

ELECTRON MICROSCOPE AUTORADIOGRAPHIC STUDY OF INTESTINAL ABSORPTION OF DECANOIC AND OCTANOIC ACIDS IN THE RAT

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ABSTRACT

Intestinal absorption of [^3H]octanoic acid and [^3H]decanoic acid was investigated in the rat by electron microscope autoradiography. The common duct (bile and pancreatic common duct) of the rats was diverted and a loop of the duodenum was cannulated 24 h later. The lipid mixture to be investigated was introduced into each experimental loop, and after 15 min or less the loop was removed. One part of each loop was used to determine the distribution of radioactivity in different lipid fractions, and an autoradiographic study was performed on the other part of the loop.

Radioactivity distribution studies confirmed that medium chain fatty acids are absorbed in their nonesterified form and established that these fatty acids are absorbed much more rapidly than oleic acid. Autoradiographic studies indicated that the medium chain fatty acids are taken up in a molecular or aggregate molecular form, leave the epithelial cells by way of the lateral plasma membrane, and are next found in the blood capillaries. Our results suggest that the Golgi complex does not play an important role in the absorption of unesterified fatty acids.

It has been shown that the absorption of fatty acids, which occurs in the upper part of the small intestine, differs according to the chain length of the fatty acids. Long chain fatty acids are esterified into triglycerides and delivered to the lymph in the form of chylomicrons. Short chain fatty acids are transported by the portal blood as free fatty acids bound to albumin. Fatty acids of intermediate chain length are transported by both the lymph and the blood, in a ratio related to the number of carbon atoms (3, 4, 5, 13, 19).

The different pathways of fatty acid absorption suggest the involvement of different subcellular components. Electron microscopy has been used by several groups (7, 17, 18, 25, 36) to study the

structures involved in the intestinal absorption of fats and particularly of long chain fatty acids. These studies, together with biochemical investigations, demonstrated that the apical vesicles of the absorptive cell are the site of triglyceride resynthesis (28, 29, 30, 31). Electron microscope autoradiography indicated that long chain fatty acids are taken up from a molecular or micellar dispersion (34, 35). Studies by Dermer (15) and from our laboratory (8, 11) have shown that long chain fatty acids enter the cell by simple diffusion. These studies have especially confirmed that the endoplasmic reticulum and the Golgi apparatus are involved in the intracellular transport of long chain fatty acids.

It appeared of interest to extend our studies to the absorption of medium chain fatty acids using electron microscope autoradiography (9) and biochemical techniques. This report deals with the absorption of [^3H]octanoic acid and [^3H]decanoic acid infused into a cannulated duodenal loop of the rat.

MATERIALS AND METHODS

Animal Preparation

Experiments were performed on male Wistar rats weighing 200–250 g. Bile and pancreatic juice were diverted by cannulation of the common duct. The animals were fasted but allowed to drink ad libitum. 24 h later, a 3–4 cm segment of duodenum was cannulated under light ether anesthesia, the proximal cannulation being 2–3 cm behind the entry of the common duct. The cannulated loop was washed with 5–10 ml of Ringer's solution maintained at 37°C.

Lipid Mixture

Tritiated octanoic acid and decanoic acid, labeled in 2- and 3- positions and of specific activity 20 mCi/ μmol , were obtained from the Commissariat à l'Energie Atomique, Saclay, France; the chemical purity of these acids was verified by gas-liquid chromatography (1, 12). The labeled acids were diluted to a specific activity of 160 $\mu\text{Ci}/\mu\text{mol}$ with unlabeled acids. Sodium tauroglycocholate (from ox bile) was obtained from the British Drug Houses Ltd, Poole, Great Britain; it was incorporated (18.2 g/liter) into the Ringer's solution used as mentioned below. The lipid mixture used in the absorption experiments consisted of 30 μmol α -monopalmitin, 30 μmol oleic acid, and 30 μmol (5 mCi) of one of the two labeled acids emulsified in 1.5 ml of Ringer's solution.

Experiment Procedure

The lipid mixture was introduced into the lumen of the duodenal loop, and uptake was allowed to proceed for 1.5, 2.5, 5, and 10, or 15 min. After this time, the isolated loop was rinsed with Ringer's solution, removed and divided longitudinally into two halves: one-half was used for the autoradiographic study, the other for biochemical analysis.

Biochemical Analysis

The intestinal mucosa was scraped off and the lipids were extracted by use of dimethoxymethane-methanol (4:1 vol/vol). Lipids were separated by thin-layer chromatography on silica gel according to Stahl et al. (32); the solvent system consisted of a mixture of petroleum ether, diethyl ether, glacial acetic acid, and methanol

(90:20:2:3 vol/vol). Small amounts of either unlabeled octanoic acid or unlabeled decanoic acid were added to the appropriate tissue extracts for easier localization of the free fatty acid fraction. The distribution of radioactivity in the thin-layer chromatography fractions was determined with a "Dünnschicht Scanner" Berthold, Wildbad, Germany LB 27–20. In several experiments, analysis of the mucosal fatty acids was carried out by gas-liquid chromatography (1, 12).

Preparation of Tissue for Electron Microscopy and Autoradiography

Intestinal wall samples were fixed in 4.16% glutaraldehyde for 16 h, washed three times in 0.33 M sucrose for 1 h, and postfixed in 2% osmium tetroxide for 1 h. All solutions were buffered to pH 7.25 with 0.1 M phosphate and all these operations were carried out at 4°C. Dehydration was performed at room temperature through successive changes of 30% ethanol (10 min), 50% ethanol (10 min), 70% ethanol (10 min), two changes of absolute ethanol (10 min each), and two changes of propylene oxide (10 min each). Infiltration was carried out with two changes of propylene oxide and Epon (1:1 vol/vol then 3:7 vol/vol, 45 min each), and the samples were then transferred into two changes of pure Epon for 1 h each and left overnight in pure Epon. The tissue samples were finally transferred to a fresh Epon mixture for 1 h and embedded.

For each tritiated fatty acid and each time interval, three or four of the embedded blocks were processed for autoradiographic studies. After the proximal part of the villus was identified from these blocks by light microscopy, sections were cut on a Reichert OM U2 (American Optical Corp., Scientific Instrument Div., Buffalo, N. Y.) ultramicrotome with glass knives and those sections showing silver to gold interference colors were chosen. Ribbons of those sections were mounted on 2% collodion-coated slides and were stained with 2.5% uranyl acetate in 50% ethanol and Reynold's lead citrate (26). A layer of carbon was applied over the sections (27), which were then covered with Ilford L4 emulsion by dipping the slides into the liquified emulsion, at 40°C, diluted 1:4 with double-distilled water. Exposure was carried out in lightproof boxes at 4°C in the presence of phosphoric anhydride. The slides were exposed for 6–14 wk in the case of [^3H]decanoic acid and up to 6 mo in the case of [^3H]octanoic acid. Specimens were developed in Microdol-X (Eastman Kodak Co., Rochester, N. Y.) for 5 min at 10°C, fixed in 30% sodium thiosulfate for 9 min, and washed with double-distilled water. The collodium membrane which supported the stained sections, the carbon layer, and the developed emulsion was stripped from the slide over a surface of double-distilled water. Grids were placed under the sections, dried, then taken up and examined with a Hitachi HU 11E electron microscope (20, 24).

Planimetry and Autoradiographic Results

Grain counts were performed on electron micrographs taken at an instrumental magnification of $\times 3,400$ and further enlarged two times. Micrographs were assembled to reconstitute the section of the upper third of the proximal part of a villus together with the neighboring lumen and the lamina propria. Such a reconstitution may include 4–30 micrographs according to the importance of overlappings and situation of sections over the grids.

The intestinal epithelial cellular profiles were divided into microvilli and the terminal web (apical cytoplasm), supranuclear apical cytoplasm including the unextended Golgi complex (c), Golgi complex when extended (g), nucleus with its nuclear membrane, infranuclear cytoplasm, and intercellular spaces. Silver grains present over the absorptive cells were counted and their distribution over the different sites listed above was expressed as percent of the total.

The intensity of the autoradiographic reaction was also expressed as number of silver grains per $100 \mu\text{m}^2$. For that purpose, areas were determined with a planimeter, and their total grain count was evaluated for each reconstituted section. The intensities for absorptive cell and lamina propria were designated by E and IV, respectively. Within the epithelial cell, C and G were the intensities of the autoradiographic reaction over supranuclear apical cytoplasm and Golgi complex, respectively; within the lamina propria, the intensity over blood capillaries was designated by S. To follow the rate of absorption of the labeled fatty acids, ratios of autoradiographic intensity in different parts of the villus (G/C, E/IV, and S/IV) were used.

RESULTS

Biochemical Study

Absorption data for octanoic acid and decanoic acid, after 15 min in the intestinal loop, are presented in Table I. The results obtained show that the uptake of both medium chain fatty acids is virtually complete at 15 min and more rapid than that of oleic acid.

The radioactivity recovered in the free fatty acid fraction of the mucosal lipids (32) was 94%–95% for decanoic acid and 91%–100% for octanoic acid regardless of the absorption time interval.

Autoradiographic Study

DECANOIC ACID: Overall morphological changes and silver grain distribution were followed after uptake times of 1.5, 2.5, 5, and 10 min. Within the cell, the grain count within subcellular structure was related to that found in the entire cell. After uptake of decanoic acid, little change appeared in the distribution of radioactivity over the absorptive epithelial cell (Table II).

Silver grains were observed over the absorptive cells and the lamina propria at all of the uptake times but with a different intensity (Figs. 1 and 10). The uptake of the medium chain fatty acid by enterocytes and its passage to the lamina propria occurred apparently at 1.5, 2.5, and 5 min and

TABLE I
Absorption of ^3H -Labeled Fatty Acids from Isolated Rat Intestinal Loops

Labeled fatty acid	Radioactivity taken up from the lumen*	Radioactivity recovered in mucosa	Radioactivity absorbed†
	%	%	%
Decanoic acid C10:0	100 – 1.5 = 98.5	1.0	97.5
Octanoic acid C8:0	100 – 2.1 = 97.9 (97.0, 98.7)	0.78 (0.85, 0.72)	97.1
Oleic acid C18:1	100 – 21.1 = 78.9 (77.5, 78.0, 79.0, 81.0)	4.9 (3.0, 5.0, 5.3, 6.3)	74.0

Percent of administered radioactivity *taken up* from the intestinal lumen by the absorptive cells, *recovered* in the intestinal mucosa, and *absorbed* 15 min after instillation into an *in situ* isolated loop of a lipid mixture (monoglyceride and two free fatty acids, one labeled with tritium).

Average data from different experiments are given in each column, and in the two middle columns the values found in the various experiments are given in parentheses.

* Radioactivity taken up equals the amount introduced (taken equal to 100) minus the average recovered amount into the loop content after experiment.

† Radioactivity absorbed equals the amount of radioactivity taken up from the loop content (first column) minus amount recovered in mucosa (second column).

TABLE II
Distribution of Silver Grains over Rat Absorptive Epithelial Cells

Time <i>min</i>	Microvilli and apical cytoplasm %	Supranuclear apical cytoplasm (c) and Golgi areas (g)		Nucleus %	Infranuclear area %
		g + c %	g/c %		
1.5	9.7 (6.3-14.3)	60.2 (56.4-65.8)	—	4.5 (3.2-5.9)	25.6 (22.2-30.4)
2.5	12.1 (7.0-17.3)	64.1 (63.5-64.8)	—	4.1 (1.9-6.8)	19.5 (17.3-21.8)
5	11.7 (10.3-13.3)	52.8 (52.7-52.9)	0.09 (0.06-0.12)	10.2 (9.8-10.6)	25.2 (24.3-25.2)
10	7.4 (5.1-10.8)	58.5 (48.6-67.8)	0.11 (0.06-0.19)	6.1 (4.2-7.9)	24.6 (18.1-30.5)

Percent of silver grains over different sites of rat absorptive epithelial cells, 1.5, 2.5, 5 and 10 min after introduction into an *in situ* isolated loop of an equimolar mixture of monopalmitin, oleic acid, and [2,3-³H]decanoic acid (5 mCi). Average data and range values (in parentheses) are listed in each column.

appeared almost complete at 10 min (Figs. 1, 3, 5, 6, and 10; Table III). Table III shows a decrease in radioactivity (grains/ μm^2) over the absorptive cells (from 10.9, 9.4, and 15.9 grains/100 μm^2 , respectively, at 1.5, 2.5, and 5 min, to 7.6 grains/100 μm^2 at 10 min) and a parallel rapid increase (3.6 grains/100 μm^2 at 1.5 min, to 5.6 and 6.7 grains/ μm^2 at 2.5 and 5 min) followed by a decrease (4.8 grains/ μm^2 at 10 min) of radioactivity over the lamina propria.

In the cell cytoplasm the labeling is distributed throughout, the heaviest concentration being in the supranuclear apical region (Figs. 1 and 3). At all times sampled, silver grains were found throughout the saccules of the endoplasmic reticulum closely associated with the mitochondria (Figs. 2 and 3), but it was not possible to determine in which organelle the radioactive molecule was incorporated. We did not observe any change in this last autoradiographic reaction during the transfer.

Whereas the cisternae of the Golgi apparatus become more extensively dilated (Figs. 2 and 3), the autoradiographic reaction at this level remained at a steady low level (Figs. 1-3, and 10). This is substantiated by the g-c ratios at 5 or 10

min (Table II) and the G-C ratio at 10 min (Table III).

The passage of tritiated materials over the junction of lateral plasma membranes and intercellular spaces can be seen in Figs. 1, 3, 5, and 6, and Table IV.

Within the lamina propria the lumen of the blood capillaries had consistently more radioactivity than the neighboring connective tissue and the lacteals; a ratio S-IV of 2.4 and 4.2 was observed at 1.5 min (Figs. 7 and 9, Table III).

The variations of ratios E-IV, G-C, and S-IV related to the different time intervals of tritiated decanoic acid absorption can be seen in Fig. 11 A and Table III. Examined with E and IV data, these ratios reveal that the tritiated material disappears very quickly from the absorptive cell and that the highest labeling of lamina propria as compared to epithelial cell occurs at 2.5 min after the beginning of decanoic acid absorption.

OCTANOIC ACID: The findings for octanoic acid parallel those for decanoic acid. The uptake and transfer of octanoic acid at 1.5, 2.5, 5, and 15 min appear to be very fast as seen from autoradiographs and the data shown in Table V and illustrated in Fig. 11 A. A clear decrease of the

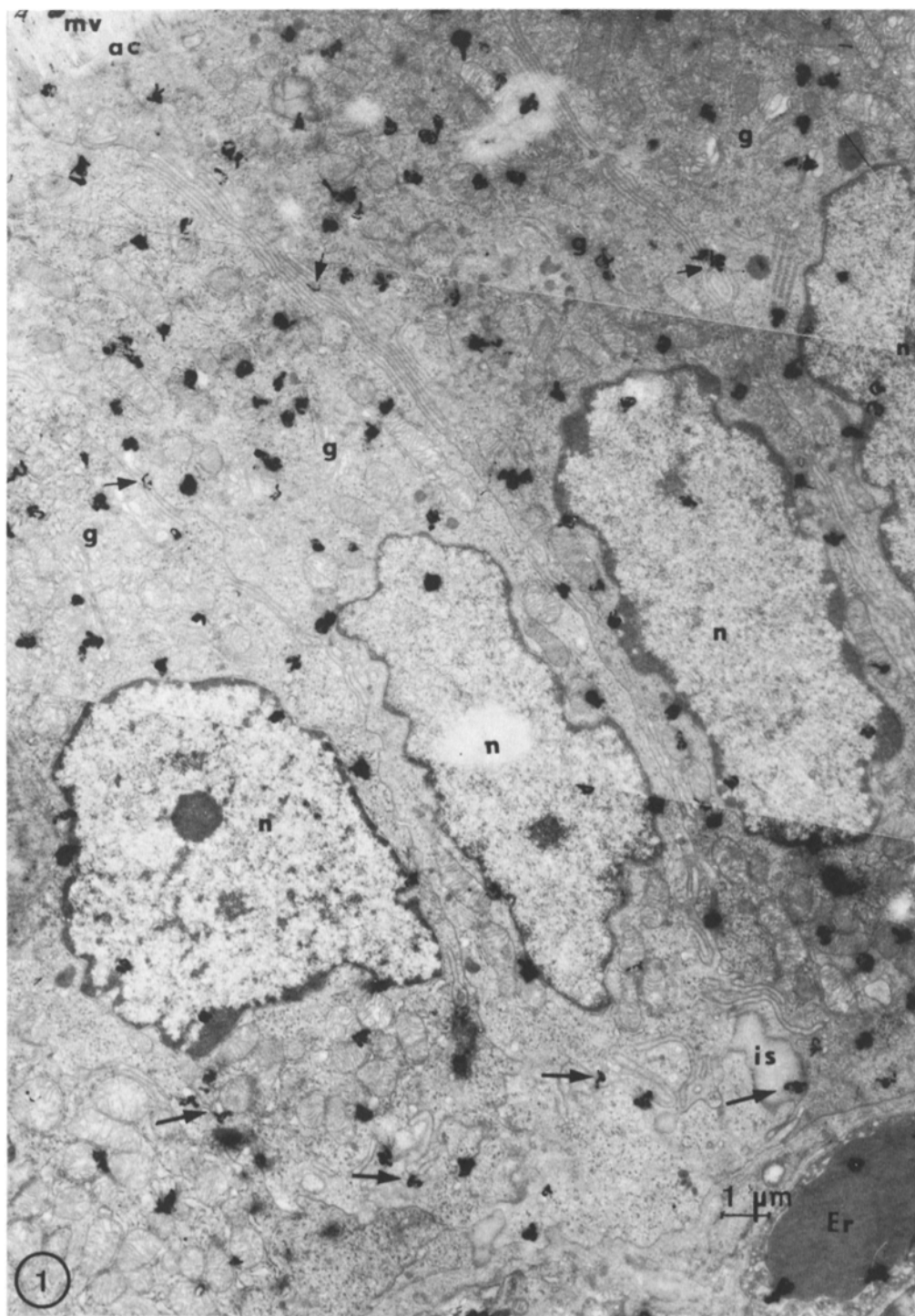


FIGURE 1 A low magnification overview of an electron microscope autoradiograph of rat duodenal absorptive cells 5 min after introduction of tritiated decanoic acid into an *in situ* isolated loop.

The heaviest concentration of silver grains is over the supranuclear region. The autoradiographic reaction appears over the microvilli (*mv*) and the adjacent apical cytoplasm (*ac*), over lateral plasma membrane and intercellular spaces (*is* and arrows) and all over the cell (nuclei (*n*) were slightly labeled). Erythrocyte (*Er*). $\times 6,800$.

TABLE III
Distribution of Radioactivity in Different Parts of the Rat Intestinal Villus

Time	Absorptive cells (E)	Lamina propria (IV)	E/IV	Supranuclear apical cytoplasm (C)	Golgi zone (G)	G/C	Blood capillaries (S)	S/IV
<i>min</i>								
1.5	8.4 (12/857)	3.7 (12/321)	2.2	11.4			15.4	4.2
	12.1 (301/2472)	4.6 (32/690)	2.6	19.5				
	11.5 (148/1258)	3.3 (33/1006)	3.5	20.0			8.0 (9/117)	2.4
	11.8 (379/2352)	2.9 (10/345)	4.0	17.5				
2.5	6.8 (284/4164)	4.7 (27/576)	1.45	9.4			(9/138 and 3/65)	1.0
	11.1 (72/648)	6.9 (15/218)	1.6	17.9			12.5 (4/32)	1.4 1.8
	10.3 (61/593)	5.2 (29/556)	2.0	13.9			10.0 (11/112)	1.9
5	15.8 (274/1735)	7.5 (113/1508)	2.1	21.3	33	1.5	11.9 (14/118)	1.6
	14.2 (187/1313)	6.7 (8/116)	2.1	17.1	37	2.1		
	19.1 (433/2264)	7.8 (81/1043)	2.4	24.3	17.8	0.7	12.3 (24/196)	1.6
	14.5 (377/2602)	5.0 (119/2354)	2.9					
10	9.5 (148/1559)	6.8 (84/1243)	1.4	13.2	24.4	1.8	10.6 (18/170)	1.6
	5.8 (122/2114)	3.5 (67/1892)	1.6	7.3	13.5	1.8	7.1 (47/660)	2.0
	7.0 (194/2763)	4.4 (85/1943)	1.6	9.0	17.4	1.9	9.1 (47/516)	2.1
	8.0 (190/2380)	4.4 (26/592)	1.8	10.8	7.6	0.7		

Distribution of radioactivity (number of silver grains per 100 μm^2) over absorptive rat epithelial cells (E) and over lamina propria (IV); over supranuclear apical cytoplasm (C) and Golgi zone (G) of absorptive cells and over blood capillaries (S), 1.5, 2.5, 5, and 10 min after introduction into an *in situ* isolated loop of an equimolar mixture of monopalmitin, oleic acid, and [2,3- ^3H]decanoic acid (5 mCi).

The radioactivity found over the intestinal lumen was 0.5–1.3 grains per 100 μm^2 . All these experiments were performed simultaneously with the same Ilford L4 emulsion. The number of silver grains encountered and the actual area measured in μm^2 are given in parentheses for (E), (IV), and (S) together with the average data for the first two columns.

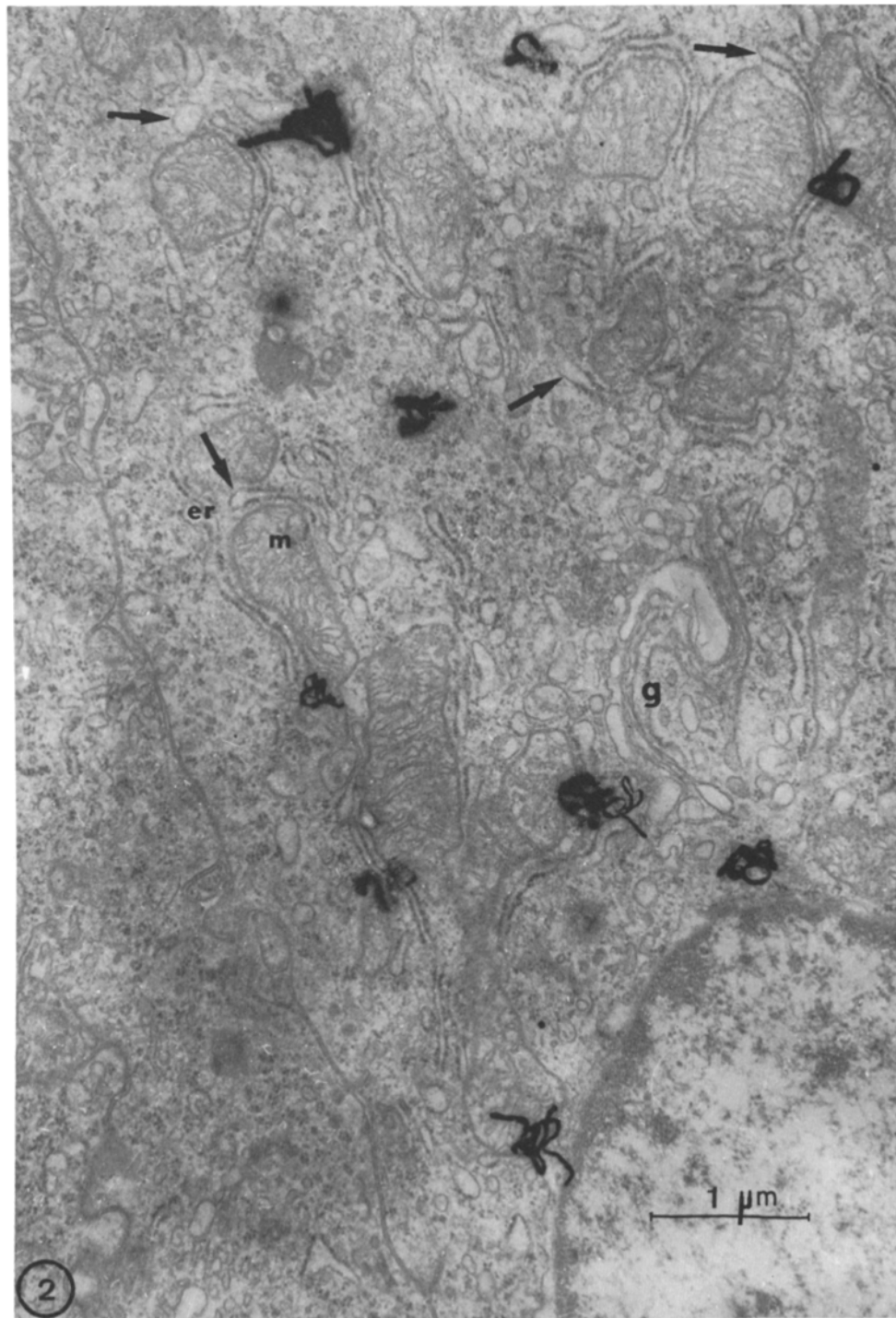
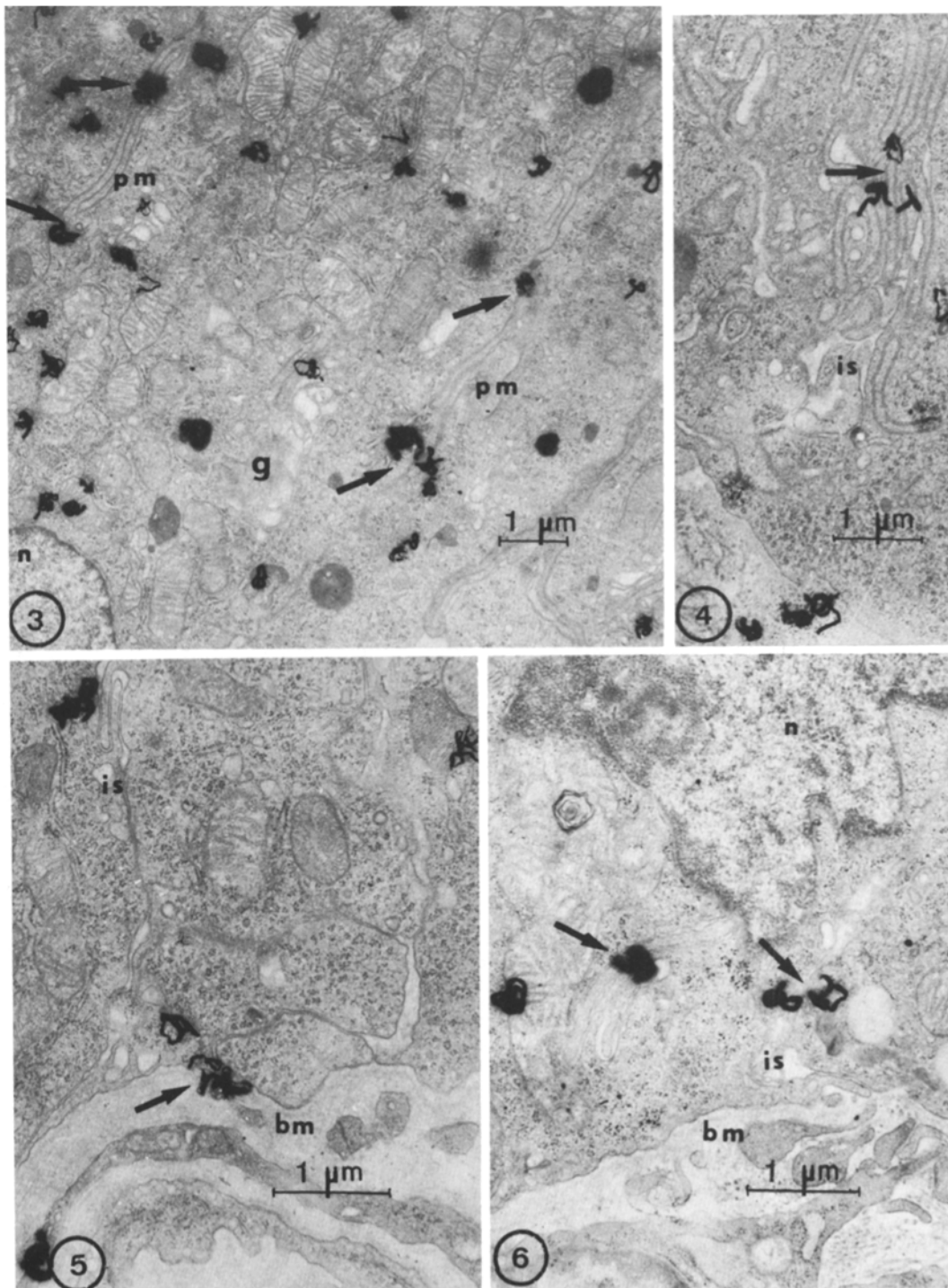


FIGURE 2 Electron microscope autoradiograph of the apical supranuclear cytoplasm of rat duodenal absorptive cell, 2.5 min after introduction of tritiated decanoic acid into an *in situ* isolated intestinal loop. Uptake of unlabeled monopalmitin, oleic acid at the same time as labeled decanoic acid can explain some ultrastructural modifications: endoplasmic reticulum is noticeably vesiculated with an irregular number of attached ribosomes (→); and the Golgi apparatus (g) shows a great number of vesicles. Associations of the endoplasmic reticulum saccules (er) and mitochondria (m) are visible. Silver grains can be seen all over the elements of the supranuclear area but are lacking over the Golgi apparatus. $\times 23,000$.



FIGURES 3-6 Electron microscope autoradiographs of rat duodenal absorptive cells 2.5 min (Fig. 5) and 5 min (Figs. 3 and 6) after tritiated decanoic acid uptake, and 15 min. (Fig. 4) after tritiated octanoic acid uptake. No accumulation of silver grains can be seen over the Golgi apparatus (*g*). Silver grains are visible over the lateral plasma membrane (*pm*), and at the supranuclear and Golgi area levels (\rightarrow) (Fig. 3). Nuclei (*n*) are present in Figs. 3 and 6. The autoradiographic reaction is seen also over the intercellular spaces (*is*) and lateral plasma membrane at the base of the epithelium where the basement membrane (*bm*) is visible, but never over the basal plasma membrane (Figs. 4-6). Fig. 3, $\times 10,000$; Fig. 4, $\times 14,000$; Figs. 5 and 6, $\times 17,000$.

TABLE IV
Output of Labeled Fatty Acid over Lateral and Basal Plasma Membranes

Time	Lateral silver grains (l)	Basal silver grains (b)	Percent of lateral silver grains*
min			
1.5	103	12	89.6 (75.0-96.0)
2.5	47	4	92.8 (84.6-100)
5	226	22	91 (85.7-100)
10	66	9	88 (85-95.4)

Silver grain distribution over lateral plasma membranes and intercellular spaces, and over basal plasma membranes of rat absorptive epithelial cells 1.5, 2.5, 5, and 10 min after introduction of an equimolar mixture of monopalmitin, oleic acid, and [2,3-³H]decanoic acid (5 mCi) into an *in situ* isolated intestinal loop.

* Percent of lateral silver grains obtained from 100 (l/l + b), the extreme values obtained are in parentheses.

silver grain density over absorptive cells can be observed while no significant increase appeared over the lamina propria (Table V).

Tritiated molecules were encountered over the lateral plasma membrane and intercellular spaces of the absorptive cells (Fig. 4), but were never found over the basal plasma membrane. After 5 min, blood capillaries were markedly labeled (Fig. 8) and autoradiographic micrographs revealed only a slight labeling of the Golgi complex.

DISCUSSION

Analysis of Labeled Mucosal Lipids

Experiments were performed with isolated intestinal loops left *in situ*, i.e. near-physiological conditions (14). This could explain the rapid fatty acid uptake and transfer by the absorptive cell (8). The 24-h fasting of animals before experimentation may also have contributed to the high absorption rate.

The data reported here corroborate previous reports that octanoic and decanoic acids are taken up from the intestinal lumen at a much higher rate than long chain fatty acids (palmitic or oleic acid)

(21). Furthermore, the radioactivity recovered in the mucosa reveals that the net absorption is also greater for the medium chain fatty acids (21). The presence of most radioactivity in the free fatty acid fraction of the mucosal lipids agrees with reports of an absence of esterification of medium chain fatty acids during intestinal absorption (3-5, 13).

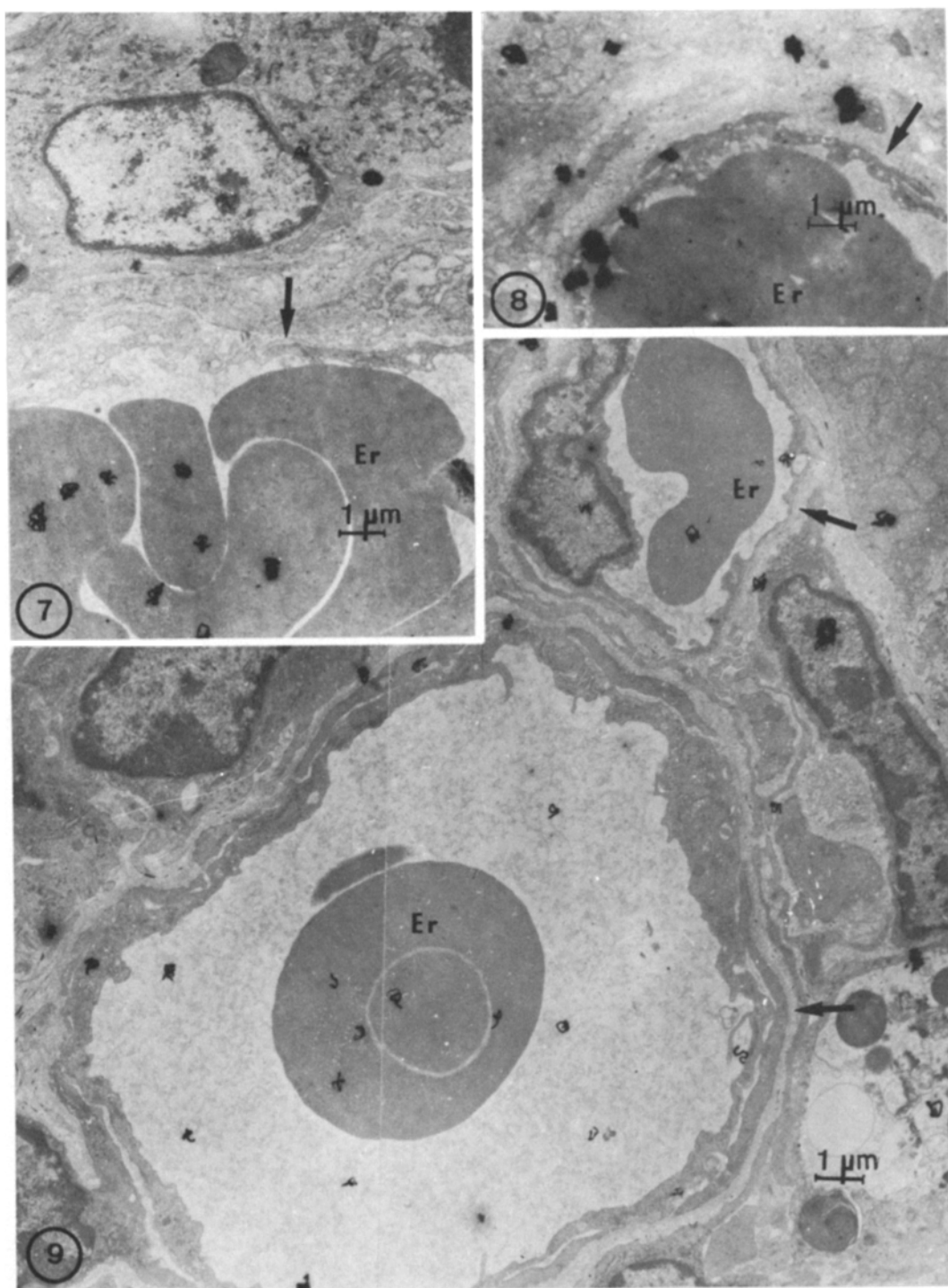
Autoradiographic Study of Decanoic and Octanoic Acid Absorption

VALIDITY OF RESULTS:—The silver grain density of the duodenal lumen at the edge of the villus sections under study was taken as the background level. This background level depends upon the effectiveness of washing and especially upon possible diffusion of labeled lipids during the infiltration and embedding procedures. In the diffusion in Epon was negligible during infiltration and embedding, the silver grain density over the edge lumen of the enterocytes should reflect accurately the true background level. In most cases the background level found was greater than the true one. From data reported in Tables III and V, the silver grain density of the lumen was very low: 3 grains or less per 100 μm^2 .

Assays were made to identify in chemical terms the labeled lipids responsible for the autoradiographs. Data from different workers (33, 37) and those from our laboratory (9) permitted us to anticipate a recovery of not less than 20% of the free medium chain fatty acids (9) and of 50% or more of the glycerides (37) during preparation of the tissue for electron microscopy.

Based upon the recoveries anticipated and upon the distribution of radioactivity in thin-layer chromatograms, it can be estimated that 87%–93% of the labeling was free decanoic acid when the uptake time did not exceed 10 min. Because of the small amount of glycerides synthesized, we conclude that the electron microscope autoradiographs reveal decanoic acid to be in the free form.

By the same reasoning, more than 90% of the radioactive silver grains represent free octanoic acid when the uptake time is 5 min or less. At longer uptake times (15 min and 20 min), a noticeable amount of label (9% at 15 min and 14% at 20 min) is encountered in the esterified lipids. This label was shown by gas-liquid chromatography to represent long chain fatty acids of 14 and 16 carbon atoms (9). It can be estimated that at these longer uptake times, only 71%–78% of the labeling was octanoic acid. Because of the presence of label



FIGURES 7-9 Electron microscope autoradiographs of the rat intestinal lamina propria 10 min after tritiated decanoic acid uptake (Figs. 7 and 9) and 5 min after tritiated octanoic acid uptake (Fig. 8). Whereas the autoradiographic reaction is slight over the neighboring lamina propria connective tissue, it is significantly apparent over the capillaries (erythrocytes [Er] and basement membrane [→]). Figs. 7-9, $\times 6,800$.

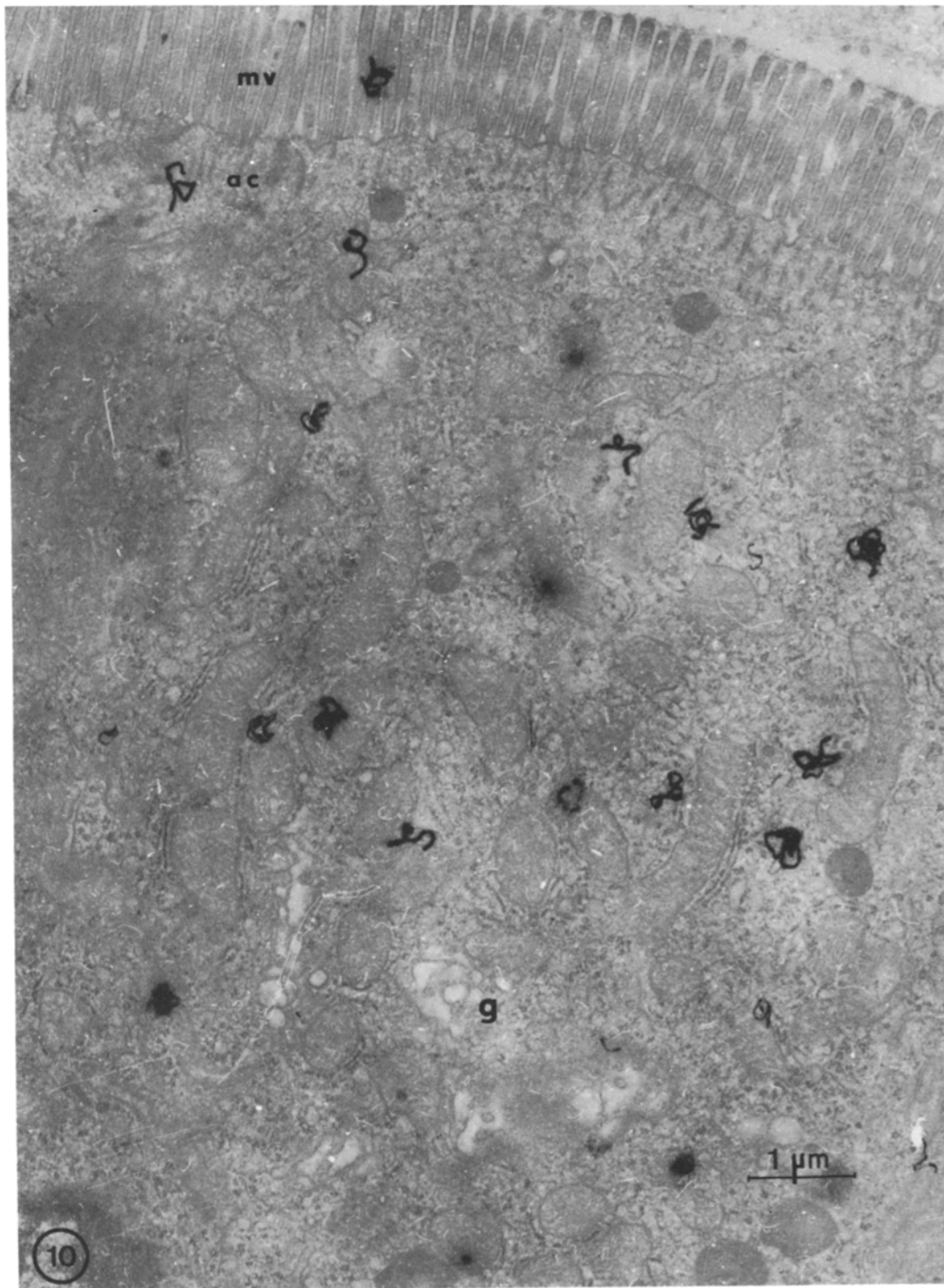


FIGURE 10 Electron microscope autoradiograph of the upper part of rat duodenal absorptive cells, 10 min after tritiated decanoic acid uptake. Silver grains are seen over microvilli (*mv*) and over adjacent apical cytoplasm (*ac*), and all over the supranuclear apical cytoplasm but are lacking over the Golgi area (*g*). $\times 16,300$.

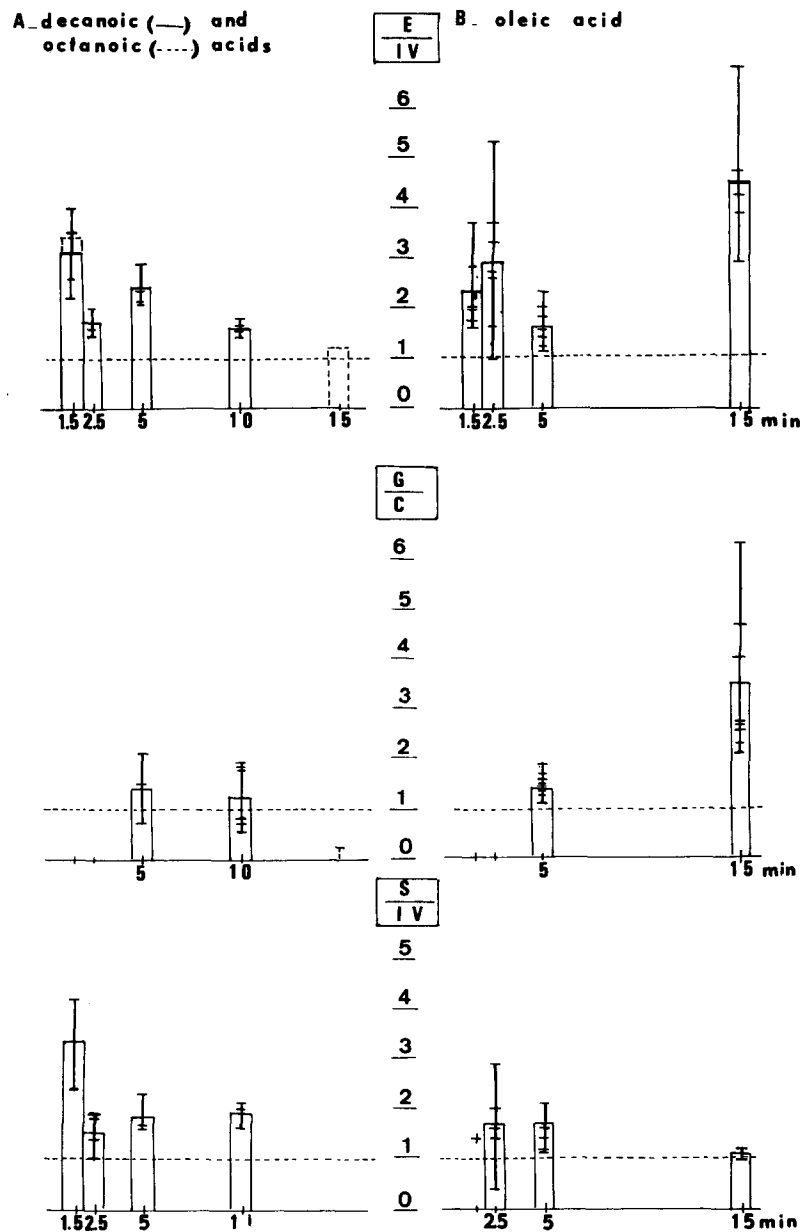


FIGURE 11 Change of E-IV, G-C, and S-IV ratios at 1.5, 2.5, 5 min, and either 10 or 15 min during the absorption of free fatty acids. *A* After introduction of an equimolecular mixture of monopalmitin, oleic acid, and either [^3H]decanoic acid or [^3H]octanoic acid (5 mCi) into an *in situ* isolated intestinal loop. *B* After introduction of an equimolecular mixture of unlabeled monopalmitin and palmitic acid and of [^3H]oleic acid (5 mCi) (8, 10 and unpublished observations). The bar represents the arithmetic average of the different values that are drawn as dashes along the vertical line (see Tables III and V).

TABLE V
Distribution of Radioactivity in Different Parts of
the Rat Intestinal Villus during Absorption of
[³H]Octanoic Acid

Time	Lumen	Absorptive cells (E)	Lamina propria (IV)	E/IV)
<i>min</i>				
1.5	3	18.3 (1206/6600)	5.4 (15/352)	3.4
15	1.7	7.5 (309/4120)	6.1 (32/560)	1.2

Radioactivity distribution, in number of silver grains per 100 μm^2 , over rat absorptive epithelial cells (E), and over lamina propria (IV) 1.5 and 15 min after introduction of an equimolar mixture of monopalmitin, oleic acid, and [2,3-³H]octanoic acid (5 mCi) into an *in situ* isolated intestinal loop.

The number of silver grains encountered and the surface area measured and expressed in μm^2 are given in parentheses. (For these experiments, the ratio g-to-c was always found to be equal to 0.03).

in long chain triglycerides, the interpretation of the results obtained for free octanoic acid, at longer uptake times, becomes doubtful. Therefore, to study transfer of free octanoic acid, only autoradiographic data at early time intervals were taken into consideration and only to confirm the results obtained with [³H]decanoic acid.

AUTORADIOGRAPHIC RESULTS:—Whatever the uptake time, silver grains were always found over microvilli and apical cytoplasm. The silver grain density was, at any time, significantly superior to that observed over the neighboring intestinal lumen. The silver grain density over microvilli and apical cytoplasm of the absorptive cells, which was low when compared to that of the supranuclear cytoplasm area, could be explained either by a more intense extraction of the labeled fatty acids at this level during tissue preparation as suggested by Buschmann and Taylor (6), or by a more rapid transfer of fatty acids through this part of the cell. This latter interpretation is supported by the fact that the autoradiographic reaction over microvilli and apical cytoplasm (terminal web) was greater at 2.5 min and lower at 10 min in the experiments with decanoic acid.

The ratio E-IV demonstrates that the autoradiographic reaction occurs predominantly over epithelial cells. Due to the unlabeled long chain fatty

acids in the lipid mixture, dilatation of endoplasmic reticulum saccules of the supranuclear apical cytoplasm, development of the Golgi apparatus, and evolution of the rough endoplasmic reticulum into a smooth one were observed on micrographs during absorption (7, 8, 25). This last transformation was more evident when the lateral plasma membranes were approached. The accumulation of tritiated material over the Golgi apparatus, which we and others had observed with oleic acid (8, 11, 15), was not observed with decanoic acid; in fact, the Golgi apparatus was even less labeled than the neighboring supranuclear apical cytoplasm. This result suggests that the Golgi complex does not play the same role in the absorption of long and of medium chain fatty acids (8).

It is pertinent to mention that, as soon as uptake begins, there is labeling of interdigitating membranes between adjacent absorptive cells, from the apical junction complex to the basal membrane. Such a result demonstrates a preferential channeling through the lateral plasma membranes by decanoic acid.

The autoradiographic reaction observed over the lamina propria indicates how rapidly the events of intestinal absorption and transport occur. This result, however, is consistent with the rapid uptake of long chain fatty acids and the release of triglycerides observed in the liver (23). The rapid early absorption via the blood is in agreement with previous biochemical reports: thus, Hashim (22) observed a radioactive peak in the portal vein at 15 min when sodium [¹⁴C]octanoate was administered to a dog by intraduodenal instillation, and Bloch et al. (2) observed such a peak 4 min after [¹⁴C]octanoate introduction into a rat jejunum segment. The radioactive reaction obtained with octanoic acid supports the observations obtained with decanoic acid and shows an extremely rapid uptake of this free fatty acid from the intestinal content, in agreement with the biochemical studies.

CONCLUSION

In conclusion, it is interesting to compare intestinal absorption of medium and long chain fatty acids. In this investigation, both medium chain fatty acids studied were taken from the intestinal content by the mucosa more quickly than oleic acid, a long chain fatty acid. Decanoic acid crossed the absorptive cell very quickly and was then found in the blood capillaries beneath the epithelium. This result, obtained by high resolution autoradi-

ography, emphasizes the property of decanoic acid of being absorbed via the portal venous blood in free acid form as had been biochemically established.

The pathway of oleic acid absorption seems to be different (8, 10). An output of this long chain fatty acid in the free form was suggested at the early stage of the absorption, when not masked by other phenomena and our previous results may suggest that this early absorption could occur via the blood capillaries (10). However, very soon after [^3H]oleic acid uptake, radioactivity was found in different lipid fractions of the mucosa, especially the triglycerides (8). This biochemical distribution was never seen associated with either [^3H]decanoic acid or [^3H]octanoic acid. Furthermore, the absorption of [^3H]oleic acid was accompanied by a concentration of silver grains over the Golgi apparatus and over the images of exocytosis. This observation is consistent with the view that the greater part of oleic acid is esterified, that it is involved in the formation of chylomicrons, and that their release from the absorptive cell occurred by reverse pinocytosis (11, 16, 18). In our present work with medium chain fatty acids, the autoradiographic intensity of the Golgi apparatus did not increase during the absorption, and the silver grains over the lateral membranes were never associated with images of exocytosis.

Whatever the fatty acid investigated, medium or long chain, the output from the absorptive cell occurs preferentially through the lateral plasma membrane (10). In the case of esterified long chain fatty acids, the release of chylomicrons by reverse pinocytosis seems to be located at the level of the Golgi complex. With free medium chain fatty acids, which are not esterified and not incorporated into chylomicrons, it would be of interest to establish whether the output takes place through the lateral plasma membrane immediately beneath the apical junction complex at the level of the supranuclear apical cytoplasm.

The role of the Golgi complex in the transfer process is not yet clear; according to the present autoradiographic study, this organelle seems only involved in the transfer of long chain fatty acids which are esterified and incorporated into chylomicrons.

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