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Original article

Effect of calcium and magnesium ions on the intestinal absorption of oleic acid *in vitro*

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Summary — The effect of Ca^{++} and Mg^{++} upon intestinal absorption of oleic acid was investigated using two *in vitro* models : rat isolated jejunal loops at 30°C and 37°C and mouse jejunal explants at 37°C. At 30°C or at 37°C, Ca^{++} significantly increased ^{14}C oleic acid uptake by rat isolated jejunal loops or mouse jejunal explants; at 37°C, Ca^{++} significantly enhanced lipid exocytosis in rat intestinal loops but not in mouse jejunal explants; in both models, in the presence of Ca^{++} and at 37°C, Mg^{++} significantly improved the esterification of oleic acid phospholipids and triacylglycerols, as shown by the increase in triacylglycerol synthesis in rat isolated intestinal loops or by the increase in triacylglycerols recovered from the incubation media of mouse jejunal explants; experiments carried out with rat isolated jejunal loops highlighted the determinant role of temperature in oleic acid absorption processes. The present work shows that the stimultaneous presence of Ca^{++} and Mg^{++} did not impede oleic acid absorption processes but, on the contrary, enhanced them.

Ca^{++} — Mg^{++} — temperature — oleic acid — intestinal absorption — rat — mouse

Résumé — Influence des ions calcium et magnésium sur l'absorption intestinale de l'acide oléique. Etude *in vitro*. *L'influence des ions calcium et magnésium sur l'absorption intestinale de l'acide oléique est étudiée à l'aide de 2 modèles* in vitro : *des intestins isolés de rat perfusés à 30 °C et à 37 °C et des explants jéjunaux de souris à 37 °C. A 30 °C ou à 37 °C, les ions calcium n'augmentent pas significativement le prélèvement de l'acide oléique, quel que soit le modèle utilisé. A 37 °C, les ions calcium augmentent significativement l'exocytose de l'acide oléique avec le modèle de l'anse intestinale isolée perfusée de rat, mais non significativement avec le modèle des explants jéjunaux de souris. En présence des ions calcium à 37 °C, les ions magnésium entraînent une meilleure estérification en phospholipides et triacylglycérols de l'acide oléique prélevé, comme en témoigne leur augmentation dans le transudat des intestins isolés de rat d'une part et dans les milieux d'incubation des explants jéjunaux de souris d'autre part. Les expériences conduites sur les intestins isolés perfusés de rat soulignent le rôle particulièrement déterminant de la température dans les processus d'absorption* in vitro. *Ce travail montre que non seulement la présence des ions calcium et magnésium ne gêne pas l'absorption de l'acide oléique* in vitro, *mais au contraire ces ions ont un effet synergique.*

Ca^{++} — Mg^{++} — température — acide oléique — absorption intestinale — rat — souris

Introduction

Many authors analysing the *in vitro* intestinal absorption of lipids have used a lipid mixture devoid of calcium and magnesium ions : Kern and Borgström (1965) using hamster intestinal rings and Breckenridge and Kuksis (1975) using rat everted sacs.

Accordingly, Browning and Trier (1969), studying the ability of human intestinal cells to absorb fatty acids and monoglycerides in organ culture, incubated explants in a calcium/magnesium-free medium. However, several *in vitro* studies using hamster everted sacs at 37°C (Strauss, 1977; Strauss and Jacob, 1981) or rat isolated perfused intestines at 30°C (Saunders and Sillery, 1979) have shown a significant stimulation of the rate of fatty acid absorption by calcium ions.

Considering their key roles in cell metabolism and tissue preservation, calcium and magnesium ions should theoretically be added to the media of any comprehensive cultures. They have usually been included in culture media used for intestinal explants. Rachmilewitz *et al.* (1980) and Zimmerman *et al.* (1985), analysing lipid secretion by cultured human intestinal biopsies, used a medium (RPMI) containing calcium and magnesium ions. In our *in vitro* studies (Carlier *et al.*, 1986) on fatty acid absorption using short-term incubation, we mixed lipids with Dulbecco's modified Eagle medium (DMEM) which contains calcium and magnesium ions.

To bring to light their effect at the high concentration usually employed in culture media, we tested their role in oleic acid absorption at 30°C and at 37°C using isolated perfused rat intestine, a model routinely used in pharmacological work (Richter and Strugala, 1985), and we analysed their influence on oleic acid absorption in mouse jejunal explants at 37°C.

Materials and methods

Animals

Adult male Wistar rats weighing 200—250 g and 2-month old Swiss mice weighing 45—50 g were used. They were fasted overnight, but had free access to water.

Experimental procedure

Isolated perfused intestinal loops. Under light ether anaesthesia 10 cm of the upper jejunum was isolated and a catheter (Biotrol No. 12 F-75140, Paris Cedex 03) firmly sutured at both ends. The loop was rinsed for 5 min with 10 ml of a saline buffer at 37°C, pH 7.4 : NaCl (109.4 mM), KCl (5.37 mM), $NaHCO_3$ (44 mM), NaH_2PO_4, H_2O (0. 9 mM). The jejunal segment was then mounted in the closed system of Parsons and Volman-Mitchell (1974), modified by Saunders and Sillery (1979), and immersed in liquid paraffin. A lipid mixture (A or B) was continuously perfused by recycling for 2 h at a flow rate of 2 ml•min^{-1}. The lipid mixture was emulsified in an oxygenated saline buffer (95% O_2, 5% CO_2). The 3 saline buffers used contained : (1) no Ca^{++} or Mg^{++}; (2) Ca^{++} alone ($CaCl_2$, 1.8 mM); (3) Ca^{++} ($CaCl_2$, 1.8 mM) and Mg^{++} ($MgSO_4$, 7 H_2O, 0.8 mM). Lipid droplets transuding through the intestinal wall were collected at the vessel bottom in 3 samplings at 30, 60 and 120 min. At the end of the experiment, the jejunal loop was rinsed with the buffer saline medium, disconnected from the pump and cut longitudinally. The mucosa was immediately scraped off.

Mouse jejunal explants. 1-mm^3 explants of the mouse jejunum were prepared (Ferland and Hugon, 1979; Carlier *et al.*, 1986) and randomly distributed into 3 groups. Each group of 30 explants was incubated for 15 min in 10 ml of an oxygenated saline buffer (95% O_2, 5% CO_2) with or without $CaCl_2$ (1.8 mM) or with $CaCl_2$ (1.8 mM) and $MgSO_4$, 7 H_20 (0.8 mM) and containing glucose (25 mM) in the same proportion as the DMEM, but devoid of amino acids and vitamins. Other explants were incubated in complete DMEM. An optically clear lipid mixture was added to these media.

Lipid mixtures

Two lipid mixtures were used. Lipid mixture A (^{14}C oleic acid/monooleyglycerol, 2/1 mol/mol, 7.5 mM; sodium taurocholate, NaT, 5 mM)

contained a high level of lipids. Lipid mixture B (^{14}C oleic acid/palmitic acid/monopalmitoylglycerol, 1/1/1 mol/mol/mol, 1.2 mM; sodium taurocholate, NaT 2.4 mM) was similar to the lipid mixture employed in our previous experiments in organ culture (Carlier *et al.*, 1986). ^{14}C oleic acid, 1.6—2 μCi (50 mCi/mmol), was obtained from CEA Saclay France; its radiochemical purity was checked by gas liquid chromatography.

Biochemical methods

Lipid analysis. For each experiment, the lipids were extracted from the mucosal scraping, the intestinal wall and the transudates or from mouse jejunal explants by the method of Delsal (1944). Because of the presence of salts and glucose and the low level of lipids in the perfused medium and the incubation medium, the method of Folch (1957) was used for lipid determination. The individual lipid classes were separated from the total extracts by thin-layer chromatography (Stahl *et al.*, 1956). The radioactivity of the different fractions was measured with a liquid scintillator spectrometer (Packard Prias PLD, Tricarb).

Protein assays. Proteins from the lipid extract residues (mucosa, and intestinal wall, explants) were measured using the method of Lowry *e t al.* (1951).

Electron microscope study. Some transudate samples were fixed with osmium tetroxide for 1 hr at 4°C. One drop of the fixed suspension was placed on collodion carbon grid. The particles shadowed with gold—palladium were observed with a Hitachi HU 11E electron microscope.

Expression of results and statistics. The amount of ^{14}C oleic acid recovered in the different lipid classes from the extracts was converted into nmol by using its specific activity in the lipid mixtures. Particularly in the mouse jejunal explants, the amount of ^{14}C oleic acid incorporated into phospholipids and triacylglycerols was considered as an index of lipoprotein synthesis and secretion. The results were expressed as the means of 3 experiments (SEM). Statistical analysis was carried out using : (1) an analysis of variance (two-way classification; factor 1 = temperature, factor 2 = perfused medium composition) to compare the means; (2) the Newman—Keuls test (Statitcf Logiciel, 8 av. Président Wilson, 75116 Paris) to study the interactions between the 2 factors.

Results

Isolated rat jejunal loops

General remarks. At the end of the experiments no proteins or radioactive esterified lipids were recovered in the lipid perfusion medium, indicating that the epithelial layer of the intestinal loop did not suffer a major disruption, and that very few physiologically active absorbing cells were shed during perfusion. The pH of the gassed lipid perfusion medium remained constant throughout the experiments.

The radioactivity recovered from the perfusate and from the intestinal compartments revealed a significant uptake of radioactive oleic acid from lipid mixture A (16—35%) and from lipid mixture B (24%). As shown in Table I in each experimental condition, the uptake of radioactive oleic acid was associated with exocytosis of the radioactive lipids recovered mainly in the intestinal wall. Ultrastructural controls showed the presence of lipoprotein particles in the transudates (Fig. 1). In all cases very low density lipoproteins (VLDL) and chylomicrons were visualized; a few reached diameters of 0.2 or 0.3 μm. Morever, we never observed any defect in the lipoprotein structure, even during the second half-hour of the experiments.

An analysis of the various lipid classes for each experiment showed that the yield of radioactive diacylglycerols remained low in the mucosa with a high load of radioactive triacylglycerols and phospholipids. Morever, the data of the Ca^{++} and Mg^{++} experiments at 30°C revealed that the yield of radioactive triacylglycerols and phospholipids from the mucosa to the transudates increased.

Effect of Ca^{++}. At 30°C, ^{14}C oleic acid was taken up and lipids were secreted. With the 2 Mg^{++}-free lipid media, no significant difference in uptake was measured between the Ca^{++}-enriched and

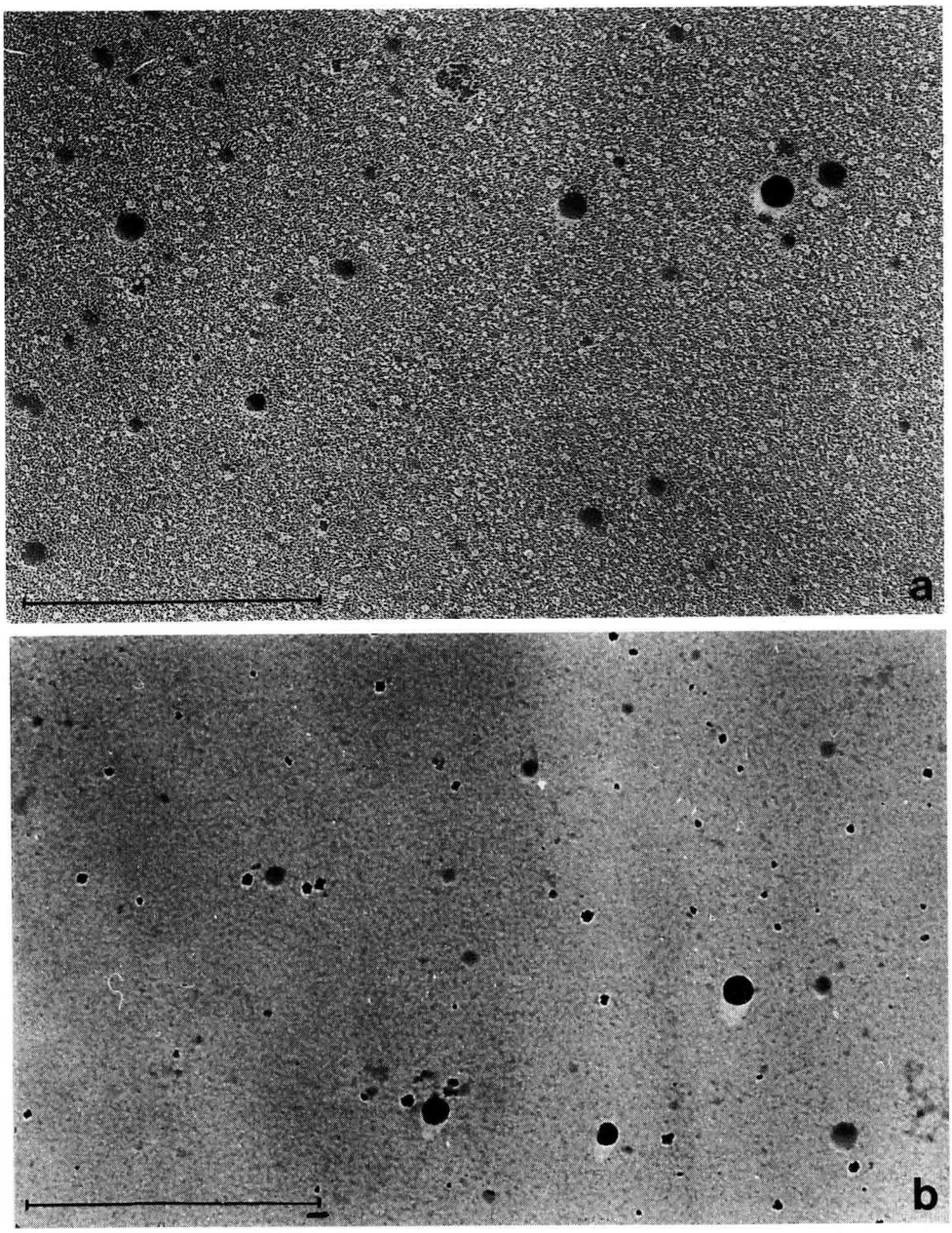

Fig. 1. Electron micrographs of lipoprotein particles of transudates from rat isolated jejunal loops, collected during the second half-hour of perfusion. The oxygenated lipid mixture perfused at 37°C, containing Ca^{++} and Mg^{++} (see Materials and methods), was 7.5 mM (Fig. 1a) or 1.2 mM (Fig. 1b) in lipids. At both lipid concentrations, well structured lipoprotein particles, VLDL (diameter inferior to 0.1 μm) and chylomicrons (diameter superior to 0.1 μm) appeared at the serosal surface of jejunum. The scale represents 1 μm.

Table I. Effect of Ca^{++}, Mg^{++} and temperature on oleic acid intestinal uptake and secretion by rat isolated intestinal loops.

Lipid mixture			*A*			*B*
Ca++		0	1.8 mM	1.8 mM		1.8 mM
Mg++		0	0	0.8 mM		0.8 mM
					Comparison of means	
Total uptake	30°C	246 $(19)_B$	226 $(3.5)_B$	313 $(24)_A$	262 ^{xxx}	—
	37°C	394 $(24)_B$	470 $(21)_A$	473 $(5)_A$	446 ^{xxx}	40.7 (19)
Comparison of means		320 ^{b}	348 ^{a}	393^{ab}	interaction $P < 0.01$	
Mucosa	30°C	218 $(19)_B$	187 $(17)_B$	265 $(9)_A$	223 ^{xxx}	—
	37°C	330 $(19)_B$	358 $(35)_B$	386 $(2)_A$	358 ^{xxx}	26.5 (2.4)
Comparison of means		274 ^{b}	272 ^{a}	325.5 ^{ab}	interaction $P < 0.01$	
Intestinal wall	30°C	23.5 $(2.1)_C$	35.4 $(2.3)_B$	45.5 $(6.3)_A$	34.8 ^{xxx}	—
	37°C	54 $(7)_B$	90 $(18)_A$	71 $(4)_A$	71.7 ^{xxx}	12 (1)
Comparison of means		38.8 ^{ab}	62.7 ^{a}	58.3 ^{b}	interaction $P < 0.01$	
Transudates	30°C	6 $(1)_A$	4.2 $(0.5)_B$	2.5 $(0.4)_B$	4.2 ^{xxx}	—
	37°C	9.8 $(1)_C$	22.5 $(4.4)_A$	15.8 $(2)_B$	16 ^{xxx}	2.2 (0.4)
Comparison of means		7.9 ^{da}	13.3 ^{ac}	9.2 ^{dc}	interaction $P < 0.01$	

Rat isolated jejunal loops were perfused for 2 h with 37.5 ml of lipid medium A, in absence (0) or in presence of Ca^{++} and Mg^{++} at 30°C or at 37°C, or with 25 ml of lipid medium B in presence of Ca^{++} and Mg^{++} ions.
Radioactive lipid recovery is expressed for 3 experiments as means (SEM) of nmol of oleic acid integrated into radioactive lipids recovered in the intestinal mucosa, in the intestinal wall and in the transudates per mg of intestinal proteins. The sum of these values is taken as the uptake.
Values with a common superscript are significantly different.
Vertical comparisons represent the effect of temperature. ^{xxx} $P < 0.001$.
Horizontal comparisons represent the effect of the composition of the perfused medium. ^{ab} $P < 0.01$; ^{cd} $P < 0.05$; $_{ABC}$ represent the classification obtained by the Newman—Keuls test.

the Ca^{++} -free media : 246 (19) and 226 (3.5) mol•mg^{-1} of protein; in the presence of Ca^{++} we observed a significant increase in lipid exocytosis (intestinal wall + transudates) : 39.6 (2.4) *vs* 29.5 (2.2) (Table I).

At 37°C with the 2 Mg^{++}-free media a significant increase in the overall uptake and in the exocytosis of ^{14}C oleic acid was seen in the presence of Ca^{++} : 470(20) *vs* 394(24). A significant increase in the transudates was measured : 22.5 (4.4) *vs* 9.8 (1) nmol•mg^{-1} of intestinal proteins.

Effect of simultaneous presence of Ca^{++} and Mg^{++}. At 30°C the simultaneous presence of Ca^{++} and Mg^{++} in the lipid emulsion caused a significant increase in uptake and in exocytosis compared to the Mg^{++}-free media (Table I), but radioactivity in the transudates was low. In spite of the increased uptake, the percentage of phospholipids and triacylglycerols was of the same order in the mucosa as in the Mg^{++}-free media (Table II) : 63 (2.1) *vs* 60 (7) and 56. 8 (3.9). Therefore, esterification was efficient since the radioactive phospholipids and triacylglycerols recovered in the intestinal wall and mucosa increased.

At 37°C, with lipid medium A containing Ca^{++} and Mg^{++} ions, no significant difference in uptake or exocytosis appeared compared to the Mg^{++}-free lipid media. There was an enrichment in phospholipids and triacylglycerols (Table II). As shown at 30°C, the effect of Mg^{++} was revealed when the yields of the total radioactive phospholipids and triacylglycerols were compared. In the presence of Mg^{++} we observed a significant increase in esterified lipids : 337 (7.5) nmol•mg^{-1} of intestinal proteins vs 210. 3 (36.2) and 286.3 (14.1).

Effect of temperature. As verified by statistical analysis, a comparison between the experiments carried out at 30°C and at 37°C, in the absence or the presence of Ca^{++} or Mg^{++}, indicated that temperature had a very determinant effect on intestinal lipid uptake and on the esterification processes and thus on exocytosis, revealed by the amounts of phospholipids and triacylglycerols recovered in the intestinal wall and the transudates (Table II). In some cases, the transudates collected during the experiments were 5—6-fold higher than at 30°C (Table I).

Interactions of temperature and perfused medium composition. Statistical studies revealed a clear interaction of the perfused medium composition with the temperature. The synergic effects on uptake (Table I), esterification (Table II) and secretion (Table I and II) were obvious.

Effect of lipid medium B. At 37°C, in the presence of Ca^{++} and Mg^{++} (Table I and II), ^{14}C oleic acid uptake was consistent with oleic acid molarity in the lipid medium, *i.e.* a ratio of 0.4/5 M/M when compared with the Ca^{++}/Mg^{++} lipid medium A. Recovery from the intestinal wall and from the transudates followed the same pattern.

Mouse jejunal explants

The explants were immersed in a lipid medium containing no Ca^{++} and Mg^{++} or enriched with Ca^{++}, or in the DMEM. In these conditions, the presence of Ca^{++} alone did not significantly increase the amount of radioactive esterified lipids measured in the explants and in the incubation medium at the end of the incubation period. The presence of Mg^{++} significantly enhanced oleic acid esterification. This enhancement was expressed by an increase in the radioactive triacylglycerols detected in the incubation medium, as noted in the isolated rat jejunal loop experiments. The same results were obtained when the Ca^{++} and Mg^{++} of the DMEM were complexed with EDTA (recent unpublished data).

Table II. Distribution of the radioactivity into phospholipids and triacylglycerols of mucosal scrapings, intestinal walls and transudates after ^{14}C oleic acid uptake by rat isolated intestinal loops.

Lipid mixture			*A*			*B*
Ca^{++}		0	1.8 mM	1.8 mM		1.8 mM
Mg^{++}		0	0	0.8 mM		0.8 mM
					Comparison of means	
% PL + TG in mucosa	30°C	56.8 (3.9)	60 (7)	63 (2.1)	59.9 (1.8)	—
	37°C	50.7 $(5.8)_B$	56.1 $(2)_B$	68.9 $(2)_A$	8.6 (5.5)	70.4 (2.8)
Comparison of means		53.8 ^{ac}	58.4 ^{bc}	65.9 ^{ab}		
% PL + TG in intestinal wall	30°C	73.5 (2.9)	66.7 (3.4)	75.3 (3)	71.8 (2.7)	—
	37°C	67.4 $(6)_B$	65 $(0.6)_B$	81.7 $(6)_A$	71.4 (5.3)	75.1 (3.5)
Comparison of means		70.4 ^{b}	65.8 ^{a}	78.5 ^{ab}		
% PL + TG in transudates	30°C	75.9 (2.7)	75.4 (4)	71.3 (4.7)	74.2 (1.5) ^{xxx}	—
	37°C	81.9 $(1)_B$	78.7$(3.5)_B$	92.3 $(1.5)_A$	84.3 (4.2) ^{xxx}	82.7 (2.6)
Comparison of means		78.9 ^{d}	77 ^{c}	81.8 ^{cd}	interaction $P < 0.01$	
Total PL + TG (nmol)	30°C	145 (5.7)	141.7 (28)	203 (4.7)	163.2 (20.3) ^{xxx}	—
	37°C	210.3 $(21)_C$	286.3 $(14.1)_B$	337 $(7.5)_A$	277.9 (37.5) ^{xxx}	27.5(2)
Comparison of means		177.7 ^{ac}	214 ^{bc}	270 ^{ab}	interaction $P < 0.01$	

Percentage of the radioactivity recovered in phospholipids (PL) and triacylglycerols (TG). The percentages are expressed as means (SEM) for 3 experiments.

Total phospholipid (PL) and triacylglycerol (TG) synthesis is expressed for 3 experiments as means (SEM) of nmol of oleic acid per mg of intestinal proteins.

Values with a common superscript are significantly different.

Vertical comparisons represent the effect of temperature ^{xxx} $P < 0.001$.

Horizontal comparisons represent the effect of the composition of the perfused medium. ^{ab} $P < 0.01$; ^{cd} $P < 0.05$; ABC represent the classification obtained by the Newman—Keuls test.

Discussion

The aim of this work was to discover whether Ca^{++} and Mg^{++}, currently added to survival and culture media, interfered with oleic acid intestinal absorption. Ca^{++} and Mg^{++} molarities were therefore chosen in reference to the culture media containing the highest amounts of these ions, such as Trowell T_8 or NCTC 135 or DMEM media. Two complementary models were used : isolated perfused intestinal loop which preserved luminal absorption polarity, and intestinal organ culture which might not completely preserve it. The mouse intestine is too fragile to be used for isolated loop study ; on the other hand, organ culture of adult rat intestine is not as yet well mastered. We therefore chose the rat intestine for isolated loop research and mouse intestine for the organ culture. The first model has not been used much for the study of lipid transport (Saunders and Sillery, 1979). Nevertheless, it has been employed to study the biotransformation of foreign compounds or the metabolism of lipophilic xenobiotics for time periods of up to 2 h (Richter and Strugala, 1985). Tso and Simmonds (1984) emphasized 2 of the advantages of Parson and Volman-Mitchell's model (1974) used by Saunders and Sillery (1979), *i. e.* the easy collection of lipid transudates and the absence of plasma protein contamination, an observation confirmed by our results. Due to the small amount of translocated lipids, these authors insisted on the use of radioactive labeled exogenous lipids. Organ culture has been widely used for investigating physiological and pathological conditions of the intestinal mucosa. With this model, several authors have analysed the uptake and metabolism of fatty acids (Browning and Trier, 1969; Rachmilewitz *et al.*, 1980; Zimmerman *et al.*, 1985; Carlier *et al.*, 1986).

To permit optimal yield of the esterification processes we used ^{14}C oleic acid in the presence or absence of another saturated fatty acid and in the presence of a monoacylglycerol. Oleic acid absorption was followed from the mucosal to the serosal side to study uptake, esterification and exocytosis of the radioactive lipids. Exocytosis was evaluated by the amounts of phospholipids and triacylglycerols in the intestinal wall after mucosal scraping and by the amount of transudates. In spite of the small amount of lipids recovered in transudates (Breckenridge and Kuksis, 1975; Tso and Simonds, 1984) their study was crucial in evaluating the esterification efficiency of the enterocytes. Electron microscope observations have demonstrated the presence of lipoprotein particles in transudates with a high proportion of VLDL at both lipid concentrations, as observed *in vivo* with the same lipid emulsions (Bernard *et al.*, 1987). The enrichment in radioactive phospholipids and triacylglycerols from the mucosal scrapings to transudates and the low yield of radioactive diacylglycerols recovered in the mucosal scrapings suggest that the normal metabolic pathways of the enterocytes were preserved during incubation. Manganaro and Kuksis (1981) observed a significant decrease of the diacyglycerol-acyltranferases when the normal structure of the enterocytes was destroyed as in homogenates and in subcellular fractions. Moreover, oxygenation of the perfused medium probably improved mucosal survival.

Table I and Figure I show that at 37°C the model behaved both physiologically and biochemically, and that lipoprotein was formed and secreted at 2 oleic acid concentrations, 5 mM and 0.4 mM; this agrees with the data of Johnston and Borgström (1964) in reference to temperature and lipid molarity. The overall results, and particularly the distribution in our

Table III. Effect of Ca^{++}, Mg^{++} on ^{14}C oleic acid absorption by mouse jejunal explants.

Ca^{++}		*0*	*1.8 mM*	*1.8mM*
Mg^{++}		*0*	*0 mM*	*0.8 mM*
Explants	PL	2.1 (0.4)	1.7 (0.1)	1.9 (0.2)
	TG	7.2 (0.4)^{a}	10.3 (0.7)^{a}	4.7 (0.8)^{a}
Medium	PL	24 (6.5)	26.1 (4.4)	20.6 (2.8)
	TG	35.4 (4.4)^{b}	39.8 (3.6)^{a}	74.4 (4.7)^{ab}
Total PL + TG		68.7 (5)$^{bc}{}_{C}$	77.9 (3.2)$^{ac}{}_{B}$	100.8 (6.8)$^{ab}{}_{A}$

Mouse jejunal explants were incubated for 15 min at 37°C in 10 ml of lipid mixture B, in absence (0) or in presence of Ca^{++} and Mg^{++} ions.
Lipoprotein synthesis is expressed for 3 experiments as means (SEM) of nmol of oleic acid integrated into phospholipids (PL) and triacylglycerols (TG) per mg of explant proteins.
Values with a common superscript are significantly different; abc $P < 0.05$; ABC represent the classification obtained by the Newman—Keuls test.

experiments of the radioactive lipids among the mucosa, intestinal wall and transudates, are in accordance with the data of Breckenridge and Kuksis (1975) and Shiau *et al.* (1978) on rat everted intestinal sacs. In experiments carried out at 30°C, in the absence of Ca^{++} and Mg^{++} or in the presence of Ca^{++} and the absence of Mg^{++}, the amount of lipids recovered in transudates is also in agreement with results presented by Saunders and Sillery (1979) on isolated rat intestinal loops.

Our present work confirms the key role of Ca^{++} in the esterification and secretion of lipoprotein particles in the intercellular spaces and thus agrees with the observations of Strauss (1977) and Strauss and Jacob (1981) using hamster everted intestinal sacs and those of Saunders and Sillery (1979) on rat isolated intestinal loops. Some of the differences observed between our two models in the present study could be explained by the higher Ca^{++} sensivity of the mucosal surface compared to the serosal surface of the intestine (Strauss and Jacob, 1981). This role of Ca^{++} is therefore necessary in every *in vitro* study of lipid absorption. On the other hand, if Mg^{++} did not always enhance lipid secretion, as mentioned by Saunders and Sillery (1979) and Strauss and Jacob (1981), that ion clearly improved triacylglycerol synthesis at 37°C in both the isolated jejunal loop and the jejunal organ culture models. Its role appears more obvious in the latter and is probably due to the synergic effect of Ca^{++} and to its availability at both the mucosal and the serosal sides of the explants (Strauss and Jacob, 1981).

The stimulating effects of temperature increase on many metabolisms is well known. Johnston and Borgström (1964) showed that low temperature impaired lipid secretion. Strauss and Jacob (1981)

emphasized its role in intestinal lipid uptake and esterification; those authors verified that if lipid secretion also took place at 30°C, it was at a low rate and that the optimal temperature for lipid secretion of everted sacs was 35°C. Our results confirm these observations of the very decisive role of temperature and the synergic effect of Ca^{++} and Mg^{++} interaction on absorption. The results also emphasize the specific role of Mg^{++} at 37°C in the esterification processes.

Conclusion

Lipid intestinal absorption involves successive events that are closely correlated and influenced by many factors. Ca^{++} and Mg^{++}, in concentrations generally found in culture media, appear to be synergically beneficial to these processes. Mg^{++} significantly improves enterocyte lipid esterification and Ca^{++} significantly enhances esterified lipid exocytosis. Temperature interferes critically with these events. In conclusion, at 37°C the presence of Ca^{++} and Mg^{++} in incubation or culture media promotes oleic acid uptake and its esterification by the intestinal mucosa. They are therefore beneficial in *in vitro* experiments on lipid absorption.

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References

Bernard A., Echinard B. & Carlier H. (1987) Differential intestinal absorption of two fatty acid isomers : elaidic and oleic acids. *Am. J. Physiol.* 253, G751-G759

Breckenridge W.C. & Kuksis A. (1975) Diacylglycerol biosynthesis in everted sacs of rat intestinal mucosa. *Can. J. Biochem.* 53, 1170-1183

Browning T. H. & Trier J. S. (1969) Organ culture of mucosal biopsies of human small intestine. *J. Clin. Invest.* 48, 1423-1432

Carlier H., Labussière H., Bernard A. & Hugon J. S. (1986) Lipid esterification and secretion by the mouse intestine in organ culture. *Comp. Biochem. Physiol.* 84A, 303-308

Delsal J.L. (1944) Nouveau procédé d'extraction des lipides du serum par le méthylal. *Bull. Soc. Chim. Biol.* 26, 99-105

Ferland S. & Hugon J. S (1979) Organ culture of adult mouse intestine. I. Morphological and cytochemical results after 24 and 48 hours of culture. *In Vitro* 15, 278-287

Folch J., Lees M. & Sloane-Stanley G. H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226, 497-509

Johnston J. M. & Borgström B. (1964) The intestinal absorption and metabolism of micellar solutions of lipids. *Biochim. Biophys. Acta*, 84, 412-423

Kern F. & Borgström B. (1965) Quantitative study of the pathways of triglyceride synthesis by hamster intestinal mucosa. *Biochim. Biophys. Acta* 98, 520-531

Lowry O. H., Rosebrough N. J., Farr A.L. & Randall R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193, 265-275

Manganaro F. & Kuksis A. (1981) Modulation of acylglycerol synthetase activities of rat intestinal mucosa by homogenization and sonication. *Can. J. Biochem.* 59, 736-742

Parsons D.S. & Volman-Mitchell H. (1974) The transamination of glutamate and aspartate during absorption *in vitro* by small intestine of chicken, guinea-pig and rat. *J. Physiol. London* 230, 677-694

Rachmilewitz D., Sharon P. & Eisenberg S. (1980) Lipoprotein synthesis and secretion by cultured human intestinal mucosa. *Eur. J. Clin. Invest.* 10, 125-131

Richter E. & Strugala G. J. (1985) An all-glass perfusator for investigation of the intestinal transport and metabolism of foreign compounds *in vitro*. *J. Pharmacol. Meth.* 14, 297-304

Saunders D. R. & Sillery J. (1979) Effect of calcium on absorption of fatty acid by rat jejunum *in vitro*. *Lipids* 14, 703-706

Shiau Y. F., Long W. B. & Weiss J. B. (1978) Effect of sugar and monoglyceride on fatty acid esterification. *Am. J. Physiol.* 234, E236-E242

Stahl E., Schröter G., Kraft G. & Renz R. (1956) Thin-layer chromatography (the method affecting factors, and a few examples of application). *Pharmazie* 11, 633-637

Strauss E. W. (1977) Effects of calcium and magnesium ions upon fat absorption by sacs of everted hamster intestine. *Gastroenterology* 73, 421-424

Strauss E. W. & Jacob J. S. (1981) Some factors affecting the lipid secretory phase of fat absorption by intestine *in vitro* from golden hamster. *J. Lipid Res.* 22, 147-156

Tso P. & Simmonds W. J. (1984) The absorption of lipids and lipoprotein synthesis. *In : Lipid Reseach Methodology* (J. A. Story ed.), Alan R. Liss, New York, pp. 191-216

Zimmerman J., Gati I., Eisenberg S. & Rachmilewitz D. (1985) Fat absorption and triglyceride synthesis and secretion by cultured human intestinal mucosa. *Israel J. Med. Sci.* 21, 962-967