protein was applied to each lane, and electrophoresed according to Methods. Gels 1 and 4, serum VLDL; gels 2 and 3, Golgi VLDL.

of lymph VLDL from cholesterol-fed and control animals that had been fasted. Furthermore, after infusion of olive oil and cholesterol, the relative composition of d < 1.006 g/ml lymph lipoproteins (VLDL and chylomicrons) from cholesterol-fed and control rats was comparable. The differences in the two studies may be the result of the diets used. The diet used in our studies contains taurocholate, to facilitate intestinal absorption of fat, and propylthiouracil, and it produces higher serum cholesterol levels than the diet used by Riley and coworkers (32). Serum cholesterol levels in the latter study averaged 95 mg/dl compared with 505 mg/dl in our studies. The differences in the two studies may also be related to the models used in the studies. As discussed earlier, intestinal lipoproteins secreted into the lymph are exposed immediately to other lipoproteins, some of which filter into the lymph from the plasma compartment. Thus, modifications in nascent lipoproteins, including lipid and protein transfer, can occur upon secretion. It is likely that both of these explanations contribute to the differences in the two studies.

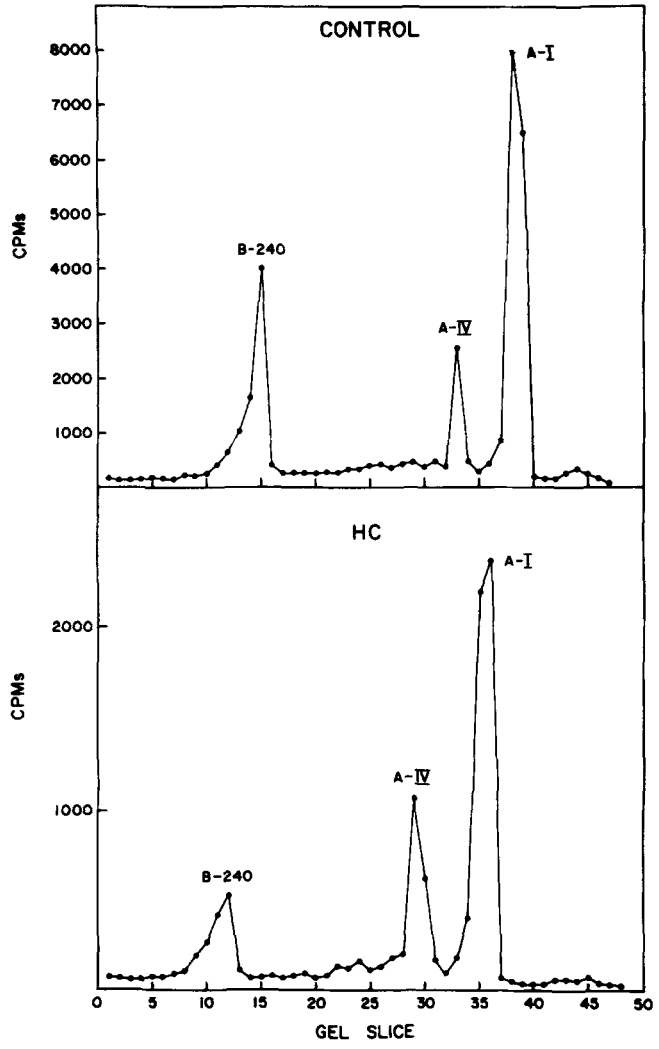


Fig. 7. Radioactivity profile of SDS polyacrylamide gels of intestinal Golgi VLDL from HC and control rats. Rats were injected intraduodenally with 200–250 μCi of [^{3}H]leucine and were killed 15 min later. Intestinal Golgi VLDL was isolated as described, and apoproteins were separated on SDS polyacrylamide gels. After electrophoresis, the gels were sliced into 2-mm sections and the amount of ^{3}H in each slice was determined.

TABLE 4. Percent distribution of ^{3}H-labeled apolipoprotein of Golgi VLDL[a]

Apoprotein	Control[b]	Hypercholesterolemic[c]	P
B-240	27.0 ± 2.6	17.0 ± 1.9	<0.01
A-IV	11.0 ± 0.9	21.6 ± 1.5	<0.01
A-I	62.1 ± 3.3	61.4 ± 2.0	N.S.

[a] Rats were injected intraduodenally with 200–250 μCi of [^{3}H]leucine and were killed 15 min later. Activity in apoproteins of Golgi VLDL was determined as described in Methods. Total activity recovered in the three apoproteins ranged from 8,000–25,000 cpms. Data are expressed as mean ± SD.
[b] Represents three gels from two experiments.
[c] Represents three gels from three experiments.

Radioisotope incorporation studies were carried out to quantitate the apoprotein distribution on Golgi VLDL from control and HC rats. We studied the relative amounts of radioactivity in the apoproteins of the Golgi VLDL 15 min after the intraduodenal administration of [^{3}H]leucine. Fifteen minutes was chosen as the time between isotope injection and killing for two reasons: *1*) Jersild (33) has shown that as early as 12 min after lipid was placed in the intestinal lumen, chylomicrons were observed in the intracellular spaces, indicating that intestinal lipoprotein formation is very rapid; *2*) the specific activity of nascent hepatic Golgi lipoprotein proteins is maximal 15 min after the intraportal injection of [^{3}H]leucine (34). In our studies greater than 85% of the total radioactivity recovered from the gels was in three apoproteins (B-240, A-IV, and A-I). No activity was found in either the apoB-335 or apoE region of the gel. The remainder of the activity was not localized in any band. Greater than 60% of the apoprotein radioactivity was found in apoA-I in both HC and control Golgi VLDL. In HC Golgi VLDL the relative amount of apoB-240 was decreased (27.0 vs. 17.0%), and there was a commensurate increase in apoA-IV (11.0 vs. 21.6%). The significance of these changes is not known at the present time. It should be remembered that these percentages represent relative amounts of radioisotope incorporated into these apoproteins within 15 min of isotope injection. Therefore the actual relative mass of apoproteins present in the Golgi VLDL may be quite different from the relative percent radioisotope incorporation. However, we have assessed the relative amounts of apoprotein present on intestinal Golgi VLDL from control rats by scanning negatives of gels and analyzing by computer the relative abundances of each apoprotein. By this technique the apoprotein composition of Golgi VLDL from control rats

was similar to the relative percent composition determined by our radioisotope incorporation studies (data not shown). Whether cholesterol feeding actually affects the relative amounts of apoproteins present on VLDL secreted by the intestine or affects the rates of synthesis of one or more of the apoproteins remains to be determined.

The apoprotein composition of Golgi VLDL from control rats as determined by these radioisotope incorporation studies is quite different from the apoprotein composition of lymph VLDL reported previously. Wu and Windmueller (10) studied the radioactivity incorporated into lymph lipoproteins collected from the isolated perfused rat intestine. Lymph was collected over a 3-hr period. Activity in lymph VLDL apoproteins was assessed on SDS polyacrylamide gels. Forty-seven percent of the total activity was found in apoB, 22.2% in apoA-IV, 27.1% in apoA-I, 3.1% in apoC, and 0.6% in apoE. Imaizumi, Fainaru, and Havel (35) analyzed the apoA-I and apoE content of small lymph chylomicrons (VLDL) from rats after intraduodenal infusion of a lipid emulsion. Radioimmunoassay of two samples revealed an average content of 38.9% apoA-I and 3.9% apoE. Fidge and McCullagh (36) reported that lymph VLDL from control rats contained 16% apoB plus higher molecular weight proteins, 19% apoA-IV, 22% apoA-I, 18% apoC, 3% apoE, and 22% apoA-V. The latter apoprotein was described as a unique protein of approximate molecular weight 59,000. This protein was found to associate primarily with triglyceride-rich lipoproteins, but its importance in lipid/lipoprotein metabolism was not defined.

We have observed small amounts of a protein of similar molecular weight (60,000) on Golgi VLDL from control and HC rats. In higher density lipoprotein fractions isolated from the Golgi apparatus, this protein is present in relatively larger amounts. Furthermore, in radioisotope incorporation studies, activity is found in this protein, suggesting it is synthesized by the intestine. Further studies are needed to identify this apoprotein synthesized by the intestine and to elucidate its function in lipoprotein metabolism.

These studies, together with our earlier studies (1), indicate that intestinal and hepatic lipoprotein synthesis is modified by diet-induced hypercholesterolemia. However, while both sites synthesize cholesteryl ester-enriched VLDL, these nascent lipoproteins are not enriched in cholesteryl esters to the degree of serum VLDL from HC rats. This indicates that altered synthesis cannot account completely for the accumulation of cholesteryl ester within the serum VLDL fraction. Other factors, such as the accumulation of cholesteryl ester-rich chylomicron remnants, as suggested by Ross and Zilversmit (37), probably also contribute to diet-induced hypercholesterolemia.

In summary, our studies have shown that the intestine of the fasted control rat synthesizes a triglyceride-rich very low density lipoprotein containing only apoproteins B-240, A-IV, and A-I. In addition, we have shown that the intestine of the HC rat synthesizes a cholesteryl ester-enriched VLDL that contains relatively more apoA-IV and less apoB-240 compared with control Golgi VLDL as determined by radioisotope incorporation studies. Thus the intestine also contributes to diet-induced hypercholesterolemia. The quantitative and qualitative significance of these diet-induced alterations in intestinal lipoprotein synthesis remains to be determined.

The technical assistance of Rita Bennett and Fred Morris is gratefully acknowledged. This work was supported by NIH grant HL 23525. Dr. Swift is an Established Investigator of the American Heart Association.

Manuscript received 26 May 1983.

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