Some factors affecting the lipid secretory phase of fat absorption by intestine in vitro from golden hamster

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Abstract We studied the secretory phase of fat absorption by sacs of the everted intestine in vitro when long chain fatty acid and monoglyceride had been taken up from a physicochemically defined bile salt micellar solution. After uptake, a sac received a supplemental incubation in a saline solution without added lipid. The presence of calcium ion in the medium during supplemental incubation was essential for the production and release of resynthesized triglyceride in the form of chylomicrons. The particles appeared to enter the serosal fluid via the lacteals. The rate of secretion of the lipid varied directly with the concentration of calcium ion in the mucosal fluid in the range 100–900 μM. At a given concentration in the serosal or mucosal fluid, the calcium ion in the latter fluid had the greater effect. The study of additional factors included the original location of the segment in the intestine of the living animal, temperature, use of D_2O instead of water, and the effects of Ba^{2+}, Sr^{2+}, Mg^{2+}, and La^{3+} on lipid secretion.—**Strauss, E. W., and J. S. Jacob.** Some factors affecting the lipid secretory phase of fat absorption by intestine in vitro from golden hamster. *J. Lipid Res.* 1981. **22:** 147–156.

Supplementary key words chylomicrons · secretion · sacs of everted intestine · calcium ion · lanthanum ion

The incubation of everted hamster intestine in a bile acid-lipid mixed micellar solution resulted in the cellular uptake of lipid, the resynthesis of TG, and the formation of cytoplasmic lipid droplets (1, 2), but not a quantitatively significant production and release of chylomicrons during the secretory stage of fat absorption. The addition of calcium ion in the incubation medium resulted in the efflux of chylomicron-like particles from the intestine of hamster (3) and the rat (4). Saunders and Sillery (4) found that apoprotein A-1 was secreted with the TG, suggesting that the intestine was producing lipoprotein. However, the precise physicochemical state of the medium containing lipid and calcium was ill-defined. The addition of calcium ion might have affected the lipid in the medium, the uptake of that lipid, as well as the secretion of the newly taken-up lipid by the cell.

In the present work, the medium contained lipid in a known physicochemical state. Sacs, uniformly taking up this lipid, were subjected to a supplemental incubation in fresh media without added lipid when calcium ion, as well as other experimental variables, might affect the secretory process. The results suggested that the addition of calcium ion was essential for the secretory phase of fat absorption. The divalent cation may promote exocytosis, as it seems to do in other types of secretory cells.

MATERIALS AND METHODS

NaTDC (Sigma Chemical Co., St. Louis, MO) was recrystallized ten times in 95% EtOH (5). Recrystallized bile salt and standards in amounts of 100 μg were applied separately onto a thin-layer plate which was developed according to the method by Hofmann (6). A single spot became visible for the recrystallized bile salt and standard. [1-^{14}C]Oleic acid (Applied Science Division, Milton Roy Company Laboratory Group, State College, PA) (sp act 50 mCi/mmol) was used as a tracer and was 99.2% pure FFA, as determined by quantitative TLC (7). Unlabeled oleic acid (Sigma) showed as a single spot of FFA upon TLC. Monoolein (Sigma) was resolved as a mixture of the 1- and 2-isomers, on boric acid-impregnated silica gel plates (8). Electrolytes and other reagents were of analytical grade. Deuterium oxide had a stated purity of 99.8% and was used as supplied (Sigma). Water was deionized and redistilled in glass.

Solutions

Each ml of bile acid-lipid, mixed micellar medium contained: 0.6 mM [1-^{14}C]oleic acid (sp act 1.7 mCi/

Abbreviations: TLC, thin-layer chromatography; NaTDC, sodium taurodeoxycholate; TG, triacylglycerol; FFA, free fatty acid; CE, cholesteryl ester; MG, monoacylglycerol; PL, phospholipid; DG, diacylglycerol; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol.

mmol), 0.3 mM monoolein, and 2.9 mM NaTDC in a bicarbonate-saline solution. The bicarbonate-saline solution consisted of: Na^+, 149.1 mM; K^+, 4.9 mM; Cl^-, 128.1 mM; HCO_3^-, 25.9 mM; and glucose, 11 mM. The pH was 7.4 after gassing the solution for 1 hr with a mixture of 95% O_2–5% CO_2 at a flow rate of 2 liters/min. Ca^{2+}, when present, was added as isotonic $CaCl_2$ to the bicarbonate-saline. In experiments with lanthanum ions, Tris (Sigma) was used to buffer a solution of the following ionic composition: Na^+, 125 mM; K^+, 5.0 mM; Cl^-, 130 mM; Tris, 5 mM; and glucose, 11 mM; the pH was 7.45 at 35°C. When isotonic $LaCl_3$ solution was included in the buffer, the pH was 7.30 at 35°C. The Tris-saline was gassed with O_2 for 1 hr at a flow rate of 2 liters/min. The saline was also used as the serosal fluid and in the preparation of the micellar incubation medium.

Experimental procedures

Male golden hamsters weighing 100 g were allowed free access to water and the regular diet (RMH 3000 Agway, Inc., Syracuse, NY). An animal was killed by a blow to the head, the peritoneal cavity opened, and the contents of the small intestine were washed out in situ with 50 ml of 0.9% NaCl. The intestine was stripped from the mesentery and placed in a Petri dish containing 0.9% NaCl. The gut was everted with a stainless steel rod and 50 ml of saline flushed through the "lumen". The intestine was washed for 30 sec in each of three dishes containing 200 ml of 0.9% NaCl, and for 15 sec in each of two beakers containing 100 ml of 0.2% NaTDC in 0.9% saline. Bile salt solution adhering to the tissue was removed by washing in 1000 ml of 0.9% NaCl, swirling with the aid of a magnetic stirrer. The everted jejunum was divided into three segments, each weighing about 100 mg, which were fashioned into sacs containing about 0.4 ml of bicarbonate-saline, with or without added $CaCl_2$, as serosal fluid. Each sac was washed vigorously for 15 sec in a beaker containing 100 ml of 0.2% NaTDC in 0.9% NaCl. Adhering bile salt solution was removed from the sac by washing in 1000 ml of 0.9 NaCl, swirling with the aid of a magnetic stirrer.

The sacs were subjected to a two-stage incubation. The first stage permitted the cells to take up ^{14}C-labeled oleic acid from a bile salt micellar medium. The second stage was used to test the effects of calcium ion or other experimental variables upon the secretory phase of fat absorption.

A sac was placed in a 25-ml Erlenmeyer flask containing 3 ml of a bile salt micellar solution. The container was gassed for 30 sec with a mixture of 95% O_2 and 5% CO_2 at a flow rate of 5 liters/min. The flask was stoppered tightly and incubated in a Dubnoff shaker, 95 oscillations/min, at 35°C. At the end of 5 min, the sac was removed from the flask, washed lightly for 5 sec in 1000 ml of 0.9% NaCl to remove adhering micellar solution, and drained on a hard filter paper. The serosal fluid was not changed. The sac was placed in a second flask of 25 ml or 1 liter capacity containing 3 or 300 ml, respectively, of bicarbonate buffer, plus 11 mM glucose, with or without added $CaCl_2$. The container was gassed for 1 min with the O_2–CO_2 mixture, and placed in the Dubnoff incubator for a supplemental period ("post-incubation") at 35°C. After 30 min, the sac was removed and the serosal fluid was aspirated by means of a 3-ml plastic syringe having a #26 gauge needle. Measurements of ionized calcium in the final mucosal fluids of sacs were performed at 23°C.

Chemical analyses

The serosal fluids were immediately extracted with chloroform–methanol 2:1 (v/v) (9). Solvents in the lipid-rich chloroform layer were evaporated under a gentle stream of N_2. The dried lipids were redissolved in 0.6 ml of benzene. Aliquots of 100 μl were added in duplicate to scintillation vials and, with TLC standards, to the origin of an activated 0.75 mm layer of silica gel G (E. Merck, Darmstadt, Germany) on a 20 × 20 cm glass plate. The plate was developed using the solvent system of Brown and Johnston (7). TG, FFA, and CE were identified and transferred into scintillation vials along with 10 ml of Aquasol (New England Nuclear, Inc., Boston MA). The amount of ^{14}C-label was determined in a liquid scintillation counter (Beckman LS 100, Beckman Instruments, Inc., Palo Alto, CA). The results in counts per min were equally quenched in part, making correction unnecessary. The recovery of ^{14}C-label from a TLC plate exceeded 95% of the amount applied originally.

Free ionized calcium determinations

A calcium-selective microelectrode and an MI-402 reference microelectrode (Microelectrodes, Inc., Londonderry, NH) were used with an Orion 801 digital pH meter in the mV mode (Orion Instruments, Inc., Cambridge, MA) to determine the concentration of free, ionized calcium.

Electron microscopy

In additional experiments, linolenic acid was substituted for oleic acid in the mucosal fluid for the first stage of incubation. The tissue was fixed in 2% osmium tetroxide in veronal-acetate buffer and processed for electron microscopy.

Statistical analyses

Differences between means were tested for statistical significance using Student's t test (10). Correla-

tion coefficients and regression line equations were calculated using regression analysis (10) and the statistical program of a TI-55 hand calculator (Texas Instruments, Inc., Dallas, TX). Statistical differences between regression lines were examined by an analysis of covariance using the F test (10).

RESULTS

Analyses of final serosal fluid

Table 1 indicates physicochemical properties of the secreted lipid. Final serosal fluid was centrifuged to yield an enriched fraction of lipid particles in the supranatant having the ultracentrifugal characteristics of chylomicrons (11). The ^{14}C-labeled lipid contained in excess of 90% TG, resembling the biochemical composition of chylomicrons (12). The ultracentrifugal procedure resulted in a three-fold concentration of lipid into the supra fraction, containing about 25–30% of the total lipid in the serosal fluid sample. The results confirm those previously presented for chylomicrons isolated from sacs (3). The infranatant fluid was not further characterized with respect to particulate lipid, but chemically resembled the supra lipid. The infra fraction probably contained incompletely recovered chylomicrons (12).

Effect of calcium ion concentration in mucosal fluid and location of segment in the intestine

The tissue specimens in various experiments possessed near-normal histological structure, except for tissues in medium without added calcium ion. In the last case, the mucosa degenerated with duration of incubation, as described previously (13).

Fig. 1 indicates lipid secretion by three sacs comprising the entire jejunum of a hamster. The secretion took place during the supplemental incubation in 3 ml of mucosal medium containing 0, 100, 900, and 2,500 μM Ca^{2+}. The serosal fluid consisted of the saline without added Ca^{2+}. For a single animal, a sac derived from a proximal segment of jejunum secreted more lipid into the serosal fluid than a sac fashioned from an adjacent distal segment. Lipid secretion by the three sacs comprised three points of a line having the form of a descending gradient, for each concentration of calcium ion in the mucosal fluid. Regression lines were calculated to fit the data at each level of calcium ion. Differences between altitudes (y-intercept) of the lines were statistically analyzed. Sacs incubated with 2.5 mM Ca^{2+} and 900 μM Ca^{2+} did not secrete lipid at statistically different rates ($P > 0.5$). Sacs incubated with 900 μM Ca^{2+} in the mucosal fluid translocated considerably more lipid than sacs incubated with either 100 μM Ca^{2+} ($P < 0.005$) or no added calcium ($P < 0.001$). The rate of lipid secretion by sacs bathing in 100 μM Ca^{2+} was also significantly greater than sacs incubated without the cation ($P < 0.05$). Total lipid secreted by sacs, with or without Ca^{2+} in the mucosal fluid contained 88–95% TG.

In general, the rate of lipid secretion depended directly upon the concentration of calcium ion in the mucosal fluid, and upon intrinsic properties of the tissues relating to their location in the intestine of the living animal. Lipid secretion was maximal between 0.9 to 1.2 mM, but small amounts of lipid

TABLE 1. Composition of lipid appearing in final serosal fluid[a]

Fraction[b]	Volume	Nmol Total Lipid[c]	Nmol Lipid/ml[d]	%CE[e]	%TG[e]	%FFA[e]	%DG[e]	%MG + PL[e]
Supra-1	0.45 ml	12.6	36.0	0.4	92.2	2.3	2.9	0.6
Supra-2	0.53 ml	6.3	11.9	0.1	91.4	2.1	3.8	0.7
Infra-1	3.50 ml	35.4	10.1	0.4	92.6	1.9	2.5	0.7
Infra-2	3.50 ml	12.7	3.6	0.9	90.6	2.7	2.5	0.6

[a] Two sacs of proximal jejunum, each derived from a hamster, were subjected to a two-stage incubation for 35 min at 35°C. The mucosal fluid for the supplemental incubation was the bicarbonate saline containing 1.0 mM Ca^{2+} as $CaCl_2$ solution. The serosal fluid was the bicarbonate saline without added calcium ion. Serosal fluid, 0.6 ml, was overlaid with 3.5 ml of 195 mM NaCl in a 4-ml polyallomer tube. The tubes were centrifuged at a force of 3.6×10^6 *g* min in a swinging bucket rotor (SB 405, International Equipment Co., Needham Heights, MA).

[b] After centrifugation, approximately 3.5 ml of the "infra" fluid was gently aspirated from the bottom of the tube with a 10-ml plastic syringe having an 8-cm, #16 spinal needle. The remaining 0.5 ml of "supra" fluid was collected and combined with three 1-ml washings of the tube using bicarbonate saline.

[c] Supra and infra fractions were extracted in chloroform–methanol 2:1 (v/v), and nmol of total lipid in the extracts were determined.

[d] Concentration of total lipid in supra and infra fractions was calculated as nmol of total lipid/ml.

[e] TLC of the extracted lipids was performed. ^{14}C-label appearing as CE, TG, FFA, DG, MG, and PL was determined and expressed as a percentage of total lipid.

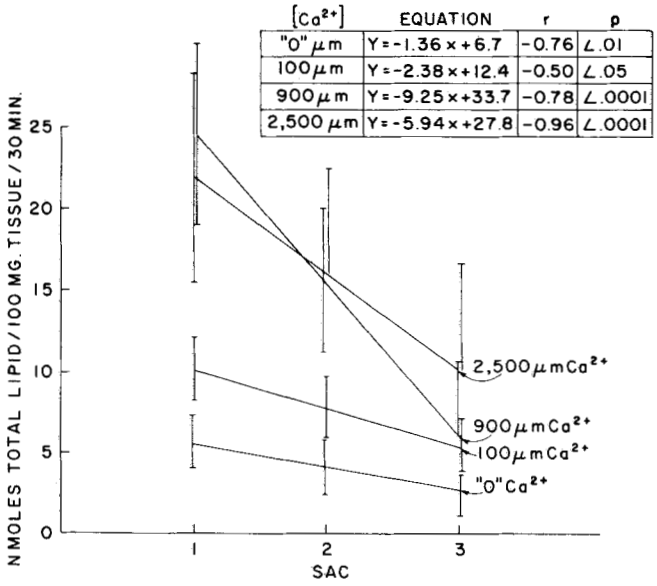

Fig. 1. Lipid secretion by everted jejunal sacs incubated with Ca^{2+} in the mucosal bathing fluid. Sac 1 was from more proximal intestine than sac 2 and sac 2 was more proximal than sac 3. Sacs were filled with bicarbonate-saline, thoroughly washed, and subjected to a two-stage incubation at 35°C under a gas phase of 95% O_2/5% CO_2. First, the sacs were incubated in a bile salt micellar solution containing [1-^{14}C]oleic acid and monoolein. After 5 min, sacs were lightly washed for 30 sec in 0.9% NaCl and then incubated for a supplemental period of 30 min. The mucosal bathing fluid during the postincubation was bicarbonate saline containing 0, 100, 900, and 2,500 μM $CaCl_2$. [1-^{14}C]Oleic acid appearing in the final serosal fluids was determined. Lipid secretion was expressed as nmol total lipids secreted by 100 mg wet weight of tissue in 30 min. Regression lines were calculated for lipid secretion by three sacs incubated with four levels of Ca^{2+} in the postincubation period. Correlation coefficients (r) for regression lines were all statistically significant at $P < 0.05$ or less. The number of points (N) used to calculate the regression lines were as follows: 0 μM Ca^{2+}, N = 6; 100 μM Ca^{2+}, N = 16; 900 μM Ca^{2+}, N = 15; and 2,500 μM Ca^{2+}, N = 6. Vertical bars represent 1 S.E.M.

appeared in the serosal fluid even when the mucosal fluid contained no added calcium ion. The ileum secreted very little lipid.

Time course for lipid secretion

Fig. 2 demonstrates the rates at which ^{14}C-labeled total lipid and TG appeared in the serosal fluid during the initial incubation, successive 10-min periods of supplemental incubation, and the total translocation for the entire period. Approximately 3.5% of the ^{14}C-labeled oleic acid in the initial micellar solution was translocated into the final serosal fluid, even though the period for uptake was only 5 min. Almost no lipid appeared in the serosal fluid during the first stage of incubation. The earliest appearance of TG in the serosal fluid was during the first 10 min of the supplemental incubation. If calcium had been included in the micellar medium, then translocation might have occurred during the early minutes after uptake, as indicated previously (3). The current data suggest that secretion was maximal at 15–25 min of incubation (10–20 min of the supplemental incubation). The time required for the appearance of chylomicrons in the serosal fluid might be partly accounted for by the time required for transport via the lacteals (**Fig. 3**).

The present results differ from the previously demonstrated time course, when lipid secretion increased progressively over a 1-hr period (3). The earlier experiment, however, used Ca^{2+} in a single-stage incubation having continuous uptake of lipid.

Criteria of effectiveness of an experimental variable

In the following experiments, we wanted to find out the effect of a specific variable or condition during "postincubation" on lipid secretion. The assay depended upon showing that, for a single animal, secretion rates of sacs 1, 2, and 3 did not have the relationship: sac 1 > sac 2 > sac 3 (see Fig. 1). If the variable significantly affected lipid secretion, then the

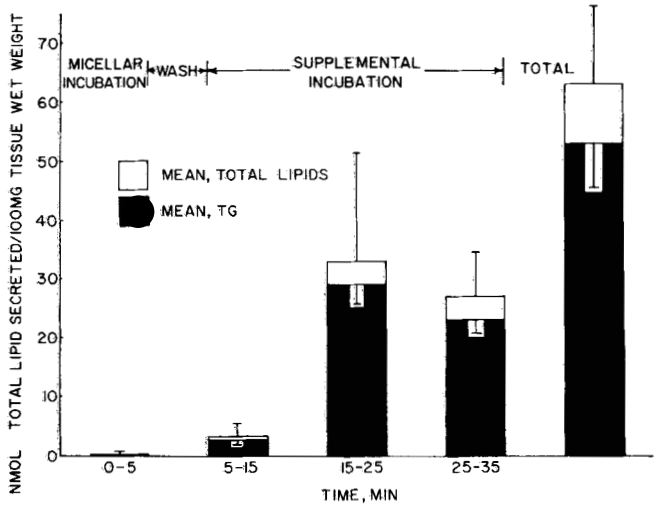

Fig. 2. Time course for secretion of total lipids and triglycerides. A segment of everted jejunum, corresponding in size and location to Sac 1, was cannulated with glass tubing and incubated in two stages as described in Experimental Procedures. The mucosal fluid during the supplemental incubation consisted of the bicarbonate saline with 1.0 mM $CaCl_2$. The serosal fluid was bicarbonate saline without added calcium ion. The serosal compartment was drained of its contents, washed three times with 1.0-ml volumes of bicarbonate saline, and filled with fresh serosal medium at the end of the preliminary incubation period and at consecutive 10-min intervals during the supplemental incubation period. Total lipids and triglycerides in the serosal fluids and washings were determined as detailed in Chemical Analyses. Lipid secretion was expressed as nmol of total lipids and triglycerides translocated by 100 mg wet weight of tissues ±1 S.E.M. The experiment was performed three times.

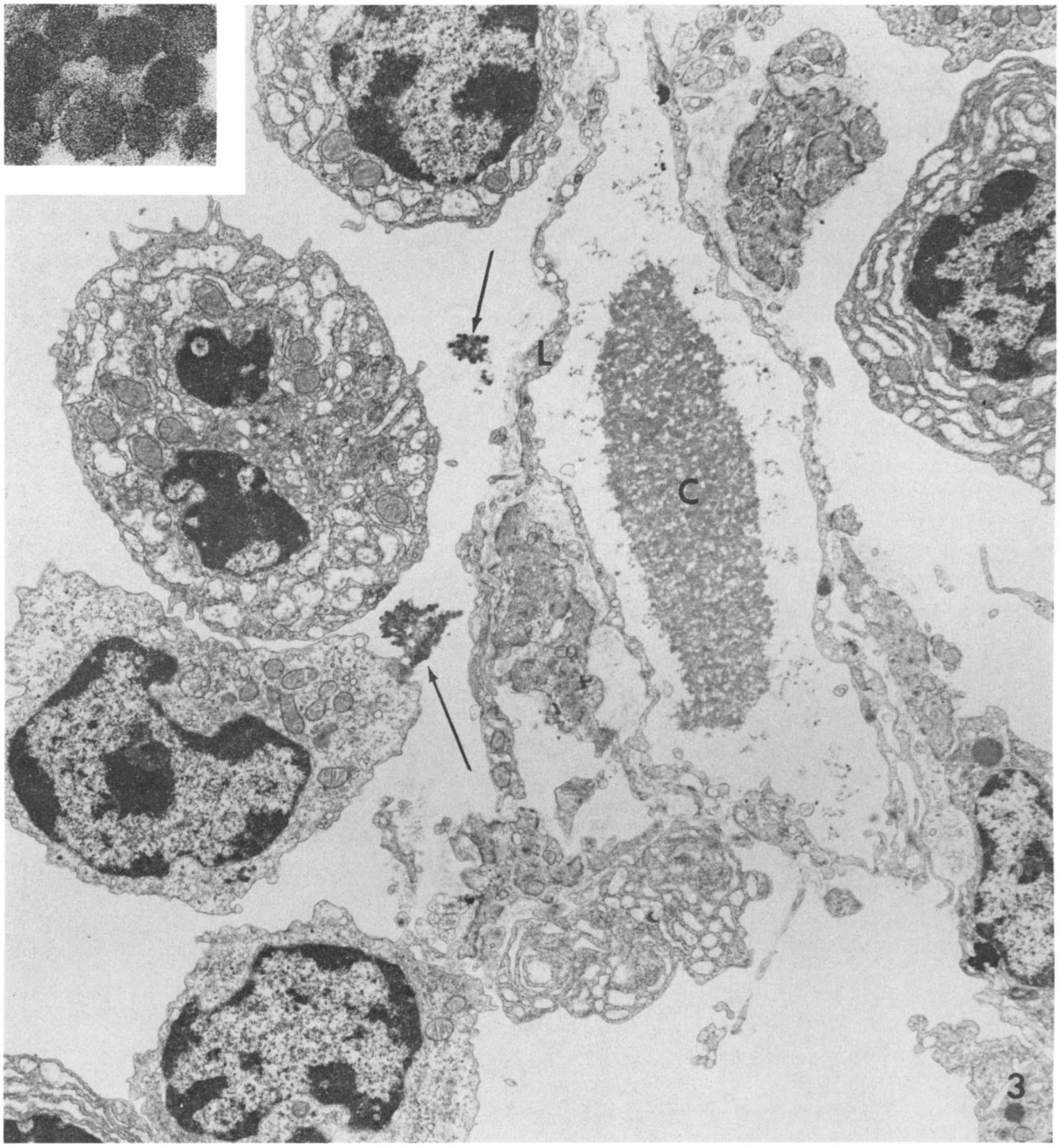

Fig. 3. Chylomicron-like particles (C) within the confines of a lymphatic capillary (L) in the lamina propria mucosae of a villus of hamster jejunum. The lacteal is distinguishable from a blood capillary because it has no basement lamina, and the wall is more attenuated and may have larger pores. Particles entered the lumen of the lacteal from the surrounding intercellular fluid where clumps of the particles of varying density (arrows) intermingle between lymphocytes and plasma cells. The particles may flow along the length of the vessel to enter the serosal fluid. The micrograph was taken from a sac of hamster jejunum that was initially incubated with linolenic acid-NaTDC micellar solution and supplementally incubated in medium containing added calcium ion at 35°C. Linolenate was used instead of oleate in the micellar medium to increase the electron density of the lipid particles after osmification. Magnification is approximately ×8,400. The inset is an enlarged region in Fig. 3 depicting the particles having a dense, granular rim (approximately ×70,000).

relationships between the sacs would be: sac 1 < sac 2 > sac 3, or sac 1 > sac 2 < sac 3.

Effect of temperature on lipid secretion

The optimal temperature for lipid secretion by the everted sac was 35°C. Lipid secretion also took place at 30°C, but at a lower rate. Practically no TG release took place at 23°C or lower temperatures.

Effect of D_2O upon lipid secretion

The effect of deuterium oxide upon the lipid secretory mechanism was tested by including nearly 100% D_2O in the mucosal and serosal fluids of a supplemental incubation. A bicarbonate-saline solution containing 1 mM $CaCl_2$ was prepared, as described in Materials and Methods, except that the solution used either D_2O or H_2O as solvents. D_2O severely impaired lipid secretion. The average lipid secretion for sacs 1 and 3, incubated with D_2O, was 1.3 ± 0.4 nmol of total lipid/100 mg wet weight of tissue (n = 4), compared with 14.4 ± 1.8 nmol (12.6 and 16.2) of total lipid/100 mg of tissue for sac 2, incubated with H_2O.

Effect of serosal calcium ion on lipid secretion

Addition of calcium ion to the serosal fluid enhanced lipid secretion by sacs (**Table 2**). Sacs incubated with 2.5 mM Ca^{2+} or 100 μM Ca^{2+} in the serosal fluid secreted considerably more lipid than sacs incubated without added calcium ion ($P < 0.02$ and $P < 0.005$, respectively). However, lipid secretion by sacs incubated with calcium ion in the serosal fluid was always less than that by sacs incubated with a similar concentration of calcium ion in the mucosal fluid (Table 2). The findings appeared to indicate that the mucosal surface was more sensitive to calcium ion than the serosal surface during lipid secretion.

It was unlikely that the lesser effect of Ca^{2+} in serosal fluid resulted from leakage of Ca^{2+} from serosal to mucosal fluid during the first incubation period. Using ^{45}Ca as tracer in the serosal fluid, less than 3% entered the micellar solution.

Conjecturally, the serosal insensitivity might have been absolute, with only the mucosal surface, presumably the microvilli, displaying sensitivity to calcium ion during lipid secretion. That is, leakage of calcium ion into the mucosal compartment might have been the mechanism of the serosal effect. To clarify the problem, we increased the mucosal volume from 3 ml to 300 ml. If there were leakage, then the large mucosal volume would minimize changes in calcium ion concentration that might enhance lipid secretion. Lipid secretion, under these conditions, would be due to an action of serosal calcium ion on the basolateral aspects of the absorptive cell.

Using 2.5 and 0.9 mM calcium ion in the serosal fluid, stimulation of lipid secretion was clear-cut, even though mucosal calcium ion concentrations were less than 10 μM (**Table 3**). When the serosal calcium ion concentration was 100 μM, there was very little lipid secretion and the final concentration of calcium ion in the mucosal fluid was again much less than 10 μM (Table 3). Histologically, the tissue deteriorated like the specimens that were incubated in mucosal fluid without added calcium ion. The observations indicated that the serosal surface was sensitive to calcium ion during lipid secretion. The reasons were not clear for the greater sensitivity of the mucosal surface.

Effects of barium, magnesium and strontium on lipid secretion

Barium chloride, when added to the mucosal bathing medium without $CaCl_2$, in concentrations of 0.09, 0.24, and 2.55 mM did not enhance lipid secretion. In levels above 0.39 mM, Ba^{2+} in the mucosal fluid,

TABLE 2. Effect of calcium ion in mucosal or serosal fluids

Sac[a]	[Ca^{2+}]	Location[b]	Lipid Secretion[c]
1	2.5 mM	Serosal	23.8 ± 8.8 (9.4,22.3,39.9)
2	2.5 mM	Mucosal	29.8 ± 10.1 (11.4,31.6,46.4)
3	2.5 mM	Serosal	11.6 ± 4.4 (6.1,8.4,20.2)
1	0.1 mM	Mucosal	19.1 ± 1.1 (18.0,20.1)
2	0.1 mM	Serosal	7.1 ± 1.1 (6.0,8.2)
3	0.1 mM	Mucosal	16.3 ± 0.5 (15.8,16.8)

[a] Sac 1 was more proximal than Sac 2, and Sac 2 more proximal than Sac 3.

[b] Ca^{2+} was included in the mucosal postincubation fluid or in the serosal fluid.

[c] Lipid translocation by sacs is expressed in mean nmol of total lipid/100 mg wet weight of tissue/30 min ± S.E.M. Individual values for lipid secretion are given in parentheses.

TABLE 3. Lipid secretion with 300 ml mucosal volumes

Sac[a]	Initial $[Ca^{2+}]$[b]	Location[c]	Lipid Secretion[d]	Final $[Ca^{2+}]$[e]
1	2.5 mM	Mucosal	41.7 ± 0.3 (41.4,41.9)	2.4 ± 0.1 mM (2.4,2.4,2.5)
2	2.5 mM	Serosal	13.4 ± 5.6 (7.5,19.2)	<10 μM [3]
3	2.5 mM	Mucosal	12.9	2.5 ± 0.1 mM (2.4,2.5,2.5)
1	0.9 mM	Mucosal	53.4 ± 4.4 (49.0,57.8)	—
2	0.9 mM	Serosal	14.5 ± 4.3 (10.2,18.8)	—
3	0.9 mM	Mucosal	17.0 ± 7.9 (9.1,24.8)	—
1	0.1 mM	Mucosal	22.0 ± 5.0 [5]	55 ± 15 μM (40,70)
2	0.1 mM	Serosal	1.9 ± 0.6 [5]	≪10 μM [2]
3	0.1 mM	Mucosal	16.7 ± 6.8 [5]	83 ± 3 μM (80,85)

[a] Sac 1 was more proximal than Sac 2, and Sac 2 was more proximal than Sac 3.
[b] Initial concentration of calcium ion present in the mucosal fluid at the beginning of the supplemental incubation, and in the serosal fluid at the beginning of the preliminary incubation.
[c] The calcium-bicarbonate medium was either the mucosal or serosal fluid.
[d] Lipid secretion was expressed as mean nmol of total lipids transported by 100 mg wet weight of tissue in 30 min ± 1 S.E.M. Individual values for lipid secretion are given in parentheses. Number of experiments is given in brackets.
[e] Concentration of calcium ion in 300 ml of mucosal fluid at the end of 30 min of supplemental incubation, as mean ± 1 S.E.M. Individual values are given in parentheses. Number of experiments is given in brackets.

without added calcium ion, caused severe degenerative changes in intestinal absorptive cells. Low concentrations of Ba^{2+} (0.09 mM) did not inhibit the secretory response to 1.2 mM Ca^{2+}.

Strontium ion enhanced the secretion of lipid, but less so than equimolar calcium ion (1.2 mM). Morphology of intestine incubated with strontium was normal.

Substitution of 1.0 mM Mg^{2+} for Ca^{+} did not enhance lipid secretion. Mg^{2+} (1.0 mM) and 0.1 mM Ca^{2+} did enhance lipid secretion, but the effect of the ion mixture was attributable to calcium ion.

Effect of lanthanum on lipid secretion

Lanthanum ion, in concentrations of 0.1, 0.5, and 1.0 mM in the mucosal medium, strongly inhibited the secretory response to 1.0 mM Ca^{2+} (**Table 4**). The trivalent cation alone did not enhance lipid secretion in concentrations of 0.5 or 1.0 mM in the mucosal fluid. Incubations with lanthanum were performed with a Tris-buffered saline to avoid the precipitation of lanthanum carbonate. Secretion of lipid by intestine incubated with Tris-saline was similar to that of tissue treated with the normal bicarbonate saline. Tissue incubated in Tris-buffered saline containing calcium or calcium and 0.1 mM La^{3+} had near-normal histology. The morphology of the villi that had been treated with 1.0 mM La^{3+} and Ca^{2+} suffered mildly.

Lipid esterification

The absorptive mucosa and final incubation media were combined and extracted for total lipids and analyzed for their content of ^{14}C-labeled TG after a supplemental incubation in Hepes-buffered saline with the following additions: *1*) 1.0 mM Ca^{2+}, *2*) no additions, *3*) 1.0 mM Sr^{2+} *4*) 1.0 mM Mg^{2+}, *5*) 1.0 mM Ca^{2+} + 100% D_2O, *6*) 0.3 mM La^{3+} + 1.0 mM Ca^{2+}, and 7) 0.3 mM Ba^{2+}. The degree of esterification was nearly constant under all conditions with 84 ± 2.5% (S.E.M.) of the total tracer as TG, suggesting that the ions affected transport rather than esterification.

Electron microscopy indicated many lipid droplets in enterocytes during the first stage of incubation. When there was secretion, the droplets disappeared.

TABLE 4. Effect of added lanthanum and calcium ions on lipid secretion

Sac[a]	$[La^{3+}]$[b]	Lipid Secretion[c]
1	1.0 mM	5.1 ± 2.6 (2.5,7.6)
3	1.0 mM	1.9 ± 0.1 (1.8,2.0)
1	0.5 mM	3.7 ± 1.2 [4]
3	0.5 mM	1.0 ± 0.2 [4]
1	0.1 mM	13.9 ± 2.0 (11.9,15.9)
3	0.1 mM	10.7 ± 6.4 (4.3,17.1)
2	0 mM (1.0 mM Ca^{2+})	16.5 ± 3.7 [6]

[a] Sac 1 was more proximal than Sac 2, and Sac 2 was more proximal than Sac 3.
[b] The concentration of lanthanum added to the mucosal fluid during the supplemental incubation only. The fluids also contained 1.0 mM Ca^{2+}.
[c] Lipid secretion was expressed as mean nmol of total lipids translocated by 100 mg wet weight of tissue in 30 min ± 1 S.E.M. Individual values for lipid secretion are given in parentheses. Number of experiments is given in brackets.

DISCUSSION

The secretory phase of fat absorption was analyzed advantageously by incubating the everted jejunum from hamster in entirely synthetic media. Perhaps the most important of the results was that the presence of calcium ion in the medium was essential for the secretion of TG in chylomicron-like particles. When Ca^{2+} was deleted, secretion ceased and cells of the absorptive epithelium deteriorated (13). It was clear that the calcium ion played a general supportive role for the intestinal mucosa. Did the calcium ion also exert a specific effect upon lipid secretory mechanisms in the cell? Certain of the data suggest an affirmative reply.

The structure of the intestine was microscopically near-normal when the medium contained calcium ion over the range 0.1–5.0 mM. Electron micrographs were typical for the secreting enterocyte, including the exocytosis of chylomicron-like particles (14). The concentration of Ca^{2+} was related directly to the lipid secretory rate, over the range 0.1–0.9 mM. This suggested that Ca^{2+} might have specifically affected the cellular mechanisms for transferring lipid. The effective range for secretion was approximately similar to the range, 0.5–1.3 mM, determining directly the contraction of the isolated heart of the frog (15). The location of an intestinal segment in the living animal also determined the rate of secretion, even at concentrations of calcium which were optimal for secretion (Fig. 1). This influence of location must have resulted from intrinsic properties of the tissues (16).

The experiments using La^{3+} provided a second indication that calcium ion stimulated lipid-transfer mechanisms in the cell. When the intestine was incubated in medium containing the antagonistic calcium and lanthanum ions, lipid secretion was sharply curtailed, while the tissue integrity was maintained. Of the assayed ions, only La^{3+} inhibited the action of Ca^{2+}. The specific and general effects of Ca^{2+} were dissociated. The lanthanum ion might have prevented uptake of Ca^{2+} from the medium or binding of the divalent cation to the plasmalemma. Theoretically, La^{3+} and Ca^{2+} acted similarly on membranes, but the binding of lanthanum was much stronger (17). Experimentally, lanthanum ion stops flux of Ca^{2+} across various biological (18–21) and artificial (22, 23) membranes, and displaces Ca^{2+} from binding sites on the plasmalemma (18, 19, 21, 24, 25), apparently without the lanthanum entering the cell (26). Therefore, the lanthanum experiments suggested that Ca^{2+} stimulated lipid secretion by acting within or on the enterocyte.

The stimulation of secretion by calcium ion in other types of cells provides a third reason favoring a specific role of Ca^{2+} in the secretion of lipid by intestinal cells. Douglas and Rubin (27) call the action of the ion on cells of the adrenal medulla, "stimulus-secretion coupling". One class of secretory cell took up external Ca^{2+} after neural or hormonal stimulation (28). Conversely, pancreatic acinar cells utilized the calcium in cytoplasmic stores after stimulation (28–33). The lipid secretion by intestine appears unique, utilizing external calcium ion for secretion without any apparent neural or hormonal stimulus.

Lipid secretion also seemed unique, judging by the effects of other divalent cations. The intestinal production was not inhibited by Mg^{2+} or stimulated by Ba^{2+}. In contrast, Mg^{2+} inhibited and Ba^{2+} stimulated secretion of catecholamines, acetylcholine, vasopressin, oxytocin, TSH, LH, ACTH, prolactin, and insulin (34). On the other hand, Mg^{2+} and Ba^{2+} apparently had similar effects on lipid secretion by the enterocyte and protein secretion by the pancreatic acinar cell (30). La^{3+} inhibited secretion by cells of adrenal medulla (35), exocrine pancreas (21), and intestine.

Reducing the temperature of incubation to less than 35°C, or substituting deuterium oxide for water in the incubation medium impaired lipid secretion. However, more than one component of the lipid secretory mechanism in the enterocytes might have been affected. Electron microscopy of absorptive cells at 23°C indicated that the temperature did not permit the transfer of lipid from endoplasmic reticulum to Golgi complex.[1] This transfer was a prerequisite for secretion. Microtubules are important for fat absorption in the living rat (36, 37). The inhibitory action of deuterium oxide on lipid secretion may be explained by the stabilizing action of D_2O on a microtubular-microfilamentous network mediating exocytosis (38), although actions of deuterium oxide on the endoplasmic reticulum (39) and cytosol (40) have been observed.

The action of calcium was to facilitate exocytosis in a number of secretory cells (41–43). A calcium-binding protein, such as calmodulin, may link calcium-ion shifts with secretion (44, 45). Intestinal cells contain calmodulin (46). Whether calcium directly effected exocytosis of lipid particles by the absorptive cell awaits future investigation.

This work was supported by grant AM-20392 from the National Institutes of Health, Public Health Service.

[1] Strauss, E. W. Lipid droplet formation in jejunum of the golden hamster in vitro during incubations with bile salt micellar solutions. Submitted for publication.

Manuscript received 25 April 1980, in revised form 31 July 1980, and in re-revised form 8 September 1980.

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