

Monoglyceride modification of jejunal absorption of fatty acid in the rat

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Abstract The effect of increasing the intracellular pool of monoolein upon the subsequent uptake and esterification of oleic acid was investigated using *in vitro* rat jejunal slice techniques. The mucosal pool of monoglyceride was expanded by preincubation of jejunal slices in a monoglyceride-containing bile salt medium at a temperature close to 1°C, which inhibited esterification. Subsequent incubation in micellar [¹⁴C]oleic acid was performed either at 37°C or in the cold. Monoglyceride preincubation increased [¹⁴C]oleic acid uptake by about 60% without increasing incorporation of fatty acid into triglyceride. This was not due to inhibition of esterifying capacity nor to changes in oleic acid binding to a mucosal fatty acid-binding protein. It is suggested that under these experimental conditions monoglyceride may modify intracellular pools of fatty acid. However, when monoglyceride and fatty acid were preincubated together, mucosal esterification rates during subsequent incubation at 37°C more than doubled. Implications of these data for present theories of rate-limiting steps in lipid absorption are discussed.

Supplementary key words fat absorption · intestinal absorption diffusion

The uptake of fatty acid from the intestinal lumen into the mucosal cell is believed to occur by passive diffusion across the microvillous membrane (1). The bile acid-fatty acid-monoglyceride micelle increases the solubility of fatty acid in the bulk aqueous phase of the intestinal lumen and maintains and delivers a high concentration of fatty acid molecules to the mucosal surface, thus sustaining a favorable concentration gradient for inward diffusion. Recent work by Sallee, Dietschy, and Rector (2) and Sallee, Wilson, and Dietschy (3) has reemphasized the possibility that monomer diffusion occurring across the unstirred water layer surrounding the mucosal membrane may be rate limiting for fatty acid uptake by the intestine. Fatty acid-binding proteins in the cytosol (4) or in the mem-

brane (5) of the mucosal cell may also be important in controlling fatty acid absorption.

Using *in vitro* rat jejunal slice techniques, it has been determined that initial rates of oleic acid uptake are slower than initial rates of mucosal esterification of this absorbed fatty acid to form triglyceride (6). These results implied that fatty acid uptake might be rate limiting for esterification. When the amount of fatty acid presented to the intestinal cell for esterification was decreased, the rate of esterification was also reduced. However, in previous studies the availability of substrates providing the glycerol skeleton for fatty acid esterification by the mucosa in this situation was not controlled. Since the monoglyceride pathway is the primary pathway for esterification in the intestinal mucosa in rat (7), hamster (8), and man (9), it was possible that if a large pool of monoglyceride could be introduced into the mucosa the rate of fatty acid esterification might be increased. This would be expected to diminish the tissue fatty acid pool size, thus changing concentration gradients of fatty acid relative to those outside the cell, and might increase the mucosal uptake rates of fatty acid. Monoglyceride might also alter intracellular binding or the metabolism of the absorbed fatty acid.

In the present studies, rat jejunal slices were preloaded with 2-monooleyl glycerol at a temperature close to 1°C to inhibit mucosal esterification, and then they were incubated in ¹⁴C-labeled oleic acid at 37°C. This model permitted investigation of the effect of an expanded intracellular pool of monoglyceride on the subsequent uptake and esterification of fatty acid.

MATERIALS AND METHODS

[1-¹⁴C]Oleic acid was obtained from New England Nuclear, Boston, Mass.; tri[9,10-³H]oleyl glycerol was obtained from Amersham/Searle Corp., Arlington Heights, Ill. Triolein was obtained from Sigma Chemical

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Co., St. Louis, Mo., and was found to be more than 99.5% triglyceride by thin-layer silicic acid chromatography. Oleic acid was obtained from Applied Sciences Laboratories, State College, Pa., and was over 99% pure by thin-layer silicic acid chromatography. 2-Mono[9,10-³H]oleyl glycerol ([³H]monoolein) was prepared by pancreatic lipase hydrolysis of tri[9,10-³H]oleyl glycerol by the method of Mattson and Volpenhein (10). The glycerides were separated by column chromatography using 100–200 mesh silica gel obtained from Ace Scientific Supply Co., Linden, N.J. The monoglyceride fraction generally contained 95–98% monoglyceride. Initial determinations of the isomers of the monoglyceride fraction by thin-layer chromatography indicated that between 60 and 85% was 2-monoglyceride. The chromatographic procedure may itself have enhanced isomerization. Since it was determined earlier in our studies that isomerization of 2-monooleyl glycerol to the 1 or 3 isomer occurred rapidly after warming, all monoglyceride preparations were stored at –20°C.

Sodium taurocholate was obtained from Maybridge Research Chemicals, Tintagel, Cornwall, England, and was over 98% pure by thin-layer chromatography; Cotazym was obtained from Organon Inc., West Orange, N.J.

Thin-layer chromatography was performed on 250- μ m-thick silica gel plates (Analtech, Inc., Newark, Del.) that had been soaked in a 2.5% solution of boric acid in ethanol (w/v) for 2 min, dried at room temperature, and then activated at 100°C for 35 min (11).

General experimental technique

Male Wistar rats weighing 200–250 g were starved for 24 hr and killed by cervical dislocation. The abdomen was opened and the jejunum was isolated, rinsed with cold saline in situ, and removed to an ice-cooled glass plate. The jejunum was everted and slices weighing approximately 50–75 mg were cut into beakers containing chilled calcium-free Krebs phosphate (12) incubation solution containing glucose, 50 mg/100 ml of solution. The incubation medium was gassed with 5% CO₂ in O₂ for 30 min on the night before use, refrigerated, and regassed for 30 min the morning of use; the final pH was 6.7 \pm 0.1.

The basic experimental design utilized an initial "preincubation" period, during which the tissue was "preloaded" with a large excess of monoglyceride, and an "incubation" period, during which the effect of the monoglyceride on subsequent fatty acid uptake was observed. The slices were preincubated for 11 min with constant stirring in an incubation medium containing sodium taurocholate (12.5 mM) and [³H]monoolein at a temperature of 1–4°C¹ on an SK12 Thermoelectrics Unlimited,

¹ The temperature was usually 1–2°C, which completely blocked esterification (6). The recorded temperature did not fall below 1°C and occasionally approached, but did not reach, 4°C. Experiments performed at these temperatures are referred to as "in the cold."

Inc., heat exchanger. Three slices of intestine were used per milliliter of medium. Of the monoglyceride absorbed during this time, more than 99.5% remained unesterified. The tissue was then washed with warm (37°C) incubation medium over gauze, rapidly transferred to warm (37°C) incubation medium containing sodium taurocholate (12.5 mM) and [¹⁴C]oleic acid, and incubated for timed periods up to 10 min with constant agitation using a Dubnoff shaker. The incubation was terminated by rinsing the tissue in ice-cold saline acidified with HCl to a pH <1.0. The tissue was gently blotted and weighed in tared weighing bottles containing 1 ml of acid-saline. Tissue from the same animal was used for control and experimental incubations.

Analytical procedures

Weighed tissue slices were homogenized in 2 ml of acid-saline and extracted in 6 ml of toluene-ethanol 2:1. Aliquots of the toluene phases were counted in Bray's solution (13) in a Beckman 250 liquid scintillation system; all radioactivity determinations were normalized to dpm using an external standard. Other aliquots were assayed by thin-layer chromatography on boric acid-silica gel plates using a chloroform-acetone solvent system (96:4), and the radioactivity in the lipid fractions was determined (6).

Total intestinal tissue lipids were quantified by thin-layer chromatography and densitometry using a Photovolt Densicord 542A with a Photovolt TLC Scanner 53C, according to the method of Nutter and Privett (14) as modified by Ziminski and Borowski (15). Oleic acid, monoolein, and triolein (Hormel Institute, Austin, Minn.) were used as standards.

Calculation of intestinal uptake and esterification rates

Total "mucosal uptake" was calculated by regression analysis of total ³H-labeled and ¹⁴C-labeled lipid in homogenized tissue slices at timed periods up to 10 min standardized to constant wet weight of intestine. Since adsorption of lipids might result in an overestimation of the in vitro uptake rates of test fats, regression analysis of multiple points during short incubation periods has been used to quantify fatty acid uptake rates in these experiments and in previous studies from this laboratory (6). The point at which the regression line crossed the Y axis was used to estimate adsorbed fatty acid, and this amount was subtracted from the apparent total fatty acid uptake for the subsequent calculations. A recent study using extracellular markers has confirmed the adequacy of this method (3).

Esterification rates were usually calculated by regression analysis of the slopes of ¹⁴C-labeled lipid present as triglyceride by methods analogous to those used for calculating total lipid uptake without subtracting the calculated adsorbed lipid. In addition, initial esterification rates were

calculated in some studies by measuring the tissue substrate concentration ($[^{14}\text{C}]$ oleic acid) and product concentration ($[^{14}\text{C}]$ triglyceride) in individual experiments at 0, 1, 2, 3, and 5 min of incubation at 37°C . The lipid newly esterified from the immediately preceding incubation period was then calculated. Between 40 and 50 points at fatty acid substrate concentrations between 50 and 300 nmoles/g of tissue were available for calculation of esterification rates under different experimental conditions.

Since in most studies under all experimental conditions less than 10% of the tissue ^{14}C -labeled lipid was present in fractions other than unesterified fatty acid and triglyceride, only the data for incorporation into triglyceride was used for calculation of esterification rates.

Statistical analysis

The time period over which uptake or incorporation of fatty acid was linear was determined by inspection. In any paired experiment no more than one point was discarded. The slopes of uptake, etc., were then calculated for that period of time according to continuous simple linear regression formulas. All of the slopes of the combined experiments were compared by a paired t test, except when the effect of temperature upon fatty acid uptake was investigated. In those calculations, a t test comparing the difference between two sample means of equal or unequal number was used.

RESULTS

Jejunal monoglyceride uptake in the cold

Representative rat jejunal slices contained 834 ± 184 nmoles/g wet weight of endogenous monoglyceride and 1140 ± 340 nmoles/g wet weight of fatty acid (means \pm SEM) prior to incubation. Jejunal uptake of $[^3\text{H}]$ monoolein from the preincubation medium was linear for at least 11 min. After a 15-min incubation in 3 mM $[^3\text{H}]$ monoolein solution, tissues contained a mean of 743 nmoles of ^3H -labeled lipid. From 1 mM $[^3\text{H}]$ monoolein, about 250 nmoles/g wet weight of tissue was taken up in the same time, suggesting that the rate of uptake in the cold paralleled the medium concentration. Separation of strips of jejunum into mucosa and submucosa after incubation in $[^3\text{H}]$ monoolein showed that over 90% of the ^3H was present in the mucosal portion. Over 99.5% of the ^3H -labeled lipid recovered from the jejunal tissue was present as monoglyceride. The distribution of the monoglyceride isomers in the tissue after preincubation and during the first 3 min of incubation paralleled that in the medium.

Effect of monoglyceride preincubation on subsequent fatty acid uptake (Expt. I)

Preloading mucosal slices with monoglyceride in the cold resulted in a significantly increased subsequent up-

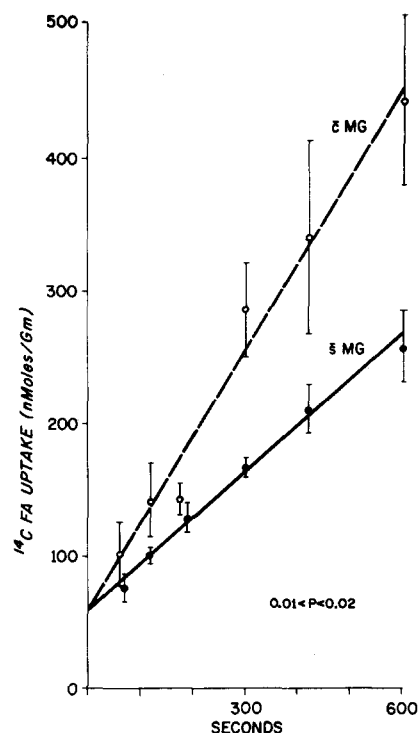


Fig. 1. Expt I: effect of monoolein preincubation on subsequent uptake of $[^{14}\text{C}]$ oleic acid (FA) at 37°C ; ●, without monoolein; O, with monoolein. Preincubation medium contained 3.0 mM monoolein; 12.5 mM sodium taurocholate; phosphate buffer, pH 6.7; temperature, "in the cold." Incubation medium contained 0.33 mM $[^{14}\text{C}]$ oleic acid; 12.5 mM sodium taurocholate; pH, 6.7; temperature, 37°C . Means \pm SEM of seven animals; with statistical comparison of slopes. MG, monoglyceride.

take of $[^{14}\text{C}]$ oleic acid at 37°C ($0.01 < P < 0.02$, Fig. 1). Fatty acid uptake per unit wet weight of intestinal tissue was linear in both control and experimental tissues, and extrapolation to zero time indicated a common intercept on the vertical axis. This intercept probably represented the amount of adsorbed or loosely bound fatty acid (≈ 62 nmoles/g wet weight); the rest was presumed to have entered the tissue.

The increased uptake in the presence of monoglyceride was clearly apparent within 120 sec after incubation at 37°C . However, there was no difference in esterification (as measured by incorporation of ^{14}C -labeled fatty acid into triglyceride) between tissues preloaded with monoglyceride and control slices (Fig. 2). At each time point the extra ^{14}C was found predominantly as unesterified fatty acids.

Effect of preincubation with monoglyceride or with fatty acid plus monoglyceride on subsequent fatty acid uptake (Expt. II)

In order to exclude the possibility that the large intramucosal pool of monoglyceride had inhibited the esterifying enzyme system, tissues were preincubated either in $[^3\text{H}]$ monoolein (1 mM) plus $[^{14}\text{C}]$ oleic acid (0.33 mM)

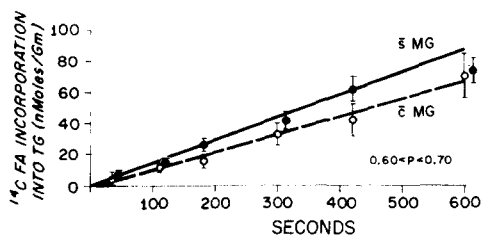


Fig. 2. Expt. I: effect of monoolein preincubation on esterification of [^{14}C]oleic acid at 37°C ; ●, without monoolein; ○, with monoolein. Experimental details as in Fig. 1. TG, triglyceride; MG, monoglyceride.

or in [^3H]monoolein alone. The amount of oleic acid taken up by the tissue during preincubation in the cold is indicated by the separation of the intercepts in Fig. 3. The subsequent uptake of [^{14}C]oleic acid during incubation at 37°C , as shown by the slopes in Fig. 3, was not significantly affected by the presence of the fatty acid already taken up from the preincubation medium. However, the fatty acid esterification rate under these conditions was significantly greater in the tissues preloaded with fatty acid plus monoglyceride ($0.025 < P < 0.05$, Fig. 4) than with monoglyceride preloading, implying that the large pool of monoglyceride did not suppress the esterifying system. Direct comparison of data between experiments I and II is somewhat hazardous because they involve different groups of rats. However, the percentage of [^{14}C]oleic acid incorporated into triglyceride after 5 min of incubation was greater in tissues preincubated with oleic acid plus monoglyceride (42% of total tissue ^{14}C) than in tissues preincubated with [^{14}C]oleic acid-free medium (37% of total tissue ^{14}C) despite the much greater tissue

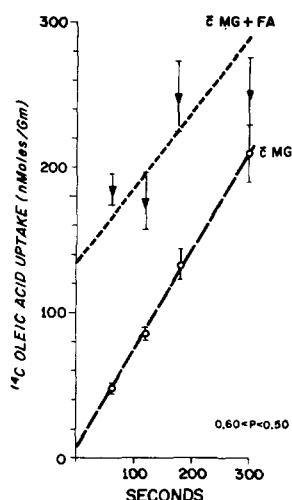


Fig. 3. Expt. II: effect of monoolein or [^{14}C]oleic acid plus monoolein preincubation on uptake of [^{14}C]oleic acid at 37°C ; ▼, monoolein plus oleic acid; ○, monoolein. Experimental details as in Fig. 1 except preincubation medium contained 1.0 mM monoolein or 0.33 mM [^{14}C]oleic acid plus 1.0 mM monoolein. Means \pm SEM of four animals. MG, monoglyceride.

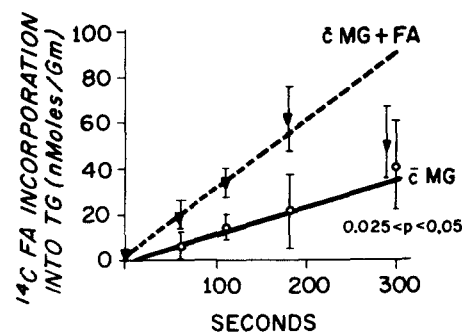


Fig. 4. Expt. II: effect of monoolein or [^{14}C]oleic acid plus monoolein preincubation on esterification of [^{14}C]oleic acid; ▼, monoolein plus oleic acid; ○, monoolein. Experimental details as in Fig. 3. TG, triglyceride; MG, monoglyceride.

pool of fatty acid available for esterification at the onset of incubation.

Effect of preincubation with monoglyceride on subsequent uptake of fatty acid in the cold (Expt. III)

In order to evaluate whether the increased uptake of fatty acid by tissues preincubated in monoglyceride was energy independent, experiments were performed in which the uptake of 0.33 mM [^{14}C]oleic acid was measured in the cold after monoglyceride preincubation at the same temperature. Control flasks were preincubated in taurocholate-containing incubation medium without monoglyceride. After monoglyceride preincubation, increased fatty acid uptake occurred also in the cold (Fig. 5). Six experiments were performed, and the difference in oleic acid uptake after preincubation with monoglyceride approached a significance level close to 0.05. One of the six experiments gave a result opposite to that of the others, and when this experiment was excluded, the significance level (P) was between 0.005 and 0.01.

Effect of micellar monoglyceride on efflux of fatty acid from jejunal slices (Expt. IV)

Since the capacity of expanded micelles to solubilize fatty acid is greater than that of a pure bile salt solution (16), it was possible that preincubation in monoolein-taurocholate media might have depleted freely diffusible intracellular oleic acid pools more than preincubation in taurocholate buffer alone. Such an effect might then have increased the "inward diffusion gradient" for exogenous fatty acid, giving data suggestive of enhanced oleic acid uptake. Accordingly, experiments were performed in which jejunal slices, preincubated in 0.66 mM [^{14}C]oleic acid were subsequently incubated at 37°C in 12.5 mM taurocholate media with and without monoolein. The rate of diffusion of [^{14}C]oleic acid out of the tissue at 37°C was not increased in the presence of monoolein. It seems unlikely, therefore, that the increase in uptake of labeled

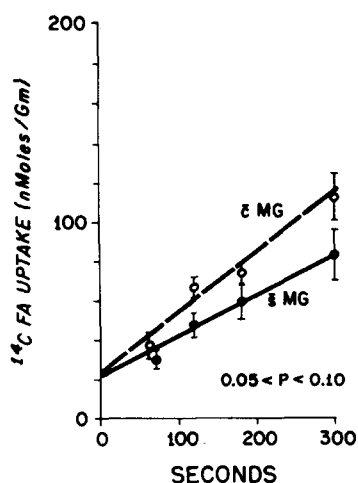


Fig. 5. Expt. III: effect of monoolein preincubation on uptake of [^{14}C]oleic acid in the cold; ●, without monoolein; ○, with monoolein. Temperature less than 4°C . Means \pm SEM of six animals. Experimental details as in Fig. 3. MG, monoglyceride.

oleic acid after monoolein preloading was effected by a decrease in a freely diffusible endogenous fatty acid pool during monoolein preincubation.

Regulation of fatty acid uptake by temperature-dependent esterification rate (Expt. V)

The uptake rates of [^{14}C]oleic acid in the cold and at 37°C are compared in Figs. 6 and 7. At 37°C , fatty acid uptake was significantly increased ($P \leq 0.005$), but the amount taken up and not incorporated into glycerides was the same at both temperatures (Fig. 7). These data are consistent with the thesis that when esterification depletes the fatty acid pool, passive inward diffusion of the substrate along concentration gradients increases.

DISCUSSION

The studies presented were designed to test the hypothesis that mucosal uptake of fatty acid occurs by passive diffusion and would be enhanced when a pool of intracellular acceptor for fatty acid esterification is provided. Initial intestinal oleic acid uptake rates were previously shown to be increased when esterification of the absorbed fatty acid was stimulated (6). In the present experiments, when esterification occurred during incubation at 37°C , total oleic acid uptake was also increased (Fig. 6). However, when esterified fatty acid was subtracted from the total [^{14}C]labeled lipid present in the tissue, the change in tissue levels of unesterified oleic acid with time was identical with that obtained during incubation in the cold (Fig. 7). These data suggest that, at the concentrations of fatty acid used in the present study, the intracellular pool of absorbed fatty acid controlled the mucosal uptake rate.

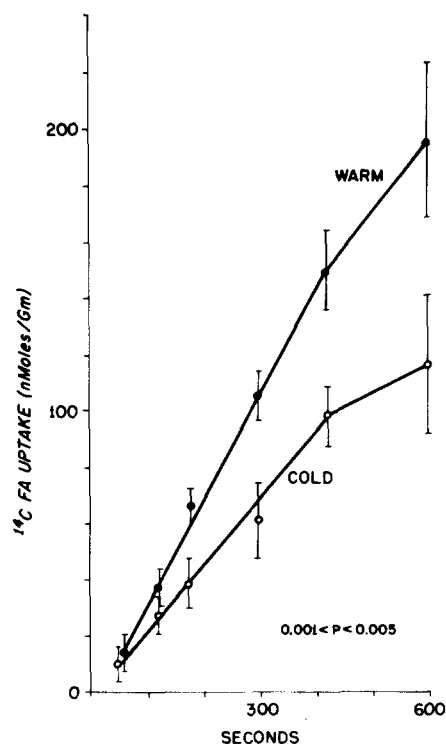


Fig. 6. Effect of temperature on total tissue [^{14}C]labeled lipid concentration during incubation. Temperature: ○, less than 4°C ; ●, 37°C . Means \pm SEM of six animals (less than 4°C) and seven animals (37°C). Data taken from studies in the absence of monoglyceride preincubation shown in Figs. 1 and 5.

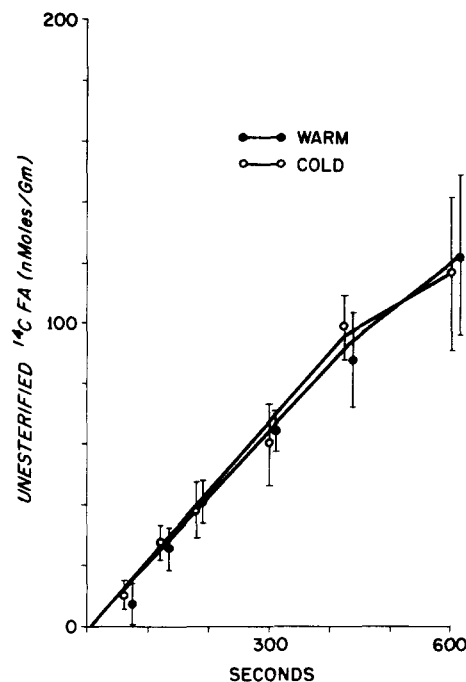


Fig. 7. Effect of temperature on tissue [^{14}C]oleic acid concentration during incubation. Temperature: ○, less than 4°C ; ●, 37°C . Means \pm SEM of six animals (less than 4°C) and seven animals (37°C). Data taken from studies in the absence of monoglyceride preincubation shown in Figs. 1, 2, and 5.

When the fatty acid pool was depleted by esterification, greater amounts were absorbed from the incubation medium.

Preloading jejunal slices with monoolein by incubation in the cold was found to enhance the rate of subsequent oleic acid uptake at 37°C by about 60%. However, this extra exogenous fatty acid was *not* incorporated into triglyceride, nor was monoolein incorporation into triglyceride at 37°C increased by previous monoglyceride preincubation. The reasons for these unexpected results were explored in several different ways. One possible explanation was that increased fatty acid "uptake" in the presence of monoglyceride did not represent true absorption into the mucosal cell but rather was due to micellar adsorption on the outside of the tissue despite the rigidly reproducible washing of the tissue after incubation in all studies. There is no satisfactory direct way of proving whether test lipids after incubation are present on the outer or inner side of the mucosal membrane. However, regression analysis of total ¹⁴C uptake crossed the Y axis at identical points during preincubation with or without monoglyceride (Fig. 1). Furthermore, one would expect that micellar fatty acid and monoglyceride adsorbed on the outer side of the membrane would diffuse away from the tissue more rapidly when transferred to a lipid-free incubation medium. The diffusion of monoglyceride from the tissue for up to 5 min after transfer into a non-monoglyceride-containing medium was found to be slow and not significantly different irrespective of the experimental conditions used. In additional studies, the diffusion of [¹⁴C]oleic acid out of the tissue and into a Krebs phosphate medium containing taurocholate (12.5 mM at 0–1°C) was measured after preincubation with or without monoglyceride and incubation in [¹⁴C]oleic acid. There was no difference in the rate of diffusion of the fatty acid "taken up" under these experimental conditions. The possibility that monoglyceride present in the preincubation medium might have increased the efflux of endogenous fatty acid from the mucosa with subsequent replacement (or exchange) by the [¹⁴C]oleic acid during incubation was ruled out by direct measurement of oleic acid efflux under the experimental conditions used. Alternatively, monoolein absorbed during preincubation might first have been esterified with endogenous unlabeled fatty acid that was then rapidly replaced by [¹⁴C]oleic acid from the medium. This was ruled out by the fact that oleic acid was taken up by mucosal tissue at a faster rate in the cold as well as at 37°C after monoolein preincubation.

The large pool of absorbed monoglyceride might also have interfered with the esterifying capacity of the tissue or might have altered intracellular binding of fatty acid. The former explanation appears unlikely because fatty acid plus monoglyceride preincubation resulted in greater

oleic acid incorporation into triglyceride by the jejunal slices. The importance of binding of fatty acid in mucosal fat absorption has attracted some attention recently. Mishkin, Yalovsky, and Kessler (17) showed that fatty acids were bound within intestinal cells with differing affinities. Changes in intracellular binding assume great importance if fatty acid uptake involves diffusion from a high to a low concentration across the lipid membrane because such binding would reduce the pool of free fatty acid on the inner side of the membrane, thereby increasing concentration gradients. Ockner et al. (4) have described a fatty acid-binding protein (FABP) in the supernatant fraction of intestinal mucosal cells that has a high binding affinity for oleic acid. This binding protein appears to be identical with the Z binding proteins fully described by Reyes et al. (18) in liver but also found in the intestinal mucosa (19). Very recently, Kessler and Mishkin (5) have presented preliminary evidence that a fatty acid-binding protein may be present in the mucosal cell membrane that during fat absorption is displaced into the cytosol, then showing some of the characteristics of the Z binding protein. In preliminary evidence that a fatty acid-binding protein may show *in vitro* binding of our mono[9,10-³H]oleyl glycerol to a mucosal fraction containing FABP. When equimolar concentrations of [³H]monoolein and [¹⁴C]oleic acid were added to mucosal FABP there was less than 15% inhibition of oleic acid binding. Thus, our data seem unlikely to be explained by direct effects of monoglyceride on such binding proteins. It appears, therefore, that the increment of fatty acid taken up after monoglyceride preloading is sequestered in a pool that neither permits rapid esterification nor interferes with further mucosal fatty acid uptake. One possible mechanism for this effect might involve a process akin to intracellular micelle formation by the large monoolein pool with subsequently absorbed fatty acid that would compete with binding by mucosal fatty acid-binding proteins. Physical binding of a portion of absorbed fatty acid might prevent access to the esterification site yet not influence further fatty acid uptake. Further detailed studies are needed to examine this possibility. Whatever the explanation, the present data give further indication that absorbed fatty acid is present in the intestinal mucosal cell in pools with different effects upon fatty acid esterification and uptake.

Fatty acid esterification occurs by both the monoglyceride and the α -glycerophosphate pathways. In previous studies of prolonged incubation (usually 30 min in duration) in hamster jejunum, esterification via the α -glycerophosphate pathway was reduced when monoglyceride was included in an incubation medium as an additional intracellular fatty acid acceptor (8). The total fatty acid uptake

² Ockner, R. K. Unpublished observations.

by the tissue from the incubation medium was similar in these experiments, suggesting that monoglyceride did not stimulate esterification capacity. In the present studies when tissues were preincubated with micellar monoolein and oleic acid, the incorporation of [¹⁴C]oleic acid into triglyceride was significantly greater than that measured in the absence of monoglyceride. Initial esterification rates of the absorbed oleic acid present in the tissue in concentrations ranging between 50 and 300 nmoles/g wet weight were calculated. In the absence of monoolein, an esterification capacity of about 0.12 nmole of oleic acid/g/sec was measured, and this rate was unaltered when monoglyceride only was present during preincubation. Preincubation with monoolein plus oleic acid resulted in esterification capacity of about 0.3 nmole of oleic acid/g/sec. These results clearly indicate that micellar monoglyceride can increase the esterification rate of absorbed unesterified fatty acid by the intestinal mucosa. However, we were unable to calculate to what extent the increased rate of esterification represented incorporation via the monoglyceride pathway because monoglyceride isotopically labeled in both glycerol and fatty acid moieties was not used in the present experiments.

Recent data clearly indicate that diffusion of long-chain fatty acids through the unstirred water layer around mucosal cells delays mucosal uptake (20). In our experiments, the conditions of stirring during incubation were so mild that the unstirred water layer must have been very large indeed. It seems pertinent, therefore, to examine where our data assists in the understanding of this potential barrier for overall fatty acid uptake. Diffusion of fatty acid through the lipid cell membrane can be sustained only by a net difference in fatty acid concentration between the outer and inner surfaces. If monomolecular diffusion through a large extracellular unstirred layer is to be rate limiting for fatty acid absorption, one might expect changes in the intracellular concentration of oleic acid to have little effect on the overall influx from the bulk phase into the cell. However, a relatively small increase in mucosal esterification rates resulted in a parallel increase in oleic acid flux from the medium in the present studies. Furthermore, in the presence of an intracellular monoglyceride pool, "excessive" amounts of oleic acid were taken up from the medium even when this fatty acid was not further esterified during the experimental period. These data are consistent with the concept that intracellular pools of oleic acid sensitively regulate oleic acid uptake under the present experimental conditions. Control might have been effected either by altering the kinetics of release of membrane fatty acid to the intracellular pool or by a change in the rate of transfer of fatty acid molecules through the membrane. Although the unstirred water layer may be a barrier to the movement of fatty acid, it

seems unlikely that it was rate limiting for overall fatty acid flux from the medium and into the cell. ■■

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