Intestinal Apoproteins during Fat Absorption

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ABSTRACT To compare the roles of apolipoprotein (Apo) A-I, B, and E (or arginine-rich apoprotein, ARP) in the intracellular production of intestinal chylomicrons (and/or VLDL), these apoproteins were localized in rat intestinal mucosa by the light microscope method of indirect immunofluorescence. In addition, tissue levels of ApoA-I and ApoB were measured during fat absorption by radioimmunoassay.

Antisera were produced using ApoA-I isolated from rat plasma high density lipoprotein, and ApoB and ARP from plasma VLDL by column chromatography. The apoproteins yielded single bands on polyacrylamide disc gel electrophoresis in urea and in sodium dodecyl sulfate. Anti-apoprotein antisera were produced in rabbits. These antisera appeared to be monospecific on double-antibody immunoprecipitation of ^{125}I-labeled apoproteins. In fasted animals granular staining of ApoA-I was noted in the supranuclear (Golgi) regions of epithelial cells in the top third of the villus. At 30 min, when fat droplets were seen in the supranuclear cytoplasm of the cells along the top two-thirds of the villus, intense ApoA-I staining surrounded droplets in the cytoplasm. At later times when epithelial cells and lamina propria both contained fat droplets, bright ApoA-I stain surrounded many droplets in the supranuclear cytoplasm of cells and in the lamina propria. Over the same period of time, tissue levels of ApoA-I rose 10-fold. The distribution and time-course of ApoB staining was nearly identical with that of ApoA-I. Concomitantly, tissue ApoB levels doubled. By contrast, in fasting rat intestine, staining of ARP was sparse, punctate, and confined to the lower quarter of the villus. After fat feeding, stained droplets were seen only in the lamina propria near the base of the villus even though abundant ARP was found in cells along most of this length of the villus. Stain was never seen to surround any droplets inside cells. Thus, ApoA-I and ApoB appeared to participate in the intracellular assemply of lipoproteins in gut, whereas ARP did not, although ARP was found within mucosal cells.

Liver and intestine differed in their stainable contents of ApoA-I and ARP. Whereas intestine stained heavily for ApoA-I and lightly for ARP, liver stained heavily for ARP and lightly for ApoA-I. Both organs stained for ApoB. These findings suggest that there may be some quantitative "specialization" of the two organs which secrete lipoproteins.

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INTRODUCTION

Dietary fat is absorbed across the intestinal epithelial cell of mammals via chylomicrons and very low density lipoproteins (VLDL)[1] (1). Several apoproteins have been identified in chylomicrons isolated from the intestinal lymph of man and rat (2–4). Among these are: (*a*) ApoA-I (5), which is the major apoprotein (Apo) of plasma high density lipoprotein (6, 7) and is an activator of lecithin cholesterol acyltransferase (8, 9); (*b*) ApoB (4), the major protein of plasma low density lipoprotein (LDL) and a major protein of ApoVLDL (6, 7), which appears to be essential for lipoprotein formation in the liver and gut (10, 11); (*c*) arginine-rich protein (ARP or ApoE) (12) which, in rat, is found in all plasma lipoproteins (6, 7) and levels of which appear to increase in VLDL and LDL in response to intakes of high fat, high cholesterol diets in several animal species (13–15); and (*d*) ApoC, which consists of three proteins that modulate the activities of lipoprotein lipase (16, 17) and of lecithin cholesterol acyltransferase (9). It is suspected that intestinal VLDL have similar apoproteins, but more studies are needed.

These apoproteins have been identified by electrophoretic and immunologic techniques in rat lipoproteins (5–7, 12, 18, 19). The electrophoretic patterns of chylomicron apoproteins isolated from intestinal lymph greatly resemble those of analogous particles isolated from the Golgi apparatus of intestinal mucosa (20). In addition, while this work was in progress, Glickman et al. (21, 22), have localized ApoA-I and

[1] *Abbreviations used in this paper*: Apo, apoprotein; ARP, arginine-rich apoprotein; LDL, low density lipoprotein; SDS, sodium dodecyl sulfate; VLDL, very low density lipoprotein.

ApoB in isolated gut epithelial cells of the rat and demonstrated that apoprotein localization is altered during fat absorption. ARP and ApoC have not been similarly studied. These findings suggest that at least the ApoA-I and ApoB of lymph lipoproteins are acquired before these particles exit from the gut epithelial cell.

During fat absorption, radioactive amino acids are incorporated into several moieties of lymphatic lipoproteins (22–24). Although the electrophoretic methods used in the elegant earlier studies do not allow for unequivocal identification of each individual apoprotein, recent findings (22) do suggest that ApoA-I and some of the other apoproteins acquired during chylomicron and/or VLDL production are newly synthesized.

We wished to compare the involvement of three apoproteins, ApoA-I, ApoB, and ARP, in the formation of intracellular lipid packaged for export. The light microscope method of indirect immunofluorescence, applied to frozen sections of rat small intestine, combined with tissue measurements of ApoA-I and ApoB have allowed us to follow the behaviors of apoproteins in the intestinal epithelial cell and along the length of the villus during fat absorption, and to distinguish between the roles of ApoA-I and ApoB on the one hand, and the role of ARP on the other. Part of this work has been presented (25).

METHODS

Male Wistar rats weighing 250–300 g were maintained on Purina Rat Chow (Ralston Purina Co., St. Louis, Mo.) and tap water ad lib. After overnight fasting, the animals were given 1.5 ml of corn oil by gastric tube without anesthesia. At time intervals thereafter the animals were anesthetized with ether and samples of proximal jejunum were taken for staining with oil red O and for indirect immunofluorescence assay. Blood samples were also taken from the inferior vena cava for analysis of lipids. Plasma triglyceride and cholesterol levels were measured by the methodology of the Lipid Research Clinics (26).

Specific proteins were detected in tissues by an indirect immunofluorescence assay on frozen section substrate. 0.5-cm lengths of intestine taken from animals under ether anesthesia were opened longitudinally, rinsed in ice cold phosphate-buffered saline (0.05 M NaCl, 0.04 M sodium phosphate buffer, pH 7.2, isotonic), quick-frozen in liquid nitrogen-cooled Freon (E.I. du Pont de Nemours & Co., Wilmington, Del.) at −158°C, and stored in screw-cap vials at −70°C until used. 4-μm thick frozen sections were cut from the tissue blocks in a microtome-cryostat; blocks were oriented so that cross sections of the intestine were provided. Sections were placed on microscope slides and air-dried for 20 min at room temperature. Sections were then fixed in methanol for 4 min at −20°C, then acetone for 2 min at −20°C, and again air-dried (21). The remainder of the indirect immunofluorescence assay was performed as previously described (27). Rabbit anti-rat apoprotein antisera (see below) and the fluorescein-conjugated IgG fraction of goat anti-rabbit IgG (heavy and light chain specific; Meloy Laboratories Inc., Springfield, Va.) were both used at dilutions of 1:100. Dilutions were freshly prepared in phosphate-buffered saline.

Controls consisted of substrate incubated on glass microscope slides as follows: (*a*) with buffer alone, i.e. without either rabbit anti-rat apoprotein antiserum or fluorescein-goat anti-rabbit IgG conjugate; (*b*) with conjugate alone; (*c*) with nonimmune rabbit serum (at same dilution as immune sera) and conjugate; (*d*) with immune sera directed against irrelevant antigens (e.g. human cell membranes) and conjugate; and (*e*) with specific anti-rat apoprotein antisera absorbed with the appropriate apoprotein antigens. Adjacent 4-μm thick sections were stained with hematoxylin and eosin, and oil red O, and examined to assist in the identification of tissue structures and to follow the progress of fat absorption.

Two or three sections at each time point and for each apoprotein were independently examined by two different observers. The entire area of each cross section of intestine was surveyed. About 60% of the gradings (0–5) were identical between the observers. The grading differed by 1 rank or less over 95% of the time. Where differences were observed the mean ranking was used.

The antisera used in the immunofluorescence studies were obtained by using antigens isolated as follows: ApoA-I was isolated from rat plasma high density lipoprotein (*d* 1.070–1.19) by column chromatography as previously described (6, 28). This material yielded a single band on sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (28). ARP was isolated by column chromatography from rat VLDL using the method of Koga et al. (6) initially, and later the method of Marsh (29). In most instances a single pass through the Sephadex G-200 (6) or the Bio-Gel A 1.5 (29) column (Bio-Rad Laboratories, Richmond, Calif.) yielded a single band on SDS polyacrylamide gel electrophoresis (Fig. 1), however, in some preparations from the Sephadex G-200 column a small, more rapidly migrating band was noted. In the latter instances, ARP was rechromatographed on the Sephadex G-200 column yielding a single band on SDS gel electrophoresis with an apparent molecular weight of ~ 35,000 (30). Two antigenic preparations were used to produce anti-ApoB antisera. First, LDL was isolated from rat plasma between the densities of 1.025–1.050 by two centrifugations at each density. These preparations formed only one precipitin line with anti-rat serum on immunoelectrophoresis and immunodiffusion. LDL was used as immunogen directly. The antisera obtained are called "anti-LDL" antisera (31). In addition, ApoB was isolated from rat VLDL by column chromatography on Sephadex G-200 (6). The material in the ApoB-containing peak (peak I) (6) did not enter SDS or urea gels (Fig. 1) (32, 33) (whereas *d* 1.025–1.050 LDL did contain small amounts of material which migrated in the arginine-rich and ApoC regions of the SDS gels). The antisera produced with ApoB are called "anti-ApoB" antisera. Antisera were produced in rabbits (28).

The specificities of antisera were evaluated by double-antibody immunoprecipitation of various test, radiolabeled apoproteins (28). We used either rabbit anti-rat apoprotein antisera and a goat anti-rabbit IgG antiserum, or the respective IgG fractions isolated from the rabbit and goat antisera by $(NH_4)_2SO_4$ precipitation followed by DEAE chromatography (34). To assure maximum precipitation of rabbit IgG by goat antibodies, rabbit IgG was iodinated with ^{125}I and lactoperoxidase (28, 35) and purified by column chromatography (1.5 × 30 cm column, 0.05 M barbital buffer, 1 mM EDTA pH 8.6). ^{125}I-Rabbit IgG was added as a tracer to rabbit antisera or to rabbit IgG preparations (~ 150,000 cpm/ml), and increasing amounts of goat anti-rabbit IgG antiserum or goat IgG were added to the tracer-containing rabbit anti-rat antibody preparations. Tubes contained 150 μl of 0.05 M barbital, 1 mM EDTA, pH 8.6, 3% bovine serum albumin (bovine serum albuminbarbital), 100 μl of rabbit antiserum (final dilution 1:1,000– 1:16,000) or rabbit IgG (final concentration 1.5–50 μg/ml, equivalent to ~

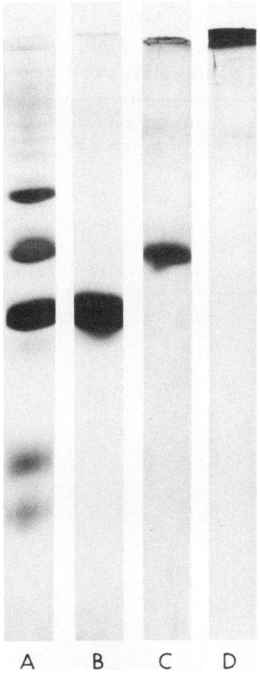


FIGURE 1 Apoproteins of high density lipoproteins ApoA-I, ARP, and ApoB in SDS gel electrophoresis (A–D, respectively).

dilution of 1:10,000 to 1:200 of original antiserum), and 100 μl of goat anti-rabbit IgG antiserum (dilution 1:20–1: 100) or goat IgG (70–120 μg/ml). Incubations were for 16 h at 4°C. Tubes were centrifuged, precipitates were "washed" by resuspension and recentrifugation in barbital buffer at 4°C, and precipitates were counted in a Packard Autogamma spectrometer (Packard Instrument Co. Inc., Downers Grove, Ill.). In the rabbit IgG/goat IgG system mass ratios ≤1/20 (rabbit/goat) yielded maximum precipitation of label. In the rabbit antiserum/goat antiserum optimal ratios were ≤1/50 (vol/vol).

To assess the specificities of the rabbit anti-rat apoprotein antisera, assay tubes contained 250 μl of bovine serum albumin-barbital (0.05 M barbital pH 8.6, 3% bovine serum albumin), 100 μl of rabbit antiserum or rabbit IgG (dilution 1:1,000– 1:4,000 or 4.6 μg/ml) and 100 μl of the appropriate ^{125}I-labeled apoprotein (~ 15,000 cpm). ApoA-I was labeled with lactoperoxidase (28, 35). LDL, ApoB, and ARP were labeled with chloramine-T (36) as modified by us (37). Incubations with first antibody were carried out for 2 days at 4°C. Goat anti-rabbit IgG antiserum or goat IgG (50 μl) was then added in optimal concentrations and incubation was continued for another 16 h at 4°C. Tubes were centrifuged and counted as described above. "Nonspecific" tubes were included in each assay. In these tubes, appropriate amounts of nonimmune rabbit serum or nonimmune rabbit IgG were substituted for immune sera or IgG. Results are expressed as $B_0 \times 100/T$, where B_0 = the precipitated counts (minus the counts in the "nonspecific" tubes) divided by T, the total counts added (minus the counts in the "nonspecific" tubes).

Tissue contents of ApoA-I (28) and ApoB (31, 37) were assessed by radioimmunoassay. Mucosal scrapings of proximal jejunum were homogenized in three volumes of 0.05 M barbital pH 8.6, 1% Triton X-100 and centrifuged at 105,000 *g* for 30 min. A floating fat cake, a clear midzone "supernate", and a pellet were obtained. Known amounts of ^{125}I-LDL and ^{125}I-ApoA-I were placed in separate aliquots of homogenates before centrifugation. More than 95% of each label was recovered in the "supernate" after centrifugation. In the assays, dilutions of supernates prepared in the assay buffer (bovine serum albumin-barbital 0.1% Triton X-100) displaced ^{125}IApo/I and ^{125}I-LDL in parallel with the appropriate standards. Coefficients of variation in these assays averaged 10%. Thus, the ApoA-I and ApoB contents of tissues could be measured with precision. Results are given as nanograms of apoprotein per milligram of homogenate protein (38).

RESULTS

Antisera. Each of the anti-ApoA-I antisera bound large proportions of the added ^{125}I-ApoA-I (Tables I and II). Antiserum R 150-1 and the IgG isolated from it appeared to precipitate ^{125}I-ApoA-I almost exclusively. R 150-1 was chosen for the immunofluorescence experiments.

Anti-ARP antibodies bound ^{125}I-ARP, but not ^{125}I-ApoA-I (Tables I and II). However, they did bind large proportions of ^{125}I-LDL. On the other hand, ^{125}I-ApoB was bound only minimally, suggesting that the anti-ARP antisera (and IgG preparations) were binding to ^{125}I-ARP and not the ^{125}I-ApoB determinants of ^{125}I-LDL. Antiserum R 178-3 was used in the immunofluorescence experiments.

Anti-LDL antisera contained antibodies which were able to bind both ^{125}I-LDL and ^{125}I-ApoB and small

TABLE I
Specificities of Rabbit Anti-Rat Apoprotein Antisera

Antisera	^{125}I-ApoA-I	^{125}I-Labeled antigen precipitated		
		^{125}I-ARP	^{125}I-LDL	^{125}I-ApoB
Anti-ApoA-I				
R 149-1	86–88	22	12–16	—
R 150-1	70–85	<5	5–7	—
R 186-2	85–87	16	11–16	—
Anti-ARP				
R 178-2, –3	<5	53–73	47–51	7–11
R 179-1	10	49–55	73–77	—
Anti-LDL				
R 134-1, –2, –3	<5	6–23	74–88	41–47
R 135-1, –2, –3	<5	20–23	76–81	—
Anti-ApoB				
R 176-1, –2, –3, –4	<5	3–9	73–87	65–69
R 177-1, –2, –3, –4	<5	4–8	77–83	—

Specificities were determined by double-antibody immunoprecipitations using whole antisera from rabbits and goats. Results of two to three experiments are given as ranges of $B_0 \times 100/T$. Final dilutions of rabbit anti-rat antisera were 1:2,000–1:4,000. Precipitations (percent of added) in the presence of nonimmune rabbit sera (instead of rabbit antisera) were as follows: ^{125}I-ApoA-I, <3%; ^{125}I-ARP, 8%; ^{125}I-ApoB, 9%; ^{125}I-LDL, <3%.

TABLE II

Specificities of IgG Fractions of Rabbit Anti-Rat Apoprotein Antisera

IgG fraction	^{125}I-ApoA-I	^{125}I-Labeled antigen precipitated		
		^{125}I-ARP	^{125}I-LDL	^{125}I-ApoB
Anti-ApoA-I R 150-1	77–88	<1	3–7	<1
Anti-ARP R 178-3	<1–3	51–67	30–43	<5
Anti-LDL R 134-1	2–3	8–10	80–83	38–42
Anti-ApoB R 176-1	<1–3	2–3	76–77	63–67

Specificities were determined by double immunoprecipitation using IgG preparations isolated from rabbit and goat antisera. Results of two to four immunoprecipitations are given as ranges of values for $B_0 \times 100/T$. Precipitations of added labels (percent added) when a pool of nonimmune rabbit IgG was substituted for specific immune IgG were as follows: ^{125}I-ApoA-I, 1–3%; ^{125}I-ARP, 4–7%; ^{125}I-LDL, 4–7%; and ^{125}I-ApoB, 4–5%. Rabbit and goat IgG final concentrations were 4.6 and 93.1 μg/ml, respectively.

proportions of ^{125}I-ARP but not ^{125}I-ApoA-I. The anti-ApoB antisera had similar specificities for ^{125}I-LDL and ^{125}I-ApoB but they bound virtually no ^{125}I-ARP or ^{125}I-Apo-I. R 134-1 and R 176-1 were used.

Apoprotein localization. After the intragastric administration of corn oil, plasma triglyceride levels increased from 25–30 to ~ 250 mg/dl over the 6 h of the experiment, whereas cholesterol levels stayed relatively constant between 60 and 70 mg/dl (two to three rats per time point). Oil red 0 staining of the small intestines of these animals revealed the following (not shown): in fasting rats virtually no staining was detected either in the epithelial cells, or in the lamina propria. At 30 min, many fat droplets were seen particularly in the apical portions of epithelial cells, involving cells in the top two-thirds of the villus. A slight amount of staining was seen at the bases of cells and in the lamina propria. At 3–6 h both the apical and basal regions of epithelial cells and the lamina propria along the entire length of the villus were engorged with fat droplets (Table III).

In the fasted state, ApoA-I granular fluorescence was present in the supranuclear regions of epithelial cells in the upper three-fourths of the villus (Fig. 2A and Table III). At 30 min, the intensity of staining of the apical cytoplasm increased and fluorescence surrounded droplets in both the supranuclear and infranuclear regions of cells in the top two-thirds of the villus (Fig. 2B), whereas, granular staining appeared in cells down to the bottom of the villus. The lamina pro-

TABLE III

Specific Staining of Apolipoproteins in Jejunum during Fat Absorption

Stain	Time	Intensity of stain				
		Epithelial cell cytoplasm			Lamina propria	
		Diffuse	Golgi	Grains	Peripheral interstitium	Lacteal lumen
	h					
Oil red O	0	0	0		0	0
	0.5	2	2		1	1
	1	4	4		2	2
	2	5	5		4	4
	3	4	4		4	3
	4	5	5		4	3
	5	2	2		2	5
	6	2	2		2	2
ApoA-I	0	0	2	0	0	0
	0.5	1	4*	0	1*	2*
	1	1	4*	0	1*	4*
	2	1	5*	0	3*	4*
	3	3*	5*	0	3*	4*
	4	3*	4*	0	3*	4*
	5	3*	4*	0	3*	4*
	6	3*	4*	0	3*	4*
ApoB	0	1	2	0	4	4
	0.5	1	3*	0	4*	4*
	1	1	3*	0	4*	4*
	2	1	3*	0	4*	4*
	3	2*	3*	0	4*	4*
	4	2*	2*	0	4*	4*
	5	2*	2*	0	4*	4*
	6	2*	2*	0	4*	4*
ARP	0	0	0	1 (1/8)	0	0
	0.5	0	0	4 (1/4)	0	0
	1	0	0	4 (1/4)	0	0
	2	0	0	4 (1/4)	2*	0
	3	0	0	4 (2/4)	2*	0*
	4	0	0	4 (3/4)	2*	2*
	5	0	0	4 (1/4)	2*	2*
	6	0	0	4 (1/4)	2*	2*

This table summarizes the mean gradings from three separate experiments. ApoA-I, ApoB, and ARP were stained and analyzed by indirect immunofluorescence as described in Methods. Intensity was graded from 0 to 5. Diffuse stain refers to smooth staining throughout the apical cytoplasm of cells in the upper three-fourths of the villus. Golgi corresponds with granular staining in areas of supranuclear cytoplasm of cells in the upper three-fourths of the villus. Grains refers to punctate staining throughout the cytoplasm of cells in the crypts. The fraction in parentheses indicates the proportionate distance from base of crypt to tip of villus in which cells contained staining. The asterisk refers to sections in which stain clearly surrounded fat droplets.

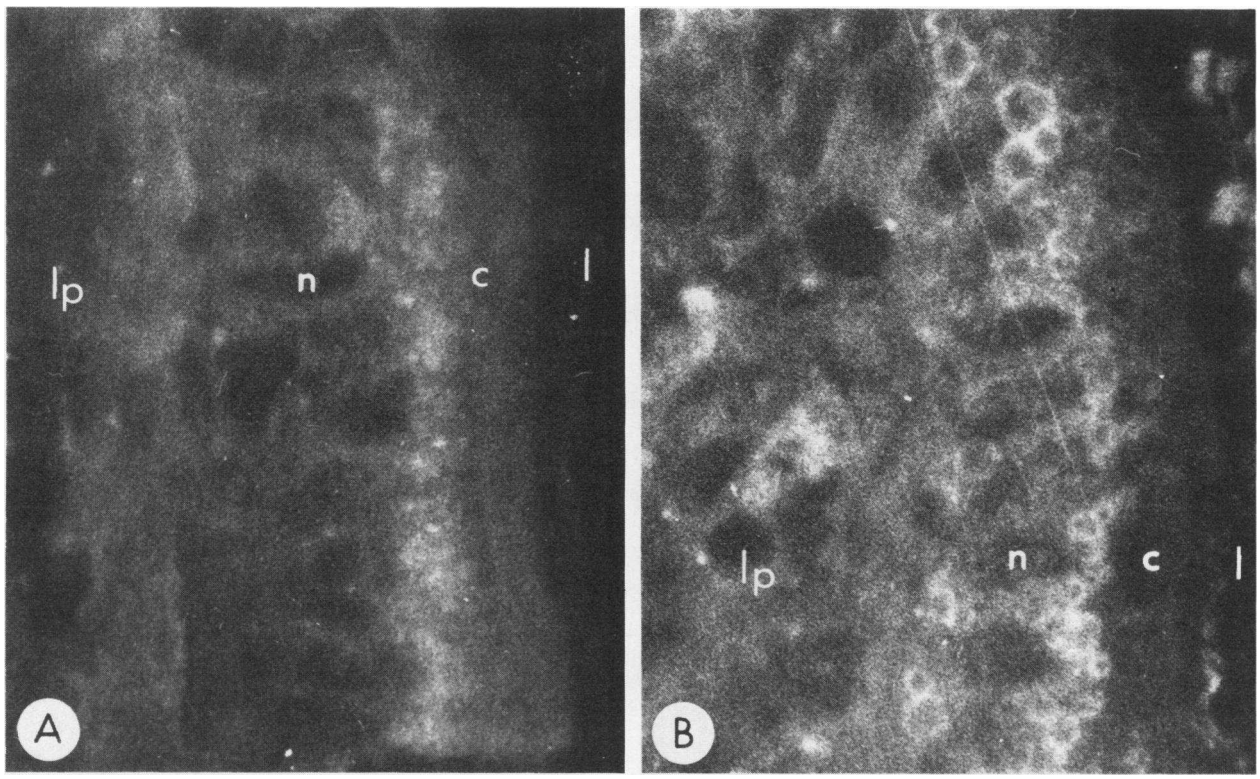


FIGURE 2 Immunofluorescent localization of ApoA-I near the tip of a jejunal villus. ApoA-I immunofluorescence in granules is apparent in the supranuclear regions of epithelial cells in the fasted rat (A). At 30 min (B), ApoA-I staining surrounds fat droplets primarily in the supranuclear regions of epithelial cells. Droplets are also seen in the infranuclear regions of epithelial cells. Nonspecific staining can be seen in the lamina propria. *l*, lumen; *n*, nucleus; *c*, cytoplasm; *lp*, lamina propria. Antiserum R 150-1, ×1,250.

pria also demonstrated some specific fluorescence surrounding fat droplets (not shown). At 3–6 h (not shown), there were many more bright droplets in both the apical and basal portions of epithelial cells and in the lamina propria, even in cells and lamina propria at the base of the villus. Over the same period of time, ApoA-I contents of jejunal mucosal scrapings rose from 70 to 414 ng/mg protein, whereas plasma levels of ApoA-I increased insignificantly by less than 1.5-fold (Table IV).

The fasted state and time sequence of immunofluorescence for ApoB was nearly identical to that seen with ApoA-I (Fig. 3 and Table III). At 30 min fluorescence surrounded fat droplets in the supranuclear portions of the cells (Fig. 3B). The degree of fluorescence was not so intense as with ApoA-I (Table III). Tissue ApoB levels rose only approximately twofold whereas plasma levels remained constant (Table IV).

The behavior of ARP was quite different. In the fasted state, there was no immunofluorescence in cells near the villus tip (Fig. 4A). Punctate immunofluorescence due to ARP was confined to epithelial cells at the base of the villus and in the subvillus glands (not

TABLE IV
Apoprotein Levels in Rat Jejunum during Fat Absorption

	Jejunum		Plasma	
Time	ApoA-I	ApoB	ApoA-I	ApoB
h				
0	70	117	22	32
0.5	73	177	17	22
1	110	237	27	29
2	170	224	31	18
3	312	199	28	22
4	414	302	27	19
5	533	278	29	22
6	592	257	26	18

Plasmas and homogenates of mucosal scrapings (105,000-g supernates) were analyzed for their apoprotein contents by radioimmunoassay. Tissue results are given as nanograms of apoprotein per milligram homogenate protein. Plasma values are milligrams per deciliter. Each time point represents a single animal. Similar data from 0 to 4 h were obtained from another set of animals.

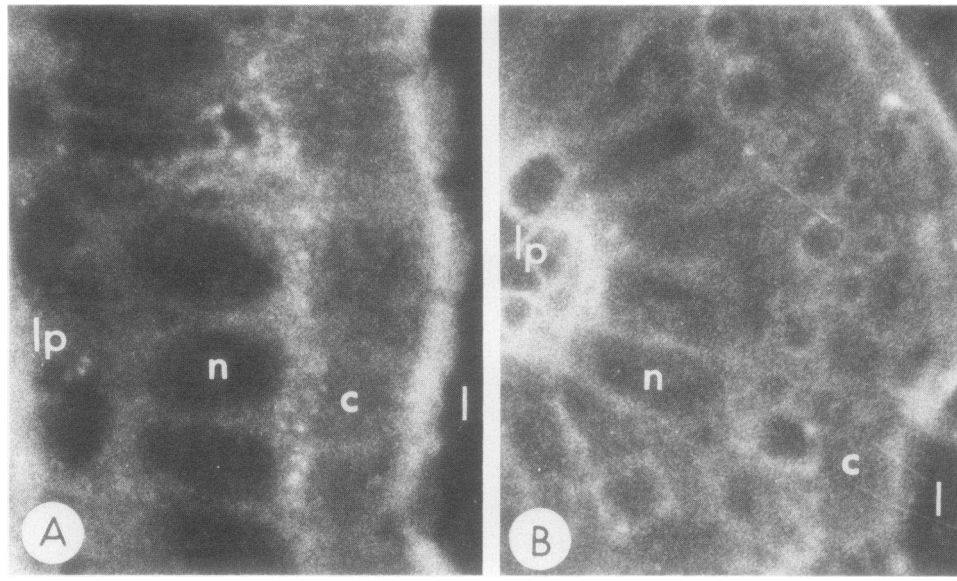


FIGURE 3 Immunofluorescent localization of ApoB at the tip of a jejunal villus. In the fasted rat (A), ApoB is seen as a granular stain primarily in the supranuclear regions of epithelial cells. There is nonspecific staining of the apical surface of the epithelial cells as well as the lamina propria in these sections. At 30 min (B), ApoB staining is seen surrounding droplets primarily in the supranuclear cytoplasm. Labels are the same as in Fig. 2. Antiserum R 176-1, ×1,650.

shown). Even at 30 min (Fig. 4B), ARP staining was still punctate and confined to the cells along the lower fourth of the villus (Table III and Fig. 5A). There was no staining around the intracellular fat droplets nor in cells at the villus tip. At 3–6 h, punctate staining involved more of the villus length (Fig. 5B) being seen in cells over as much as the lower three-fourths of the villus. However, even at that time when cells were engorged with fat and ApoA-I and ApoB fluorescence around fat droplets was intense, there was still no ARP staining around the intracellular droplets. Specific ARP staining was seen around fat droplets in only the lamina propria (Fig. 4C). The intensity of fluorescence after fat feeding was comparable to that seen for ApoA-I and ApoB (Table III).

In all control preparations, no granular staining in cells, or staining or droplets within cells or in the lamina propria was noted (Fig. 6). A variable amount of staining of the apical surface of mucosal cells and of the interstitial structure of the lamina propria was seen. The outline of fat droplets never demonstrated immunofluorescence when normal rabbit serum was used (Fig. 6B). These apoproteins were similarly identified with the above techniques in fasting rat duodenum and ileum, but not in stomach or colon.

The immunofluorescent localization of apoproteins in liver differed from that found in the intestine. Whereas ApoA-I staining in the intestine was bright, in liver the stain was only moderate in intensity (Fig. 7A). Carbohydrate feeding, which increases VLDL secretion by liver, did not alter the staining of ApoA-I. ApoB and ARP were both stained brightly in the hepatocyte, particularly near the bile canalicular portions of the cell (Fig. 7B, C).

DISCUSSION

Unequivocal identification of individual apoproteins in tissues requires that antisera be monospecific. It is helpful to this end that immunizing antigens be pure, and it is essential that the specificities of the antisera obtained be adequately characterized. The antigens used here were homogeneous in two or three electrophoretic systems (Fig. 1). Similar results have been reported by others (6, 7, 17, 18, 39). The specificities of the antisera were tested by double-antibody immunoprecipitations (Tables I and II), and in the immunofluorescence assay before and after absorption by appropriate apoproteins. By both of these criteria specificity appeared to be adequate. The adequacy of the immunofluorescence assay itself was tested by including several commonly accepted controls. Thus, the specificity of the localizations appears to be established.

Chylomicron and VLDL formation in the intestinal epithelial cell during fat absorption is a complex process requiring: (*a*) the esterification of lipids, (*b*) the synthesis of new proteins, (*c*) the assembly of lipids and apoproteins, and (*d*) the secretion of the assembled product (1). On electron microscopy (40–42), within minutes of the ingestion of a fat-containing meal, lipid

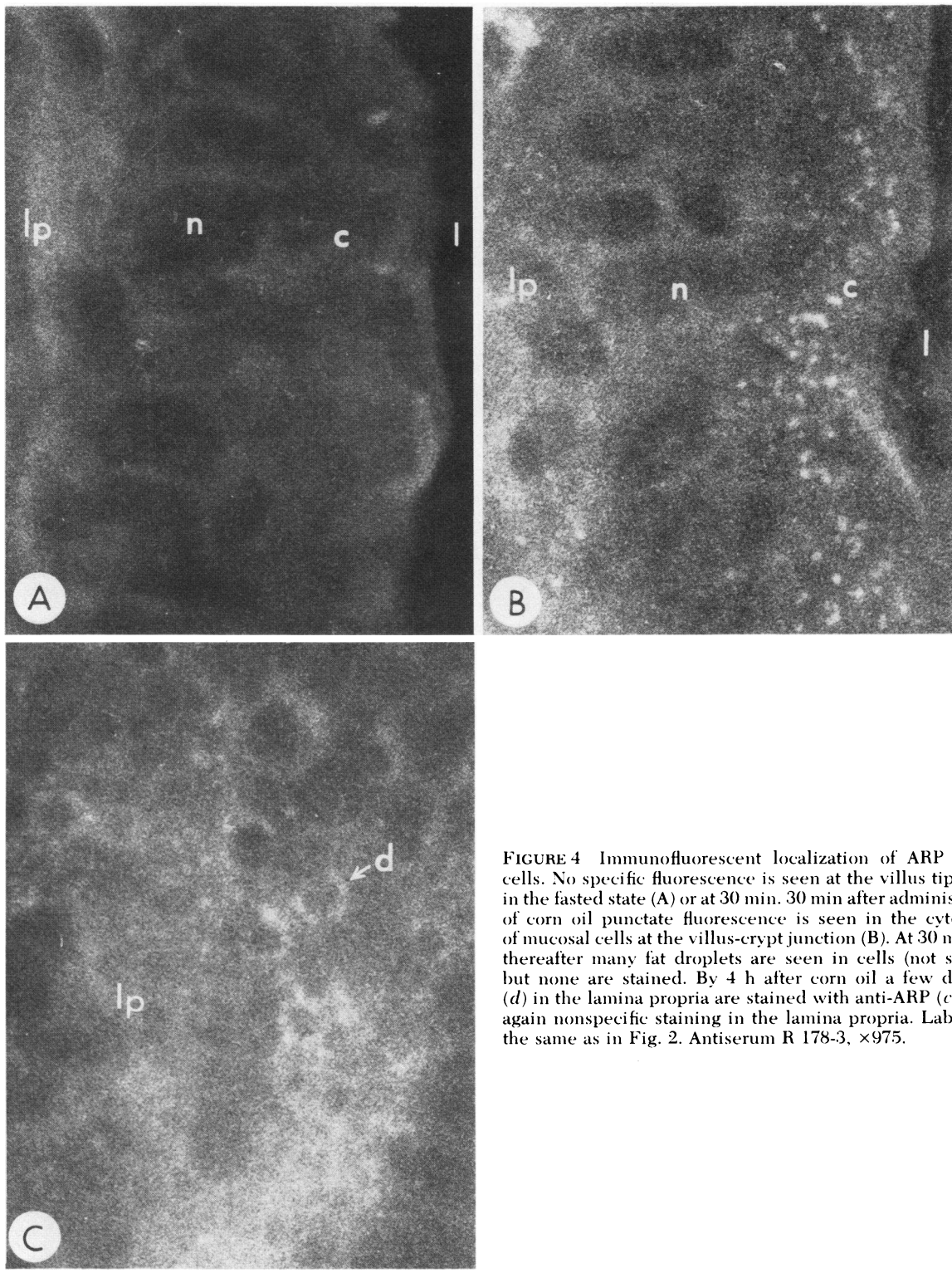


FIGURE 4 Immunofluorescent localization of ARP within cells. No specific fluorescence is seen at the villus tip either in the fasted state (A) or at 30 min. 30 min after administration of corn oil punctate fluorescence is seen in the cytoplasm of mucosal cells at the villus-crypt junction (B). At 30 min and thereafter many fat droplets are seen in cells (not shown), but none are stained. By 4 h after corn oil a few droplets (*d*) in the lamina propria are stained with anti-ARP (*c*). Note again nonspecific staining in the lamina propria. Labels are the same as in Fig. 2. Antiserum R 178-3, ×975.

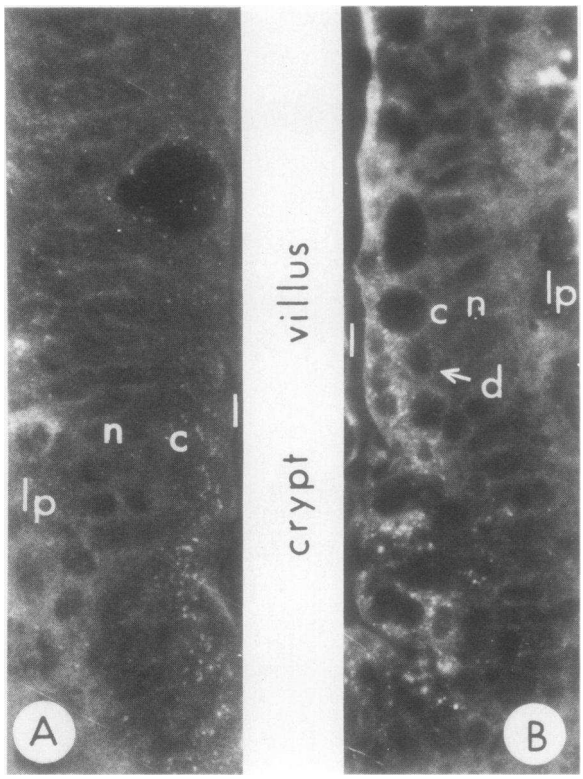


FIGURE 5 Immunofluorescent localization of ARP along the villus. At 30 min after corn oil intracellular granules specific for ARP are seen in the mucosal cells of the crypt and along the lower one-fourth of the total villus-crypt height (A). By 4 h, granules are seen in cells further up the villus (B). The area illustrated corresponds to about one-fourth of the entire villus-crypt height (average 10 cells). Cells at the villus tip were never seen to stain for ARP. Even at 4 h, no fat droplets (*d*) were seen to be surrounded by specific staining. Nonspecific staining of the lamina propria can be seen. Labels are the same as in Fig. 2. Antiserum R 178-3, ×875.

globules are seen beneath the brush border membrane. These migrate toward the supranuclear region of the cell accumulating in the various portions of the Golgi apparatus, including the secretory vesicle. Lipoproteins are extruded from the latter structures into the lamina propria, where they find their way into the lacteals. It is not known whether the two lipoproteins are distinct classes under independent control, or merely two ends of a broad density continuum. Indeed, it has not been possible to distinguish the intracellular production of chylomicrons and VLDL from each other. Therefore, our findings may be applicable to one or both of the lipoprotein classes under discussion.

ApoA-I and ApoB were clearly demonstrable in intestinal mucosa by radioimmunoassay and in the Golgi region of the gut epithelial cells by immunofluorescence, even in the fasted state when VLDL secretion proceeds at a low rate and chylomicron formation is minimal (43). Obviously even this low level of lipoprotein production is sufficient to maintain detectable pools of apoproteins within the cell. Fat feeding rapidly altered the picture. Initially, fluorescent staining of the cell increased and intracellular fat droplets were surrounded by apoproteins. Later, stained droplets were seen throughout the width and height of the villus. There were concomitant rises in tissue levels of ApoA-I and ApoB which indicate that the increases in fluorescence were indeed due to increased tissue contents rather than to changes in the immunoreactivities of the apoproteins. These findings suggest that: (*a*) ApoA-I and ApoB were acquired by lipoproteins before they entered the Golgi regions and (*b*) that lipoprotein lipids were accompanied by ApoA-I and ApoB as they were secreted from the cells. The rises in tissue levels are also compatible with the notion that both apoproteins were newly synthetized during fat absorption, although the magnitude of the changes may represent both synthesis and recycling of apoproteins from plasma. Incorporation of radioactive amino acids into ApoA-I (22, 44) and what may be ApoB (23) provide further proof for apoprotein synthesis by intestinal cells.

The contrasting behaviors of ApoA-I and ApoB on the one hand and Arp on the other are striking. Little ARP staining was seen in the intestinal epithelial cells of fasting animals, and what there was of it seemed to be confined to the lower parts of the villi and to the glands. No stain was ever seen to surround droplets inside of cells even at the height of chylomicron formation. This was so despite the fact that the degree of ARP fluorescence within cells was significant after fat feeding and exceeded that of ApoB. Although ARP levels in tissue cannot be measured at this time, it is reasonable to assume that fluorescence and tissue levels would be correlated, as in the case of ApoA-I and ApoB (Tables III and IV). Droplets stained with ARP were seen only in the lamina propria.

Clearly the roles of ApoA-I and ApoB differ from that of ARP. ApoA-I and ApoB seem to be intimately connected with fat absorption and lipoprotein production, and the intestine may contribute importantly to the circulating pools of these apoproteins (21, 23). ARP may not be involved in chylomicron and VLDL formation under these conditions although staining within mucosal cells increases with fat feeding. The stain seen in the lamina propria may be due to ARP secreted separately from the intestinal cell, or from ARP secreted by liver and found in intestinal lymphatics.

We found ApoA-I fluorescence in abundance in the intestinal epithelial cell, but little ApoA-I staining in hepatocytes. Recently Felker et al. (45), reported that the rat liver secretes approximately 10 times as much ARP as ApoA-I. The intestine stains heavily for ApoA-I

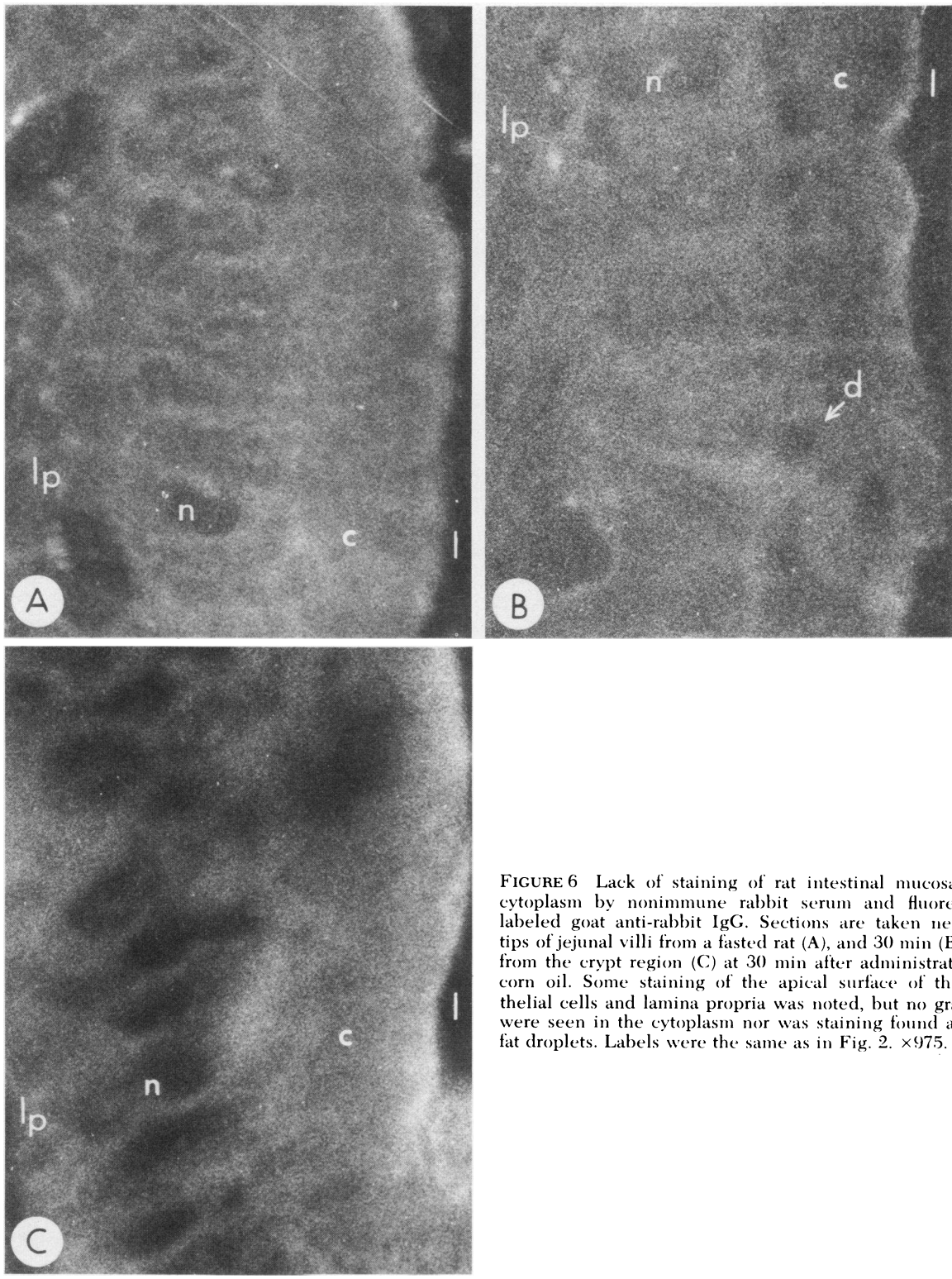
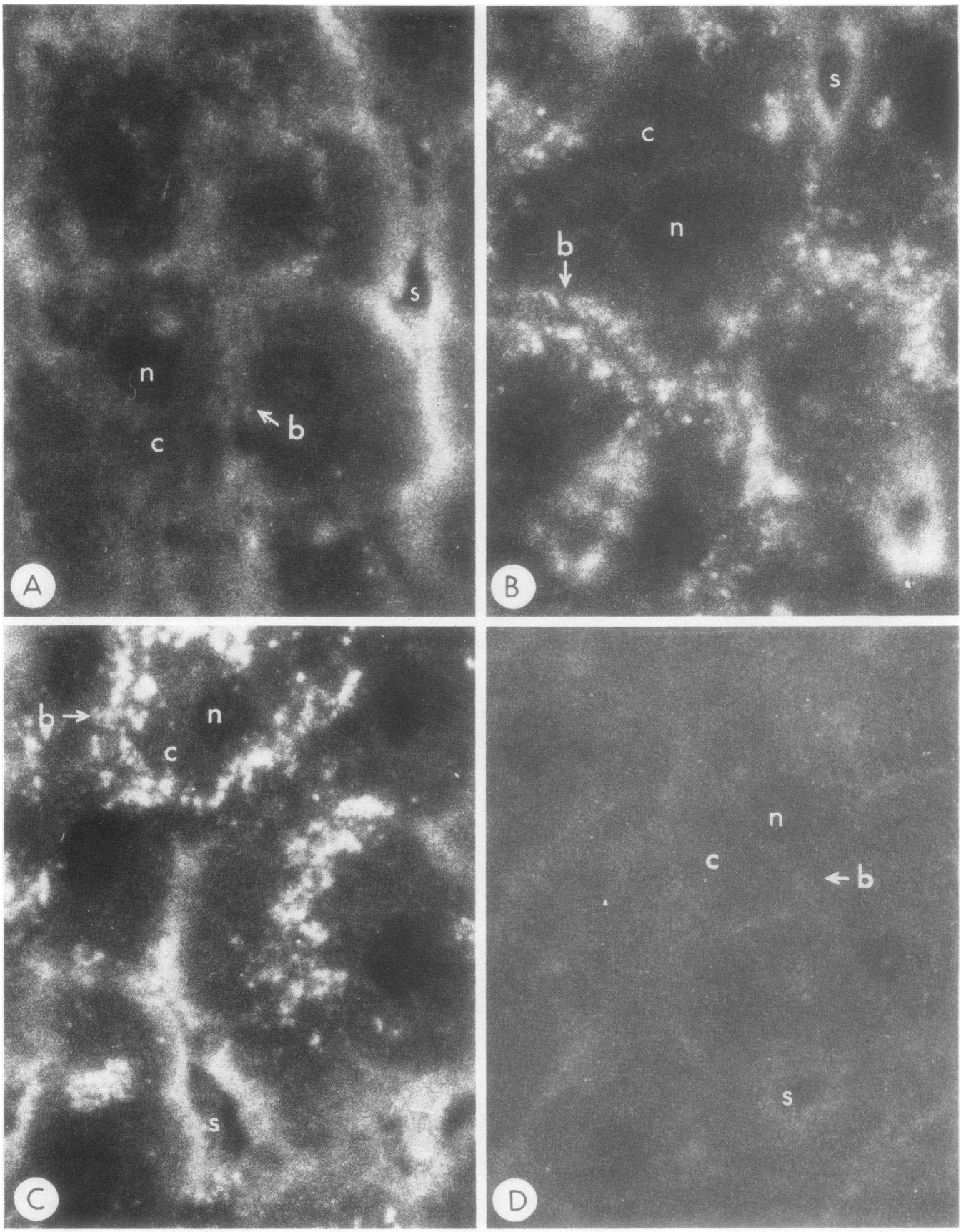


FIGURE 6 Lack of staining of rat intestinal mucosal cell cytoplasm by nonimmune rabbit serum and fluorescein-labeled goat anti-rabbit IgG. Sections are taken near the tips of jejunal villi from a fasted rat (A), and 30 min (B), and from the crypt region (C) at 30 min after administration of corn oil. Some staining of the apical surface of the epithelial cells and lamina propria was noted, but no granules were seen in the cytoplasm nor was staining found around fat droplets. Labels were the same as in Fig. 2. ×975.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and only very lightly for ARP. Glickman et al. (4, 22), found the predominant apoprotein of chylomicrons to be ApoA-I, whereas ARP was present but much less abundant. Similar findings have been reported by Fainaru et al. (5, 12). These data support the idea that under the conditions studied, the gut secretes ApoA-I preferentially, whereas the liver preferentially secretes ARP. The findings of Rooke and Skinner (44) that both tissues are capable of synthesizing ApoA-I suggest that the differences between gut and liver may be quantitative rather than qualitative.

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FIGURE 7 Immunofluorescent localization of ApoA-I (A), ApoB (B), and ARP (C) in liver taken from a 14-h fasted rat. Identical results were obtained at various times after intragastric administration of corn oil. The antisera used were R 150-1, R 176-1, and R 178-1, respectively. ApoA-I stain in hepatocytes is faint and granular, whereas ApoB and ARP staining is prominent and granular (*n*, nucleus; *c*, cytoplasm). Much of the stain is concentrated near the bile canalicular regions of the cells (*b*). Bright, diffuse staining of sinusoids (*s*) is seen in all sections. Nonspecific staining with normal rabbit serum (D) is faint. No granular fluorescence is seen. ×1,250.

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