A dual, concentration-dependent absorption mechanism of linoleic acid by rat jejunum in vitro

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Abstract Linoleic acid absorption was studied using everted rat jejunal sacs. At low concentrations (42–1260 μM), the relationship between linoleic acid concentration and its absorption rate fitted best to a rectangular hyperbola. At high concentrations (2.5–4.2 mM) the relationship between the two parameters was linear. The separate additions of 2,4-dinitrophenol, cyanide, or azide, or decrease in the incubation temperature from 37 to 20°C did not change the absorption rate of linoleic acid. Absorption rate of linoleic acid at low concentrations increased as the hydrogen ion and taurocholate concentrations were increased or as the unstirred water layer thickness was decreased. Linoleic acid absorption rate was decreased after the additions of lecithin, oleic, linolenic, and arachidonic acids or the substitution of taurocholate with the nonionic surfactant Pluronic F 68. These observations indicate that a concentration-dependent, dual mechanism of transport is operative in linoleic acid absorption. Facilitated diffusion is the predominant mechanism of absorption at low concentrations, while at high concentrations, simple diffusion is predominant. At low concentrations, the absorption rate of linoleic acid is influenced by the pH, surfactant type and concentration, the simultaneous presence of other polyunsaturated fatty acids, and the thickness of the unstirred water layer.—**Chow, S-L., and D. Hollander.** A dual, concentration-dependent absorption mechanism of linoleic acid by rat jejunum in vitro. *J. Lipid Res.* 1979. **20:** 349–356.

Supplementary key words essential fatty acids · intestinal absorption · surfactants · bile salts · unstirred water layer

Linoleic acid, the most common essential fatty acid in the mammalian diet, can be synthesized only by plants. Intestinal malabsorption or dietary deficiency of the fatty acid can result in reduced growth rate, a wide variety of skin disorders, increased susceptibility to infections, reduced myocardial contractility, increased platelet aggregation, and decreased prostaglandin synthesis (1). After its absorption, linoleic acid is converted into dihomo-gamma-linoleic acid and arachidonic acid, both of which can serve as precursors for prostaglandin synthesis (2). The essential fatty acids also serve as a structural component of cell membranes (3). Finally, increased dietary ingestion of polyunsaturated fatty acids, such as linoleic acid, has been strongly advocated as a means of lowering blood cholesterol and triglyceride levels in order to reduce the risk of atherosclerotic cardiovascular disease. Despite the biological importance of linoleic acid, its mode of absorption by the small intestine and the factors that may influence its absorptive process have not been investigated. We studied linoleic acid absorption by using everted small intestinal sacs of the rat. We have characterized the influences of other polyunsaturated fatty acids and of metabolic inhibitors and uncouplers on the absorption of linoleic acid. We have examined the effects of incubation temperatures, the thickness of the unstirred water layer, the presence of ionic and nonionic surfactants, the incubation solution pH, and phospholipid content on the absorption of this important polyunsaturated essential fatty acid.

METHODS

Materials

[1-^{14}C]Linoleic acid (New England Nuclear, Boston, MA) with sp act 50.6 mCi/mmol was used as a tracer compound. The radiochemical purity of the compound was found to be greater than 98% by thin-layer chromatography (TLC) on silica gel G developed in hexane–diethyl ether–acetic acid 70:30:1. Nonradioactive linoleic acid (Sigma Chemical Co., St. Louis, MO) had less than 1% impurities. [^{3}H]Inulin (Amersham/Searle Corp., Arlington Heights, IL) with sp act 0.9 Ci/mmol and radiochemical purity greater than 99% was used as a nonabsorbable marker (4). Purified grade sodium taurocholate (Calbiochem Co., San Diego, CA) was found to have less than 1% impurities by TLC (5). In some experiments, a nonionic surfac-

Abbreviations: TLC, thin-layer chromatography; DNP, 2,4-dinitrophenol; FABP, fatty acid-binding protein.

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tant, Pluronic F 68 (BASF Wyandotte Corp., Wyandotte, MI), was used for solubilizing linoleic acid. Oleic, linolenic, and arachidonic acids with purity greater than 99% were obtained from Sigma Chemical Co. L-α-Lecithin, type III-D from egg yolk, was purchased from Sigma Chemical Co. Purified grade potassium cyanide (Fisher Scientific Co., Fairlawn, NJ), sodium azide, and 2,4-dinitrophenol (Sigma Chemical Co.) were used as metabolic inhibitors and uncouplers. Analytical reagent grade sodium dihydrogen phosphate and disodium hydrogen phosphate (J. T. Baker Chemical Co., Phillipsburg, NJ) were used as buffer components. A micellar solution of the surfactant in the phosphate buffer was prepared by ultrasound irradiation for 5 min at 70 W of power with a sonicator (Artek Corp., Farmingdale, NY). The standard micellar incubation solution had a pH of 6.5 and contained the following components at the given concentrations: linoleic acid (21 μM–4.2 mM), sodium taurocholate (10 mM), sodium dihydrogen phosphate (85.67 mM), disodium hydrogen phosphate (45.56 mM), and tracer amounts of [^{14}C]linoleic acid and [^{3}H]inulin. On separate occasions, excipients such as lecithin and fatty acids other than linoleic were added to the buffer solution at an equimolar concentration of 0.42 mM. In some experiments the pH of the incubation solution was varied between 5.4 and 7.4 by changing the relative concentration of the sodium salts of phosphate. The osmolarity of the final solution ranged from 285 to 315 mosmol per liter (6).

Everted sac preparations

Male Sprague–Dawley rats (Spartan Research Animals, Inc., Haslett, MI) weighing 200–250 g had free access to water and Purina rat chow (Ralston Purina Co., St. Louis, MO). The rats were not fasted prior to experimentation. Each rat was killed by stunning and cervical dislocation. The small intestine was rinsed in situ with chilled saline. The 15 cm of jejunal segment immediately distal to the ligament of Treitz was then removed, everted, and subdivided with sutures into 1.5-cm sacs which were identified with tags indicating their original intestinal location. Intestinal segments that contained Peyer's patches were not used. No fluid was placed in the serosal compartment since no transmural transport of linoleic acid has been found to take place in this preparation.[2]

Incubation methods

The sacs were immediately immersed in 50 ml of the micellar solution which was kept at 37°C and was contained in a Plexiglass incubation chamber with internal dimensions of 2 × 6 × 30 cm. The chamber was placed in a water bath (Precision Scientific Co., Chicago, IL) and was agitated at various rates (0–120 oscillations/min); unless specified otherwise the standard rate of oscillations was 80 per min. Preincubation samples of the solution were withdrawn in triplicate and were used for calculation of the initial specific activity of linoleic acid and inulin. After 2 min of incubation one intestinal sac was removed from the chamber every min for the next 4 min (**Fig. 1**). Each sac was immediately immersed for 15 sec in a beaker that contained 200 ml of either 1 mM sodium taurocholate or 0.1 mM Pluronic F 68 solution in order to remove some of the adherent incubation solution off the sac's surface. The beaker's contents were stirred by a magnetic stirrer at a constant rate. In preliminary experiments the rinse was found to remove some of the incubation solution that had remained adherent to the sac, leaving a more constant and a more reproducible amount of adsorbed fatty acid on the sac's surface. The sac was then gently blotted on paper towels and dried in an oven (Fisher Scientific Co., Fairlawn, NJ) at 50°C under vacuum (20 in of mercury) for 24 hr. The sutured ends of each sac were removed and the sacs were weighed in the dry state. All experimental work was performed under subdued lighting and aluminum foil cover in order to prevent structural changes of linoleic acid by ultraviolet light irradiation.

Radioactivity determinations

The radioactivity of the absorbed linoleic acid and the adsorbed inulin was assayed and separated by total combustion of the intestinal sacs by a sample oxidizer (Tri-Carb Model 306, Packard Instrument Co., Downers Grove, IL). The [^{14}C]linoleic acid and [^{3}H]-inulin were thus converted to $^{14}CO_2$ gas and tritiated water, respectively. Monophase-40 (Packard Instrument Co.) was used as a scintillator for tritiated water. Carbosorb and Permafluor V (Packard Instrument Co.) were used for $^{14}CO_2$ trapping and scintillation counting. All radioactivity measurements were carried to a counting error of ±1% by using a liquid scintillation counter (Beckman LS 250, Fullerton, CA) with automatic quench calibration at ambient temperature.

Calculations and statistical analysis

Absorption of linoleic acid by the everted intestinal sacs had to be corrected for adsorbed linoleic acid which had remained on the surface of the enterocytes despite the rinse procedure and blotting. The nonabsorbable sugar, [^{14}C]inulin, was used to determine the volume of incubation fluid that had remained adherent to the sac surface. The assumption was made that the concentrations of inulin and linoleic acid in

[2] Chow, S-L., and D. Hollander. Unpublished observations.

the adherent fluid compartment would be the same as their concentration in the incubation fluid. The net absorption of linoleic acid was derived by subtracting the amount present in the adherent mucosal fluid compartment from the total gross uptake by the following formula: $A_t = A\ (100 \div W)\ (CPM_t^h \div CPM_o^h - CPM_t^c \div CPM_o^c)$ where A_t is the net amount of linoleic acid absorbed at a given time $-\ t$ (nmol per 100 mg tissue); A is the amount (nmol) of linoleic acid in 100 μl of incubation fluid; W is the weight of the sac in mg; CPM_o^h and CPM_t^h are the specific activities of [^{3}H]linoleic acid in the pre-incubation samples and in the intestinal sacs at given time $-\ t$, respectively; CPM_o^c and CPM_t^c are specific activities of [^{14}C]inulin in the pre-incubation solution and on the intestinal sacs at specific time $-\ t$; and 100 is the correction of all the results to a constant weight denominator of 100 mg.

The data were plotted by using least-squares regression analysis (7). The absorption of linoleic acid under various experimental conditions was compared statistically to baseline data by using ANOVA and Student's t test (8).

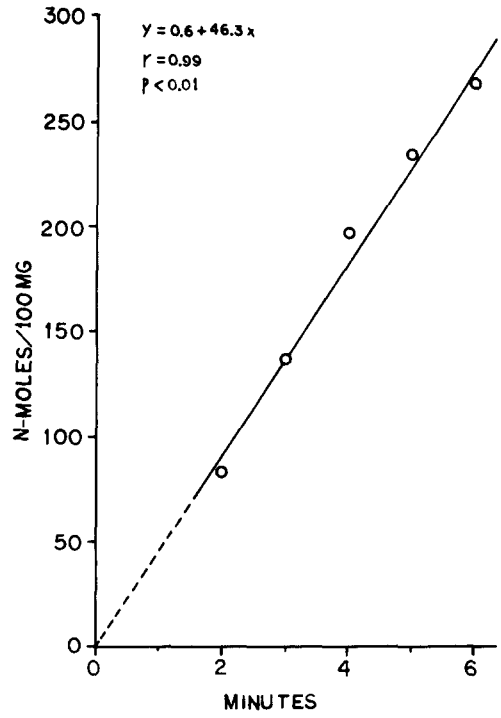

Fig. 1. The relationship between 420 μM linoleic acid absorption and time in a single experiment. The mucosal solution contained 10 mM sodium taurocholate in the standard phosphate buffer at pH 6.5 The slope was calculated by the least-squares method and was used as the value for absorption.

RESULTS

Influence of linoleic acid concentration on its absorption rate

The relationship between linoleic acid concentration and its absorption rate was studied in a micellar solution that contained linoleic acid at various concentrations and 10 mM sodium taurocholate in the standard phosphate buffer at pH 6.5. In the first series of absorption studies, linoleic acid concentration was varied from 42 to 1260 μM. From five to eight different animal experiments were performed at each linoleic acid concentration. In each set of these experiments the relationship between linoleic acid absorption and time was found to be linear during the entire 6 min of incubation. An example of a single experimental plot is shown in Fig. 1. The absorption of linoleic acid was plotted by the least-squares method, plotting the absorption vs. time and using the value of the slope as the value for absorption rate. The values for absorption from all experiments at each linoleic acid concentration were pooled and the mean ± SE rate of absorption at each concentration was plotted against the concentration. Analysis of the plot by both linear and nonlinear methods revealed that the plot fitted best to a rectangular hyperbola (**Fig. 2**) at this range of concentrations. The shape of the curve indicates that, at this range of concentrations, linoleic acid is absorbed by a saturable mechanism. On the other hand, at higher luminal concentrations (2.52–4.2 mM) the rate of linoleic acid absorption vs. its concentration fitted best to a linear plot (**Fig. 3**). This finding suggests that a passive diffusion mechanism may be the dominant mode of absorption of linoleic acid at the higher range of luminal concentrations. It is important to point out, however, that the plot of absorption vs. concentration is a continuum without an abrupt change if the data are plotted throughout the low and high ranges of concentrations.

Influence of archidonic acid on linoleic acid absorption

A constant amount of arachidonic acid (420 μM) was added to the micellar solution which contained linoleic acid at concentrations ranging from 42 to 840 μM. Linoleic acid absorption was linear with time in each experiment. The slope of absorption vs. time was plotted by the least-squares method and was used to calculate the rate of linoleic acid absorption in each individual experiment (Fig. 1). A Lineweaver–Burk plot of linoleic acid absorption in the presence and absence of arachidonic acid is depicted in **Fig. 4**. The addition of arachidonic acid to the incubation medium changed the slope describing linoleic acid absorption but did not shift the slope's intercept with the ordinate.

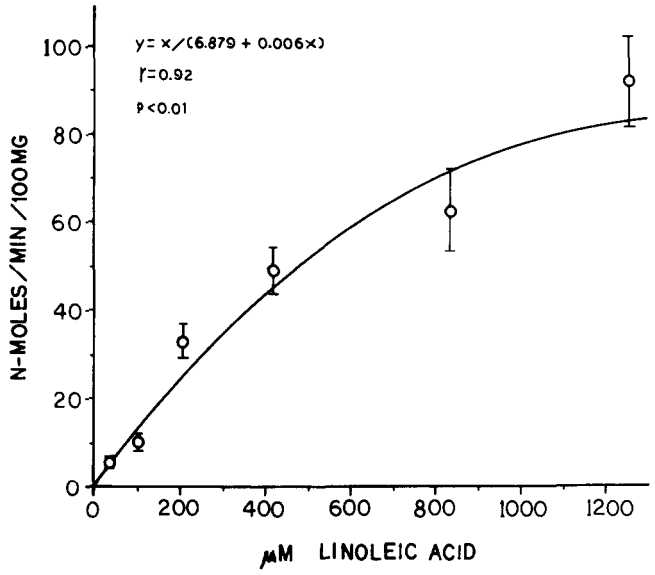

Fig. 2. The relationship between linoleic acid concentration and its absorption rate. The linoleic acid concentration was kept within the low range of intraluminal concentrations (42–1260 μM) in a micellar solution which contained 10 mM sodium taurocholate in the standard phosphate buffer at pH 6.5. The mean absorption rate of linoleic acid at each concentration was calculated from the slope of the absorption–time plots obtained by the least-squares method. The vertical bar represents standard error (SE) of the mean. The concentration rate profile fitted best to a rectangular hyperbola which was plotted with an NLIN program.

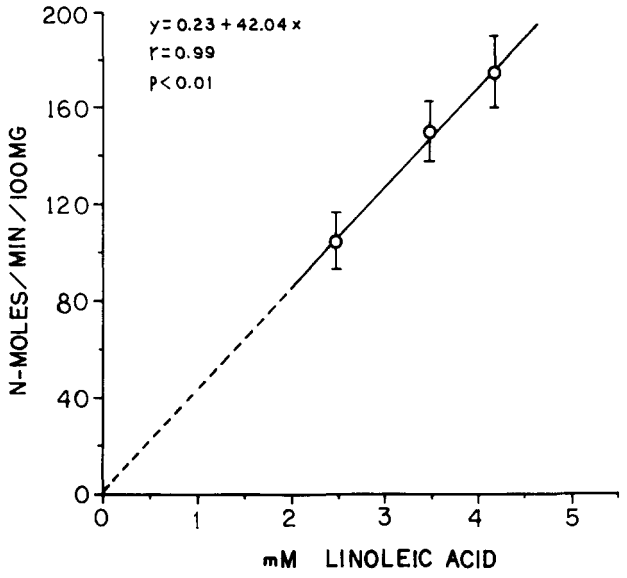

Fig. 3. The relationship between high concentrations of linoleic acid and its absorption rate. Linoleic acid concentrations were varied from 2.5 to 4.2 mM. The micellar solution contained 10 mM sodium taurocholate in the standard phosphate buffer at pH 6.5. The mean absorption rate of linoleic acid at each concentration was calculated from the slope of the absorption–time plots obtained by the least-squares method. The vertical bar represents standard error (SE) of the mean.

Thus, arachidonic acid addition changed the K_m without changing the V_m of linoleic acid absorption. The Lineweaver–Burk plot of linoleic acid absorption yielded an apparent affinity constant (K_m) of 967 μM and an apparent maximal velocity of transport (V_{max}) of 125 nmol/min per 100 mg. These values do not fit the observed experimental values (Fig. 2) because of errors inherent in the use of this plotting system in analyzing the transport of lipids such as linoleic acid (9–11). By using the Hofstee plot (10) of plotting V vs. V/C_s, where V represents the velocity of transport and C_s the concentration of linoleic acid, values for K_m and V_{max} of 478 and 92, respectively, were obtained. These values fit quite closely to the observed values for K_m and V_{max} in Fig. 2.

Effect of metabolic inhibitors and uncouplers on linoleic acid absorption

Absorption of linoleic acid in the low range of luminal concentrations was studied in the presence of a variety of metabolic inhibitors and uncouplers. The basal absorption rate of linoleic acid was compared with absorption rate in experiments in which 0.1 mM 2,4-dinitrophenol (DNP), 1 mM potassium cyanide, or 1 mM sodium azide were added separately to the standard micellar solution containing 420 μM linoleic acid. The addition of these inhibitors and uncouplers (**Table 1**) did not change the rate of linoleic acid absorption from its rate of absorption under basal conditions ($P > 0.05$).

Effect of temperature on linoleic acid absorption

The absorption rate of linoleic acid was studied at incubation temperatures of 37, 28, and 20°C. Separate

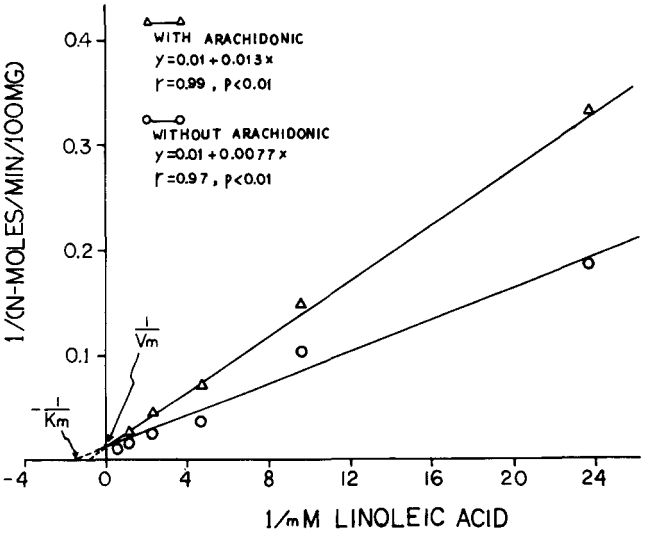

Fig. 4. Lineweaver–Burk plots of linoleic acid in the presence and absence of 420 μM arachidonic acid. The addition of arachidonic acid changed the K_m value without changing the V_m value of linoleic acid absorption.

TABLE 1. Influence of metabolic uncoupler and inhibitors on linoleic acid absorption

Inhibitor	No. Animals	Absorption Rate[a]	P^b
mM		*nmol/min/100 mg*	
none	8	46.2 ± 4.2	
DNP (0.1)	5	41.8 ± 4.3	>0.05
KCN (1.0)	5	47.3 ± 6.6	>0.05
NaN_3 (1.0)	5	44.6 ± 2.3	>0.05

[a] Values are mean ± SE of jejunal absorption rate of 420 μM linoleic acid at pH 6.5 and 37°C. Absorption rate was calculated from the slope of the absorption–time plot obtained by the least-squares method.

[b] Statistical comparison was performed by analysis of variance and *t* test.

experiments were conducted at low (420 μM) and high (2.52 mM) concentrations of linoleic acid dissolved in the standard micellar incubation solution containing 10 mM sodium taurocholate. No change ($P > 0.05$) in the absorption rate of linoleic acid at either concentration was observed at the different incubation temperatures (**Tables 2** and **3**).

Effect of unstirred water layer on linoleic acid absorption

In order to evaluate the influence of the thickness of the unstirred water layer on linoleic acid absorption, the oscillation rate of the incubation chamber was varied from 0 to 120 oscillations per min. Linoleic acid (420 μM) was solubilized in the standard micellar solution at a pH of 6.5. The absorption rate of linoleic acid increased with each increase in the oscillation rate (**Table 4**).

Influence of surfactants on linoleic acid absorption

The absorption of 420 μM linoleic acid was studied in the presence of 2, 6, or 10 mM sodium taurocholate in the standard phosphate buffer solution at pH 6.5. The absorption rate of linoleic acid in the 2 mM sodium taurocholate solution was used as a basal value. When compared to the basal value (**Table 5**), a significant increase in the absorption rate of linoleic acid was observed at sodium taurocholate concentrations of 6 and 10 mM.

Since sodium taurocholate is an anionic surfactant, absorption experiments were also performed in the presence of 2 mM Pluronic F 68, which is a nonionic surfactant composed of a mixture of polyoxyethylene–polyoxypropylene ethers with an average molecular weight of 8350 (12). The absorption rate of linoleic acid solubilized in 2 mM Pluronic F 68 was lower than its absorption rate in the 2 mM sodium taurocholate solution (Table 5).

Effect of pH on linoleic acid absorption

The pH of the incubation medium was varied from 5.4 to 7.4 by changing the relative amounts of the monobasic and dibasic salts of phosphate in the incubation solution. The incubation fluid contained 420 μM linoleic acid and 10 mM sodium taurocholate. A significant increase in the absorption rate of linoleic acid was observed as the incubation pH was decreased (**Table 6**).

TABLE 2. Influence of temperature on linoleic acid (420 μM) absorption

Temperature	No. Animals	Absorption Rate[a]	P^b
°C		*nmol/min/100 mg*	
37	8	46.2 ± 4.2	
28	5	44.2 ± 6.8	>0.05
20	5	43.3 ± 4.4	>0.05

[a] Values are mean ± SE of jejunal absorption rate of 420 μM linoleic acid at pH 6.5 and 37°C. Absorption rate was calculated from the slope of the absorption–time plot obtained by the least-squares method.

[b] Statistical comparison was performed by analysis of variance and *t* test.

TABLE 3. Influence of temperature on linoleic acid (2.52 mM) absorption

Temperature	No. Animals	Absorption Rate[a]	P^b
°C		*nmol/min/100 mg*	
37	5	118.5 ± 11.5	
28	5	111.8 ± 10.3	>0.05
20	5	109.1 ± 10.7	>0.05

[a] Values are mean ± SE of jejunal absorption rate of 2.52 mM linoleic acid at pH 6.5 and 37°C. Absorption rate was calculated from the slope of the absorption–time plot obtained by the least-squares method.

[b] Statistical comparison was performed by analysis of variance and *t* test.

TABLE 4. Influence of oscillation rate of the incubation chamber on linoleic acid absorption

Oscillations per Minute	No. Animals	Absorption Rate[a]	P^b
		nmol/min/100 mg	
0	8	9.9 ± 1.0	
40	5	39.9 ± 6.0	<0.01
80	8	46.2 ± 4.2	<0.01
120	8	51.2 ± 3.1	<0.01

[a] Values are mean ± SE of jejunal absorption rate of 420 μM linoleic acid at pH 6.5 and 37°C. Absorption rate was calculated from the slope of the absorption–time plot obtained by the least-squares method.

[b] Statistical comparison was performed by analysis of variance and *t* test using the absorption rate at 0 oscillations per minute as a baseline value.

TABLE 5. Influence of surfactants on linoleic acid absorption

Surfactant	No. Animals	Absorption Rate[a]	P[b]
mM		*nmol/min/100 mg*	
Pluronic F 68 (2)	5	23.9 ± 3.2	<0.01
Taurocholate (2)	5	32.8 ± 4.0	
Taurocholate (6)	5	38.2 ± 2.6	<0.05
Taurocholate (10)	8	46.2 ± 4.2	<0.01

[a] Values are mean ± SE of jejunal absorption rate of 420 μM linoleic acid at pH 6.5 and 37°C. Absorption rate was calculated from the slope of the absorption–time plot obtained by the least-squares method.

[b] Statistical comparison was performed by analysis of variance and *t* test using the absorption rate at 2 mM taurocholate as a baseline value.

Influence of fatty acids and lecithin additions on linoleic acid absorption

The unsaturated long chain fatty acids oleic (C18:1), linolenic (C18:3), and arachidonic (C20:4) as well as the phospholipid, lecithin, were added separately at a concentration of 420 μM to assess their possible influence on the absorption of linoleic acid. The micellar incubation solution contained 10 mM sodium taurocholate and 420 μM linoleic acid in the phosphate buffer at pH 6.5. The absorption rate of linoleic acid decreased progressively as the number of unsaturated sites of the added fatty acid was increased. The absorption rate of linoleic acid was the lowest after the addition of lecithin (Fig. 5).

DISCUSSION

The mechanism of absorption of linoleic acid and the factors modifying its rate of absorption were evaluated in vitro by using rat everted jejunal sacs. The relationship between linoleic acid concentration and its absorption rate was investigated over a wide range of luminal solution concentrations. At the low range of luminal concentrations of linoleic acid (42–1260 μM), the relationship between its absorption and its concentration delineated apparent saturation kinetics (Fig. 2); thus, mediation of the absorptive process by either active transport or facilitated diffusion is likely. To explore the possibility that an active, energy-requiring, transport mechanism is responsible for linoleic acid absorption, experiments were performed in the presence of an oxidative phosphorylation uncoupler, 2,4-dinitrophenol, or in the presence of potassium cyanide or sodium azide (cytochrome *c*-oxidase inhibitors). The separate additions of these agents to the incubation medium, which contained low concentrations of linoleic acid, did not change the absorption rate of linoleic acid when compared to baseline absorption rates (Table 1). Furthermore, when the absorption rate of linoleic acid at either low or high concentrations was assessed at temperatures ranging between 20 and 37°C, the rate of absorption did not change (Tables 2, 3). The results of these two different experimental approaches lead us to conclude that the process of linoleic acid absorption does not require metabolic energy in either range of luminal concentration. The observations of apparent saturation kinetics (Fig. 2) and the lack of a requirement for energy (Tables 1–3) provide evidence that, at low luminal concentrations, linoleic acid is absorbed by a facilitated diffusion mechanism which could be carrier mediated and does not require energy.

The addition of arachidonic acid to the solution containing low concentrations of linoleic acid changed the slope of the Lineweaver–Burk plot of the results but did not change the intercept (Fig. 4). These observations suggest that arachidonic acid may inhibit linoleic acid absorption by competing for a common carrier which may be responsible for the absorption of both compounds. Since the fatty acid-binding protein (FABP) is known to have a high affinity for long chain fatty acids (13–15) and perhaps to participate in the intracellular transport of fatty acids from the cell membrane to the intracellular organelles (13, 15), it is reasonable to suggest that FABP is the common carrier involved in the intestinal absorption of linoleic acid and arachidonic acid. Therefore, the decrease in linoleic acid absorption after the addition of arachidonic acid is likely to be caused by their mutual competition for a common carrier. It should be noted, however, that the observed decrease in linoleic acid absorption following arachidonic acid addition could also be caused by enlargement of the micellar size by the addition of arachidonic acid. An expansion in the size of the micelles would decrease the micellar rate of diffusion towards the absorptive surface of the enterocytes (16, 17). Similarly, the addition of lecithin to the incubation medium may lower the absorption rate of linoleic acid (**Fig. 5**) by producing larger-sized micelles (18) which would diffuse towards the absorp-

TABLE 6. Influence of pH on linoleic acid absorption

pH	No. Animals	Absorption Rate[a]	P[b]
		nmol/min/100 mg	
7.4	5	29.3 ± 2.8	
6.5	8	46.2 ± 4.2	<0.01
5.4	5	53.4 ± 4.7	<0.01

[a] Values are mean ± SE of jejunal absorption rate of 420 μM linoleic acid at 37°C. Absorption rate was calculated from the slope of the absorption–time plot obtained by the least-squares method.

[b] Statistical comparison was performed by analysis of variance and *t* test.

tive cell membrane of the enterocytes at a lower rate. Indeed, the combination of both factors, that is, increases in micellar size and competitive binding to the FABP, may be the best explanation for the observed decrease in linoleic acid absorption following the addition of oleic, linolenic, and arachidonic acids (Figs. 4, 5). At this point it is not possible to separate these two effects and to assess the relative contribution of either mechanism to the overall influence of the fatty acids on linoleic acid absorption rate.

At high luminal concentrations (2.52–4.2 mM) of linoleic acid in the incubation medium the absorption rate was linearly related to its concentration (Fig. 3). This observation suggests that simple diffusion is the predominant mechanism of linoleic acid absorption in this range of concentrations. Absorption of linoleic acid by passive diffusion probably conceals the coexisting carrier-mediated mechanism of absorption that was dominant at the lower concentrations of linoleic acid (Fig. 2). A similar, concentration-dependent dual mechanism of transport has been previously reported for other nutrients such as retinol (19), vitamin B_{12} (20), and thiamine (21, 22), and is probably shared by other long chain unsaturated fatty acids rather than being unique for linoleic acid.

The aqueous insolubility of linoleic acid requires surfactants for solubilization of the molecule in the aqueous intestinal phase. As the surfactant concentration is increased, fatty acids shift from the oil to the micellar phase (17). In order to test the influence of the surfactant concentration on the absorption of linoleic acid we increased the sodium taurocholate concentration in a stepwise fashion from 2 to 10 mM (Table 5). The absorption rate of linoleic acid increased in parallel with each increase in the sodium taurocholate concentration. These experiments indicate that absorption of linoleic acid is directly proportional to its micellar concentration. At higher sodium taurocholate concentrations, the amount of linoleic acid in the micellar phase would increase and the result would be an increase in the driving force across the unstirred water layer towards the absorptive cell membrane (23).

The substitution of 2 mM nonionic surfactant, Pluronic F 68, for sodium taurocholate resulted in a decrease in the overall absorption rate of linoleic acid. Since the size and weight of the Pluronic micellar particles are much larger (12) than the sodium taurocholate micelles, the diffusion rate of Pluronic micelles in the aqueous phase would be lower and, consequently, absorption would be hindered significantly.

Since the unstirred water layer at the luminal cell surface is frequently a significant barrier to micellar diffusion (17, 23), we investigated its influence on the absorption rate of linoleic acid. The thickness of the unstirred water layer was presumed to vary by changes in the rate of oscillation of the incubation chamber from 0 to 120. A 5-fold increase in the rate of absorption of linoleic acid was observed at 120 oscillations per min when compared to its absorption rate at 0 oscillations per min (Table 4). If we assume that the thickness of the unstirred layer was diminished at higher oscillations, then we concluded that the thickness of the unstirred water layer influences the absorption rate of linoleic acid. The relative importance of the resistance of the unstirred water layer vs. the resistance of the cell membrane cannot be determined with the present experimental design but it is likely that both contribute to the overall resistance to linoleic acid uptake by the enterocytes.

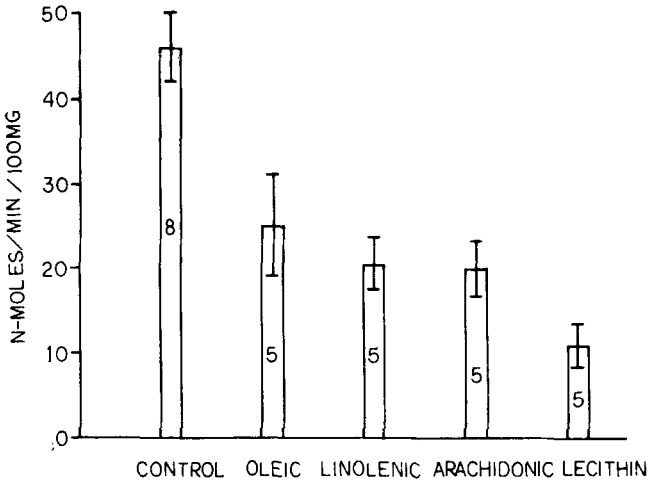

Fig. 5. The effect of other fatty acid additions on the absorption rate of 420 μM linoleic acid. Each bar represents the mean ±SE absorption rates of at least five different experiments. The figure in each bar is the number of rats used. Statistical differences between groups were calculated by comparison of slopes of the regression lines by analysis of variance. The absorption of linoleic acid in the absence of other fatty acids was used as a baseline value.

The intraluminal intestinal pH varies under normal absorptive conditions and depends on the proximity of the absorbing segment to the pylorus. We investigated the influence of the luminal pH on linoleic acid absorption by changing the incubation solution's pH from 5.4 to 7.4. An increase in the hydrogen ion concentration in the incubation fluid caused a parallel increase in the absorption rate of linoleic acid. Two separate mechanisms may account for this observation. The first mechanism has to do with the negative surface charges of both the luminal cell membrane (24) and the micellar particles (18). As the negatively charged micellar particles approach the absorptive cell membrane their surface charge increases the resistance of their diffusion towards the negatively charged absorptive cell membrane (25). An increase in the solution's hydrogen ion concentration would decrease the negative surface charge of the cell membrane, thereby lowering the

resistance to diffusion of the micellar particles towards the cell membrane and resulting in the observed increase in linoleic acid absorption (Table 6).

Changes in the ionization of the fatty acid itself offer another explanation for the increase in linoleic acid absorption at higher hydrogen ion concentrations. The pK_a of linoleic acid in sodium taurocholate micelles is 6.5 (26). Therefore, as the hydrogen ion concentration in the incubation solution is increased the proportion of linoleic acid in the protonated form would also increase. The surface charge of the linoleic acid-carrying micelles would become less negative and the result would be a decrease in diffusional resistance of the micelles towards the absorptive cell surface. Both of the above mechanisms indicate that the relative acidity of the proximal small intestine is advantageous for the absorption of linoleic acid.

Linoleic acid is an essential dietary fatty acid. Knowledge regarding the mechanism of its absorption by the small bowel and the factors modifying its absorption rate is essential for comprehension of normal nutrition and physiology. Since polyunsaturated fatty acids may retard or diminish the process of atherosclerosis, the understanding of their absorptive processes may be of therapeutic benefit as well.

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REFERENCES

1. Vergroesen, A. J. 1977. Physiological effects of dietary linoleic acid. *Nutr. Rev.* **35:** 1–5.
2. Van Dorp, D. A., R. K. Beerthuis, D. H. Nugteren, and H. Vonkeman. 1964. Enzymatic conversion of all *cis*-polyunsaturated fatty acids into prostaglandins. *Nature.* **203:** 839–841.
3. Houtsmuller, U. M. I. 1975. Specific biological effects of polyunsaturated fatty acids. *In* The Role of Fats in Human Nutrition. A. J. Vergroesen, editor. Academic Press, London, England. 331–351.
4. Esposito, G., and T. Z. Csaky. 1974. Extracellular space in the epithelium of rats' small intestine. *Am. J. Physiol.* **226:** 50–55.
5. Gregg, J. J. 1966. New solvent systems for thin-layer chromatography of bile acids. *J. Lipid Res.* **7:** 579–581.
6. Hendry, E. B. 1961. Osmolarity of human serum and of chemical solutions of biologic importance. *Clin. Chem.* **7:** 156–164.
7. Draper, N. R., and H. Smith. 1966. Applied Regression Analysis. John Wiley & Sons, New York, N.Y. 1–43.
8. Dixon, W. J., and F. J. Massey, Jr. 1969. Introduction to Statistical Analysis. McGraw-Hill, New York, N.Y. 150–215.
9. Dietschy, J. M. 1970. Difficulties in determining valid rate constants for transport and metabolic processes. *Gastroenterology.* **58:** 863–874.
10. Dowd, J. E., and D. S. Riggs. 1965. A comparison of estimates of Michaelis–Menten kinetic constants from various linear transformations. *J. Biol. Chem.* **240:** 863–869.
11. Winne, D. 1973. Unstirred layer, source of biased Michaelis constant in membrane transport. *Biochim. Biophys. Acta.* **298:** 27–31.
12. Schmolka, I. R., and A. J. Raymond. 1965. Micelle formation of polyoxyethylene–polyoxypropylene surfactants. *J. Am. Oil. Chem. Soc.* **42:** 1088–1091.
13. Ockner, R. K., and J. A. Manning. 1974. Intestinal fatty acid binding protein (FABP): Studies on physiological function. *J. Clin. Invest.* **53:** 57a.
14. Ockner, R. K., and J. A. Manning. 1974. Fatty acid binding protein in small intestine. Identification, isolation, and evidence for its role in cellular fatty acid transport. *J. Clin. Invest.* **54:** 326–338.
15. Ockner, R. K., J. A. Manning, R. B. Popenhausen, and W. K. L. Ho. 1972. A binding protein for fatty acids in cytosol of intestinal mucosa, liver, myocardium, and other tissues. *Science.* **177:** 56–58.
16. Wilson, F. A., and J. M. Dietschy. 1974. The intestinal unstirred layer; its surface area and effect on active transport kinetics. *Biochim. Biophys. Acta.* **363:** 112–126.
17. Simmonds, W. J. 1974. Absorption of lipids. *In* Gastrointestinal Physiology. E. D. Jacobson and L. L. Shanbour, editors. Physiology Series One, Vol. 4. University Park Press, Baltimore. 343–376.
18. Carey, M. D., and D. M. Small. 1972. Micelle formation by bile salts: Physical chemical and thermodynamic considerations. *Arch. Intern. Med.* **130:** 506–527.
19. Hollander, D., and K. S. Muralidhara. 1977. Vitamin A-1 intestinal absorption in vivo: Influence of luminal factors on transport. *Am. J. Physiol.* **232:** E471–E477.
20. Herbert, V. 1960. Mechanisms of absorption of vitamin B-12. *Federation Proc.* **19:** 884–886.
21. Hoyumpa, A. M., H. M. Middleton, R. A. Wilson, and S. Shenker. 1975. Thiamine transport across the intestine. I. Normal characteristics. *Gastroenterology.* **68:** 1218–1227.
22. Sklan, D., and N. Trostler. 1977. Site and extent of thiamine absorption in the rat. *J. Nutr.* **107:** 353–356.
23. Simmonds, W. J. 1976. Uptake of fatty acid and monoglyceride. *In* Lipid Absorption–Biochemical and Clinical Aspects. K. Rommel and H. Goebell, editors. MTP Press Ltd, St. Leonard's House, Lancaster, England. 51–61.
24. Schultz, S. G., and P. F. Curran. 1968. Intestinal absorption of sodium chloride and water. *In* Handbook of Physiology, Section 6, Vol III. C. F. Code, editor. American Physiological Society, Washington, D.C. 1245–1275.
25. Kruyt, H. R. 1952. *In* Colloid Science. Elsevier, New York, N.Y. 144.
26. Hofmann, A. F., and H. S. Mekhjian. 1973. Bile acids and the intestinal absorption of fat and electrolytes in health and disease. *In* The Bile Acids. P. P. Nair and D. Kritchevsky, editors. Plenum Press, New York, N.Y. 103–152.