Journal of Physiology (1990), **421**, *pp.* 463–474
With 4 figures
Printed in Great Britain

MECHANISM OF INTESTINAL FATTY ACID UPTAKE IN THE RAT: THE ROLE OF AN ACIDIC MICROCLIMATE

By YIH-FU SHIAU

From the G-I Section, Veterans Administration Medical Center and the University of Pennsylvania, Philadelphia, PA 19104, USA

(*Received 14 April 1989*)

SUMMARY

1. Micellar solubilization of lipolytic products is an important step in lipid absorption. However, micelles are not absorbed intact; dissociation of lipolytic products from bile salt micelles must occur. The dissociation of micelles has been postulated to occur in an acidic microclimate.

2. The effect of an acidic microclimate on the uptake of micellar fatty acid was examined in the rat intestine. We reported that the presence of a lower pH microclimate is associated with a higher fatty acid uptake, suggesting that a lower pH enhances fatty acid uptakes from the micelles.

3. Fatty acid uptake from solutions containing a constant amount of bile salt (10 mM) and varying amounts of fatty acid (3·3–26·4 mM) revealed a saturation phenomenon which reflects the fatty acid carrying capacity of a 10 mM-taurocholate solution.

4. There was a linear relationship between fatty acid uptake and fatty acid concentration when the micellar solutions contained a constant ratio of fatty acid and taurocholate (1·32).

5. Our results indicate that the fatty acid carrying capacity of the micelle and the number of micelles in the solution are both important determinants for the amount of fatty acids delivered to the microclimate. The amount of fatty acids derived from the dissociation of micelles within the microclimate determines fatty acid uptake by the intestine.

INTRODUCTION

Solubilization of lipolytic products by bile salt micelles is an important event in normal fat absorption (Hofmann & Borgstrom, 1962; Hofmann & Small, 1967; Hofmann, 1968, 1974, 1976; Johnston, 1968; Carey & Small, 1970; Dietschy 1978; Shiau, 1981, 1987; Thomson & Dietschy, 1981). Mixed micelles are transported from the lumen to the absorptive site. However, micelles are not absorbed as an intact structure (Johnston, 1968). The dissociation of lipolytic products from bile salt micelles must precede their absorption. Several hypotheses have been postulated to explain this event (Hofmann, 1974; Dietschy, 1978; Shiau & Levine, 1980; Chijiwa & Linscheer, 1987*b*). One hypothesis suggests that due to a random Brownian movement, micelles collide against the cell membrane and lipolytic products are

MS 7638

transferred to the cell (Hofmann, 1974). However, there were no experimental data to support this hypothesis.

The second hypothesis suggests that fatty acids are equilibrated between the micellar and intermicellar phases in the lumen (Chijiwa & Linscheer, 1987*b*). Micelles function as a transport vehicle in the lumen and fatty acid uptake occurs mainly from the intermicellar phase. Uptake of fatty acid from the intermicellar phase leads to the release of fatty acid from the micelles. Thus the shifting of micellar fatty acids to the intermicellar phase is a key event for absorption. However, the same group of investigators also reported that this model cannot explain the effect of luminal pH on oleic acid uptake (Chijiwa & Linscheer, 1987*a*). Thus fatty acid uptake cannot be explained solely by this hypothesis.

A third hypothesis involves an unstirred intermediate compartment on the surface of the intestine which forms the major diffusion barrier for the large molecular species (Wilson & Dietschy, 1972; Westergaard & Dietschy 1974, 1976; Dietschy, 1978). Since micelles are large molecular aggregates, the major barrier to their diffusion is the unstirred water layer. Within the unstirred water layer, fatty acid monomers are in equilibrium with fatty acids solubilized in micellar aggregates. Removal of fatty acid monomers from the unstirred water layer by absorption allows a continuous release of fatty acid from micellar aggregates (Westergaard & Dietschy, 1974, 1976).

A fourth hypothesis suggests that micelles dissociate upon entering a low pH compartment. Fatty acids released in the acidic microclimate are protonated; therefore they are preferentially partitioned into a lipid membrane (Shiau & Levine, 1980). This hypothesis was originally derived from data generated from a test-tube model (Shiau & Levine, 1980) and supported by the demonstration of the presence of an acidic microclimate on the surface of the intestinal lining (Lucas, Schneider, Haberich & Blair, 1975; Shiau, Fernandez, Jackson & McMonagle, 1985).

The last two hypotheses involve a three-compartment model. However, the major differences are the postulated driving force for fatty acid uptake. In the unstirred water layer model, the major driving force for fatty acid absorption is the concentration of the monomeric fatty acid in this intermediate compartment. Absorption of fatty acid monomers from the unstirred water layer allows further release of fatty acid from micellar aggregates (Westergaard & Dietschy, 1976). Thus micelles only serve as the reservoir of fatty acid. In contrast, the major driving force in the dissociation model is the total fatty acid concentration in the microclimate deriving mainly from dissociation of micelles. The number of micelles entering the microclimate and the amount of fatty acid carried by individual micelles will have a major effect on the uptake. Due to the difference in the driving force, these two hypotheses can be tested. We have confirmed that there is a low-pH compartment at the surface of the jejunal epithelium (Shiau, Fernandez, Jackson & McMonagle, 1985) and show, in the present study, that its removal decreases fatty acid uptake from fatty acid bile salt micelles. The kinetics of fatty acid uptake measured under different experimental conditions can be accounted for by the dissociation model.

METHODS

A previous report from our laboratory (Shiau, Fernandez, Jackson & McMonagle, 1985) has shown (*a*) an acidic microclimate is present on the surface of the intestine, (*b*) within the same segment of the small intestine, pH measurements obtained from different sites were identical, (*c*) the microclimate pH can be altered by using gentle shearing, and (*d*) if the initial microclimate pH of the intestine is higher than 6·2, incubating the intestine in a medium containing 28 mM-glucose can lower the microclimate pH. These features are the bases of the following experimental design.

Experimental protocol

Male Sprague–Dawley rats (Charles River Laboratory, Cambridge, MA, USA) weighing 300–400 g were used in this study. Under Nembutal anaesthesia (50 mg (kg body weight)$^{-1}$), the abdominal cavity was opened, the ligament of Treitz was identified and a small incision on the small intestine was made. The luminal contents were flushed to the distal intestine with normal saline (0·9% NaCl). A 12 cm segment of proximal jejunum (2 cm from the ligament of Treitz) was removed, everted and the luminal contents were gently washed away.

In order to correlate the microclimate pH to fatty acid uptake, a 12 cm segment of jejunum was divided into three 4 cm segments and placed in a 4 °C saline solution. Each segment of the everted intestine was secured on a metal rod (o.d. 2·5 mm) with three ligatures. The first randomly selected segment was used as the control; a 2 cm portion was removed for pH measurement and the remaining 2 cm segment secured on the rod was used for fatty acid uptake. The second randomly selected segment was spun at 1000 r.p.m. for 90 s to eliminate the microclimate. At the end of the spin 2 cm of the intestine was removed for pH measurement and the remaining 2 cm segment of the intestine was used for fatty acid uptake measurement. The last segment was spun at 1000 r.p.m. for 90 s and then incubated at 37 °C in phosphate buffer containing 5 mg ml^{-1} glucose (28 mM) for 5 min. At the end of the incubation, a 2 cm segment was removed for pH measurement and the remaining tissue on the rod was used for fatty acid uptake study. In order to eliminate the possible effect of timing on the uptake study, the order of incubation was also randomized.

Microclimate pH measurement

pH electrode. The pH-sensitive electrodes were a modification of the flat-surface electrode described by Lucas *et al.* (1975). The properties and functional details of the flat-surface electrode were reported earlier by our laboratory (Shiau, Fernandez, Jackson & McMonagle, 1985). Basically, a pH-sensitive glass membrane (Corning 015 glass) was sealed to the tip of a small glass capillary tube (o.d. 2·0 mm). After overnight hydration, the electrode was filled with 0·05 M-HCl. A restricted diffusion KCl electrode was used as the reference. The surface of the reference and the pH electrodes were placed at the same level. Ag–AgCl wires were used to connect both the pH and the reference electrodes to the potentiometer (Keithley, 602). Output from the potentiometer was connected to a recorder and the potential changes during the study were recorded graphically.

All of the electrodes used in this study exhibited a linear relationship between voltage and pH within the pH range tested. Only those with greater than 52 mV (pH unit)$^{-1}$ at room temperature were used (greater then 85% of the ideal response).

Calibration of pH electrode. The pH was calibrated at 37 °C using commercially prepared buffers at pH 5·0 and pH 7·0. The mV/pH relationship was estimated at the beginning and at the end of the experiment. Based on the mV/pH relationship, the pH of each measurement was calculated.

Tissue preparation for microclimate pH measurement. The everted intestine was opened along the antimesenteric side and then mounted on a tissue holder which was then placed between two half-chambers. These two chambers were perfused independently from two reservoirs. The bottom, serosal, compartment was circulated by a gas-lifting system. The top, mucosal, compartment was not completely sealed, circulation of the medium in the top compartment was achieved by a circulating pump. The opening of the top compartment allowed the advancement of the pH electrode towards the mucosal surface to measure the surface pH. Details of the chamber construction were shown in an earlier paper (Shiau, Fernandez, Jackson & McMonagle, 1985). Both compartments were perfused continuously with a well-oxygenated, calcium-free, phosphate buffer, pH 7·2.

The pH electrode was mounted on a micromanipulator. The electrode was advanced slowly

towards the surface of the intestinal lining. The maximum potential change was recorded and then converted to pH. The microclimate pH was the lowest pH obtained as previously defined (Shiau, Fernandez, Jackson & McMonagle, 1985).

Measurement of intestinal fatty acid uptake

Two different tissue preparations were used for fatty acid uptake studies. The preparation used to correlate the uptake and microclimate pH was a 2 cm segment of the everted intestine secured on the rod as previously described (McClintock & Shiau, 1983). The tissue was incubated at 37 °C in a test-tube with 8 ml of medium contained 3·3 mM-[^{3}H]oleic acid, 10 mM-sodium taurocholate

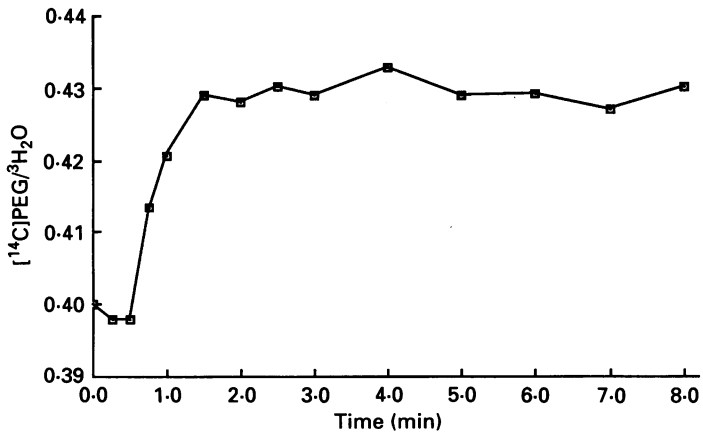

Fig. 1. Validation of intracellular fatty acid uptake measurement. [^{14}C]PEG and ^3H_2O (1/2·5 ratio) were added to the incubation medium. After incubating the medium with everted intestinal rings, the ^{14}C to ^{3}H ratio in the medium was measured over 8 min. The change in the ratio indicated that these two markers equilibrated differently. Water equilibrated into a larger water pool, presumably both intra- and extracellular space. PEG equilibrated into a lesser water pool, presumably the extracellular compartment. A steady state of equilibrium was reached after a 90 s incubation.

and a tracer amount of [^{14}C]polyethylene glycol in calcium-free phosphate buffer. The medium was pre-gassed with 100% oxygen. Uptake was measured at the end of 2 min of incubation in an unstirred condition to make sure that the microclimate was not disrupted.

At the end of incubation, the tissue was inactivated in acid saline (0·9% NaCl acidified to pH 1·0 with 12 M-HCl) and homogenized in 5 ml of calcium-free phosphate buffer. A fraction of the homogenate was used for ^{3}H and ^{14}C measurement. [^{14}C]PEG was used to correct the adherent water volume and to calculate the uptake of the tested compounds.

Validation of the everted ring preparation. ^3H_2O and ^{14}C-labelled non-absorbable marker (PEG) was added to calcium-free, phosphate buffer. The activities of ^{3}H and ^{14}C in the original buffer were measured and the ratio of ^{3}H to ^{14}C was calculated. Ten pieces of the pre-weighed everted intestinal rings (approximately 25 mg per ring) were added to 2 ml of the medium and incubated at different time intervals starting from 15 s to 8 min. At the end of incubation, the tissue and the medium was poured through a gauze filter. One millilitre of the medium was collected and counted. The ^{14}C/^{3}H ratio of the medium was plotted against time (Fig. 1). The tissues were homogenized and the extracellular space calculated. Within the first 30 s, the ratio reduced initially and increased rapidly afterwards. After 90 s, the ratio became constant. Thus by 2 min of incubation, distribution of the non-absorbable marker had reached equilibrium in the everted ring preparation. Measurements of intracellular fatty acid obtained after a 2 min incubation were valid. The change in the ratio indicated that there was an uneven distribution of water and non-absorbable marker. Water was distributed into a larger space than was PEG. The calculated extracellular space reached 23±2% of the wet weight.

Validation of the everted intestinal segments preparation. The everted intestine was secured on a

metal rod with two ligatures. The intestine was incubated at 37 °C in 8 ml phosphate buffer in an unstirred condition. The medium containing [^{14}C]PEG was pre-gassed with 100% oxygen. After the tissue was incubated in the medium for 15 s to 8 min, the tissue was removed and blotted dry on an evenly damped filter paper. The middle portion of the everted intestine was transferred to a pre-weighed vial and the tissue weight was obtained gravimetrically. Tissue was then homogenized in 2 ml of saline. A 0·5 ml volume of the homogenate and 1 ml of the incubation medium were used for scintillation counting. The extracellular space was calculated and expressed as ml(g tissue)$^{-1}$. The PEG fluid space in this preparation after equilibration was estimated to be 0·18 ± 0·02 ml (g tissue)$^{-1}$. Equilibration of PEG in the extracellular space occurred by 2 min of incubation.

Medium. The medium used for fatty acid uptake was made up of sodium taurocholate, [^{3}H]oleic acid and a tracer amount of [^{14}C]PEG 4000 in calcium-free phosphate buffer which contained (in mequiv/l): 153 sodium, 2 potassium, 143 chloride, 2 monobasic phosphate and 5 dibasic phosphate. Concentrations of bile salts and oleic acid are specified in the results section. The pH of the buffer was adjusted to 7·2. The incubation medium was pre-gassed with 100% oxygen at 2 min^{-1} for at least 30 min.

Materials

Oleic acid and taurocholate were purchased from Calbiochem (San Diego, CA, USA). Labelled oleic acid, taurocholate and PEG were all purchased from New England Nuclear (Boston, MA, USA).

Statistics

Comparison of fatty acid uptake and microclimate pH among the experimental groups were made by using one-way analysis of variance and Bonferroni *t* tests.

RESULTS

Effect of microclimate pH on fatty acid uptake (*Table* 1)

The microclimate pH of the control tissue 6·05 ± 0·05, the spun tissue 6·47 ± 0·04, and that of the tissue incubated in glucose solution was 5·96 ± 0·07. Using the Bonferroni *t* tests, the pH of the spun segment was significantly higher than that of the control and that of the segment incubated in 28 mM-glucose ($P < 0·05$). There was no difference in uptake of fatty acid from a medium containing 10 mM-sodium taurocholate and 3·3 mM-oleic acid by the control everted intestinal segment and that incubated in 28 mM-glucose (5·07 ± 0·19 and 5·29 ± 0·23 nmol (mg protein)$^{-1}$ min^{-1} respectively). The spun tissue had the least uptake (4·45 ± 0·24 nmol (mg protein)$^{-1}$ min^{-1}, $P < 0·05$), suggesting that higher microclimate pH retarded fatty acid uptake. Comparable patterns of fatty acid uptake were observed with 13·2 mM-oleic acid in 10 mM-taurocholate. These findings imply that the acidic microclimate plays an important role in fatty acid uptake.

Kinetics of fatty acid uptake

The majority of fatty acid in the intestinal lumen is carried by bile salt micelles. A small portion exists as monomers. When the amount of fatty acid exceeds the maximum solubility of bile salt micelles, fatty acids exist as emulsions. Since fatty acid can exist in several different forms, it is necessary to analyse the kinetics of fatty acid uptake under different experimental conditions. Furthermore, events occurring after cellular uptake might affect the interpretation of the uptake events. In order to differentiate these two hypotheses, the following three experiments were performed.

TABLE 1. Effect of microclimate pH on fatty acid uptake

	Basal	90 s spin	90 s spin + glucose
pH ($n = 33$)	6·05 ± 0·05	6·47 ± 0·04*	5·96 ± 0·07
Fatty acid uptake (nmol (mg protein)$^{-1}$ min^{-1})			
In 3·3 mM-oleic acid ($n = 21$)	5·07 ± 0·19	4·45 ± 0·24*	5·29 ± 0·23
In 13·2 mM-oleic acid ($n = 12$)	24·43 ± 1·25	18·36 ± 0·89*	23·43 ± 1·00

* $P < 0·05$ when compared to basal or 90 s spin + glucose.

The microclimate pH of the intestine can be altered by gentle shearing at 1000 r.p.m. for 90 s and reproduced by incubating the tissue in 28 mM-glucose sodium solution (Shiau *et al.* 1985). Using these methods to alter the microclimate pH, fatty acid uptake from micellar solutions was determined in everted intestinal rings. The study showed that fatty acid uptake is inversely related to the microclimate pH. Results are expressed as mean ± S.E.M.

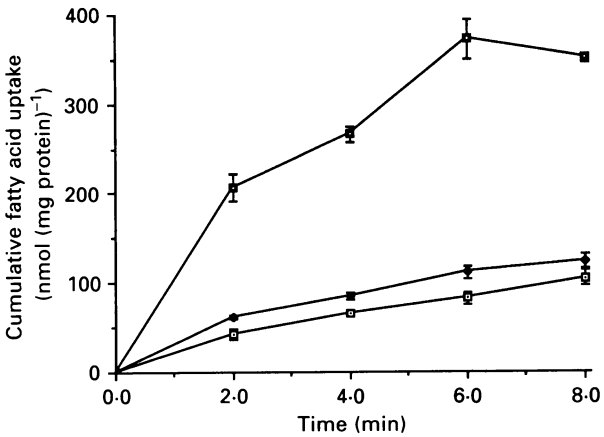

Fig. 2. Cumulative fatty acid uptake measured over 8 min of incubation. ⊡, fatty acid uptake measurements obtained with 6·6 mM-oleic acid in 5 mM-sodium taurocholate. ◆, fatty acid uptake measurements obtained with 13·2 mM-oleic acid in 10 mM-sodium taurocholate. ■, fatty acid uptake measurements obtained with 39·6 mM-oleic acid in 30 mM-sodium taurocholate. (Mean ± S.E.M. $n = 6$ for each point.)

Cumulative fatty acid uptake (Fig. 2)

This series of fatty acid uptake was studied by incubating two everted intestinal rings in 2 ml of medium containing 6·6, 13·2 and 39·6 mM-oleic acid in 5, 10 and 30 mM-sodium taurocholate solution. Incubation covered an 8 min period. Since the extracellular marker had already equilibrated with the extracellular space at 90 s, the measurements obtained after 2 min incubation are valid. Cumulative fatty acid uptake went up with respect to time. However, it was not linear suggesting that there were 'apparent' limiting events in the uptake process. The lack of linearity of uptake with respect to time might be due to a saturation of fatty acid-binding protein, an increase in fatty acid oxidation or an increase in fatty acid exit from the cell. To concentrate on studying the uptake event and to avoid the influence of cellular metabolism on our measurements, uptake was measured at 2 min incubation

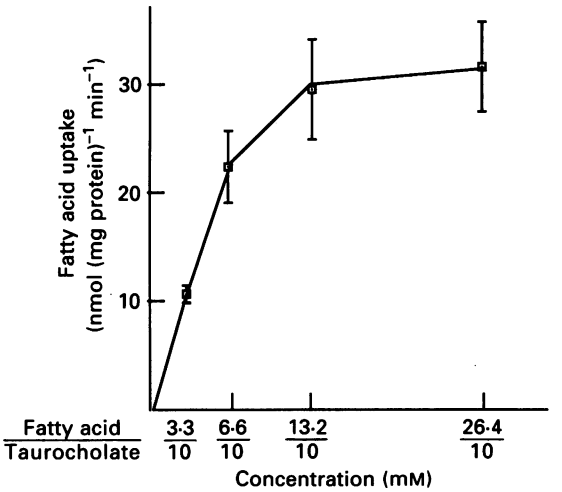

Fig. 3. The rate of fatty acid uptake measured at a constant bile salt concentration. Taurocholate concentration, 10 mM; oleic acid concentrations varying from 3·3 to 26·4 mM. (Mean ± S.E.M. $n = 6$ for each point.)

in subsequent studies. The highest intracellular accumulation of fatty acid reached 372 ± 23 nmol (mg protein)$^{-1}$ when the tissue was incubated for 6 min in a medium containing 39·6 mM-oleic acid and 30 mM-taurocholate.

Fatty acid uptake in the presence of a constant bile salt concentration (Fig. 3)

Solubilization of fatty acid by bile salt micelles may play an important role. To assess the effect of fatty acid solubility on the transport event, different amounts of fatty acid (3·3, 13·2, 26·4 mM) were added to 10 mM-sodium taurocholate solution, and fatty acid uptake by the everted intestinal rings was measured at 2 min incubation. The results were expressed as the rate of uptake (nmol (mg protein)$^{-1}$ min^{-1}). The study revealed that at concentrations above 13·2 mM, oleic acid uptake appeared to have reached a plateau (30·5 ± 3·45 nmol (mg protein)$^{-1}$ min^{-1}). Despite reaching a plateau, the cumulative fatty acid uptake was 61 nmol (mg protein)$^{-1}$ which was substantially less than the amount that could have accumulated intracellularly. When the tissue was incubated at 39·6 mM-oleic acid in 30 mM-taurocholate for 8 min the cumulative fatty acid uptake reached 372 ± 23 nmol (mg protein)$^{-1}$. Therefore, a plateau of uptake under these experimental conditions is not due to the saturation of the binding sites in the epithelial cells. It must be the result of events occurring in the luminal phase. This finding might reflect a maximum expansion of micelles.

Fatty acid uptake in the presence of a constant fatty acid to bile salt ratio (Fig. 4)

To further analyse the uptake events, fatty acid uptake was measured in micellar solutions maintaining a constant fatty acid to bile salt ratio. Concentrations of 6·6, 13·2, 26·4 and 39·2 mM-oleic acid were added to 5, 10, 20 and 30 mM-sodium taurocholate to maintain a constant fatty acid to bile salt ratio of 1·32. The rate of fatty acid uptake increased linearly with respect to fatty acid concentration

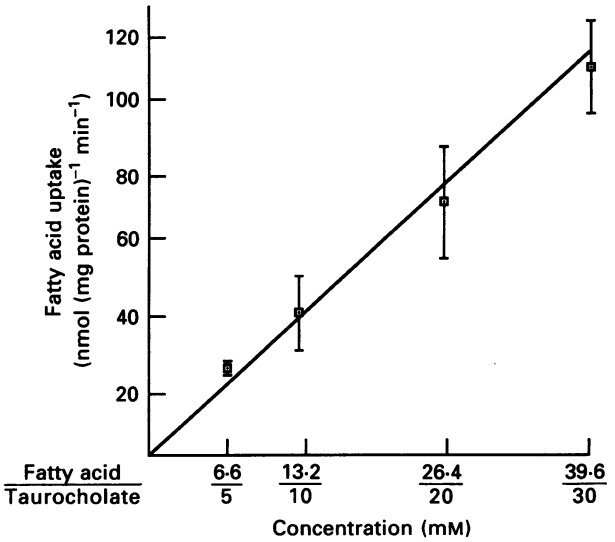

Fig. 4. The rate of fatty acid uptake measured at a constant oleic acid to bile salt ratio. Oleic acid to taurocholate ratio was maintained at 1·32; taurocholate concentration varied from 5 to 30 mM while oleic acid concentration varied from 6·6 to 39·6 mM. (Mean ± S.E.M. $n = 6$ for each point.)

confirming that the rate-limiting factor in fatty acid uptake is related to the number of micelles and the total fatty acid content of individual micelles.

DISCUSSION

The uptake of fatty acid in both the dissociation and the unstirred water layer models, involves the diffusion of fatty acid from the luminal phase into an intermediate compartment, and from the intermediate compartment into the epithelial cells. Since the uptake process involves multiple compartments, the passage of fatty acid from one compartment into another and the events occurring within individual compartments should be considered independently.

In the intestinal lumen, fatty acid can exist in several different forms; monomeric, micellar aggregates and emulsion. When different amounts of fatty acid are added to a solution containing a constant amount of bile salts, fatty acids are solubilized by bile salt micelles. When the total fatty acid concentration is less than the maximum fatty acid solubility, 10^{-6} M), the monomeric forms of fatty acids are in equilibrium with micellar aggregates (Sallee, 1974, 1978). When the maximum fatty acid solubility is reached, by definition any further increase in total fatty acid concentration is not expected to increase the monomeric fatty acid concentration (Mukerjee, 1965). In the presence of bile salt micelles, an increase in total fatty acid concentration can expand the micelles. Once the micelles are fully expanded, further addition of fatty acid will not increase the amount of fatty acid carried by bile salt micelles nor will that increase the concentration of monomeric fatty acid. Instead, the fatty acids form an emulsion (Mansbach, Cohen & Leef, 1975).

Since total fatty acid concentration in the lumen and in our experimental conditions exceeds that of its maximum solubility, for practical purposes, the monomeric fatty acid concentration is at its maximum. Therefore, the contribution of monomeric fatty acid diffusion from the bulk phase into the microclimate becomes a constant factor. Droplets of fatty acid emulsion do not interact well with water, thus limiting their ability to diffuse into the aqueous microclimate. The plateauing effect shown in Fig. 3 might indicate that fatty acid emulsion does not contribute significantly to the uptake events. Thus the major determinant for delivering fatty acid into the microclimate must come from diffusion of micellar aggregates. The amount of fatty acid delivered to the microclimate as micelles will depend on several factors: (a) the number of micelle particles in the bulk phase (the number of vehicles), (b) the fatty acid content of individual micelles (the load of the vehicles), and (c) the 'concentration' gradient of micelles between the bulk phase and the microclimate (the driving force).

The 'apparent saturation' observed in the presence of a constant taurocholate concentration (Fig. 3) is not the result of saturation of the binding sites of the fatty acid-binding protein in the cytosol, because the cumulative intracellular uptake of fatty acid can reach a level substantially higher (Fig. 2) than that observed in these experimental conditions. The 'saturation' is most likely a reflection of the maximal solubilization of fatty acid by a given number of micelles. To support this interpretation, fatty acid uptake was examined in the presence of a constant fatty acid to bile salt ratio. Under these conditions, fatty acid uptake was linearly related to its concentration (Fig. 4). When fatty acid and bile salt concentrations are increased proportionally, the number of micellar particles increases while the amount of lipid carried by the micelles remains unchanged. Therefore the increase in fatty acid uptake reflects the increase in the number of micelles in the solution. Since micellar and monomeric fatty acids both participate in delivering fatty acid to the microclimate, individual forms of fatty acid can be an independent driving force for delivering fatty acid to the microclimate. The rates of diffusion of monomeric fatty acids and micellar aggregates across the unstirred water layer have been examined extensively (Wilson, Sallee & Dietschy, 1971; Wilson & Dietschy, 1972; Westergaard & Dietschy, 1974, 1976; Dietschy, 1978; Sallee, 1979; Thomson & Dietschy, 1981). Monomeric fatty acids have faster diffusion coefficients than those of the micellar aggregates. Although the intermediate compartment is the major diffusion barrier for micelles, the high fatty acid content in micelles overcomes the slower diffusion coefficient to become the major mechanism for delivering fatty acid into the intermediate compartment.

The major differences between the dissociation and the unstirred water layer model are the events occurring in the intermediate compartment. The model postulated by Westergaard & Dietschy (1976) suggested that removal of monomeric fatty acid from the unstirred water layer by the cells leads to further release of fatty acid from micellar aggregates. Thus, the driving force for uptake in that model is the concentration of monomeric fatty acid in the unstirred water layer. Micelles function as a reservoir to supply fatty acid. Since monomeric fatty acid concentration is limited by its solubility (10^{-6} M) (Mukerjee, 1965), a rate-limiting process is expected. Thus the unstirred water layer model cannot explain the concentration-dependent

uptake event reported earlier by other investigators (Hoffman, 1970; Hoffman & Simmonds, 1971; Sallee, Wilson & Dietschy, 1972; Sallee, 1979).

In the dissociation model, fatty acid released from micelle dissociation together with the amount derived from monomeric diffusion determines the total fatty acid concentration within the microclimate. Total fatty acid concentration in the microclimate is the driving force for the uptake. The amount of fatty acid released from micelle dissociation reflects the number of micelles entering the microclimate and the fatty acid content of individual micelles. Therefore the driving force for fatty acid uptake by the cell indirectly reflects the total luminal fatty acid concentration.

In the low-pH compartment, fatty acids are protonated. Protonated fatty acids are preferentially partitioned into the lipid membrane, thus favouring cellular uptake. Removal of protonated fatty acid from the microclimate shifts the equilibrium between the ionized and non-ionized forms (Hogben, Tocco, Brodie & Schanker, 1959) in favour of continuous conversion of the ionized into non-ionized species. Therefore, the rate of fatty acid uptake by the enterocyte will then depend on the amount of fatty acid delivered to the microclimate and the permeability characteristics of the protonated species of fatty acid.

In this study, we have demonstrated that alteration of microclimate pH has a significant effect on intestinal fatty acid uptake. A lower microclimate pH is associated with a higher rate of fatty acid uptake from the micellar solution (Table 1). These observations are consistent with the prediction derived from the dissociation hypothesis. Dissociation of fatty acid from micelles in a low-pH compartment can also affect the structure of bile salt micelles. The critical micellar concentration (CMC) of bile salts is reduced in the presence of other lipids (Hofmann & Borgstrom, 1962). Thus removing these lipids from micelles could raise the CMC. When micelles diffuse into an area with a relatively low bile salt concentration and at the same time, CMC is increased, disruption of the bile salt micelle occurs. Bile salt monomers are also dissociated from micellar aggregates (Shiau & Levine, 1980). The dissociated bile salt monomers are reabsorbed passively from the microclimate (McClintock & Shiau, 1983). Passive bile salt absorption from the proximal intestine was not the part of the features of the collision model (Hofmann, 1968), the unstirred water layer model (Westergaard & Dietschy, 1976) or the intermicellar fatty acid model (Chijiwa & Linscheer, 1987*b*).

In summary, a low-pH compartment does exist on the intestinal lining (Lucas, Schneider, Haberich & Blair, 1975; Shiau, Fernandez, Jackson & McMonagle, 1985) and the low-pH compartment plays a key role in fatty acid uptake. Both the monomeric and aggregated forms of fatty acid are independent driving forces contributing to the delivery of fatty acid to the microclimate. The amount of lipid delivered to the microclimate depends heavily on the number of micelles and the amount of lipids carried by individual micelles. Upon entering the microclimate, a lower pH converts ionized fatty acids to non-ionized forms and reduces their solubility in the bile salt micelle. Thus fatty acids dissociate from bile salt micelles. Once lipids are dissociated from bile salt micelles, the CMC of bile salt is increased. Due to the increased CMC, bile salts can no longer maintain their aggregate form. Bile salt monomers are also released from micelle aggregates in the microclimate. Released lipids are passively absorbed by the intestinal epithelial cells. The rate of

absorption of individual classes of lipid is dependent on the amount of lipid delivered to the microclimate and the permeability coefficient of the lipid. Due to the differences in the permeation coefficients of individual lipids, the rate of absorption of individual lipids varies.

This study was supported by a grant (RO1-DK39316-01) from the US National Institutes of Health and a Merit Review Grant from the US Veterans Affairs.

REFERENCES

CAREY, M. C. & SMALL, D. M. (1970). The characteristics of mixed micellar solution with particular reference to bile. *American Journal of Medicine* **49**, 590–608.

CHIJIWA, K. & LINSCHEER, W. G. (1987*b*). Distribution of monomer activity of cholesterol in micellar bile salt–effect of cholesterol level. *American Journal of Physiology* **252**, G309–314.

CHIJIWA, K. & LINSCHEER, W. G. (1987*a*). Mechanism of pH effect on oleic acid and cholesterol absorption in the rat. *American Journal of Physiology* **252**, G506-510.

DIETSCHY, J. M. (1978). General principles governing movement of lipids across biological membranes. In *Disturbances in Lipid Lipoprotein Metabolism*, ed. DIETSCHY, J. M., GOTTO, A. M. JR & ONTKO, J. A. American Physiological Society, pp. 1–28. Washington DC.

HOFFMAN, N. E. (1970). The relationship between uptake *in vitro* of oleic acid and micellar solubilization. *Biochimica et biophysica acta* **196**, 193–203.

HOFFMAN, N. E. & SIMMONDS, W. J. (1971). The intestinal uptake and esterification, *in vitro*, of fatty acid as a diffusion limited process. *Biochimica et biophysica acta* **24**, 331–333.

HOFMANN, A. F. (1968). Functions of bile in the alimentary canal. In *Handbook of Physiology*, section 6, *Alimentary Canal*, vol. 5, *Bile, Digestion and Ruminant Physiology*, ed. CODE, C. F., pp. 2507–2533, American Physiological Society, Washington DC.

HOFMANN, A. F. (1974). *Lipid Digestion and Absorption in Unit V of the Undergraduate Teaching Project*, American Gastroenterological Association, Milner-Fenwick Inc., Baltimore, MD, USA.

HOFMANN, A. F. (1976). Fat digestion: the interaction of lipid digestion products with micellar bile acid solutions. In *Lipid Absorption: Biochemical and Clinical Aspects*, ed. ROMMEL, K., GOEBELLO, H. & BOHMER, R., pp. 3–18. University Park Press, Baltimore, MD, USA.

HOFMANN, A. F. & BORGSTROM, B. (1962). Physico-chemical state of lipids in intestinal content during their digestion and absorption. *Federation Proceedings* **21**, 43–50.

HOFMANN, A. F. & SMALL, D. (1967). Detergent properties of bile salts: correlation with physiological function. *Annual Review of Medicine* **18**, 333–376.

HOGBEN, C. A. M., TOCCO, D. J., BRODIE, B. B. & SCHANKER, L. S. (1959). On the mechanism of intestinal absorption of drugs. *Journal of Pharmacology and Experimental Therapeutics* **125**, 275–282.

JOHNSTON, J. M. (1968). Mechanism of fat absorption. In *Handbook of Physiology*, section 6, *Alimentary Canal*, vol. 3, *Intestinal Absorption*, ed. CODE, C. F., pp. 1353–1375, American Physiological Society, Washington DC.

LUCAS, M. L., SCHNEIDER, W., HABERICH, F. J. & BLAIR, J. A. (1975). Direct measurement by pH microelectrode of the pH microclimate in rat proximal jejunum. *Proceedings of the Royal Society* B **192**, 39–48.

MCCLINTOCK, C. & SHIAU, Y. F. (1983). Jejunum is more important than the terminal ileum in taurocholate absorption in rats. *American Journal of Physiology* **244**, G507–514.

MANSBACH, C. M. II., COHEN, R. S. & LEEF, P. B. (1975). Isolation and properties of the mixed lipid micelles present in intestinal content during fat digestion in man. *Journal of Clinical Investigation* **56**, 781–791.

MUKERJEE, P. (1965). Dimineralization of anions of long chain fatty acid in aqueous solution and the hydrophobic properties of acid. *Journal of Physical Chemistry* **69**, 2821–2827.

SALLEE, V. L. (1974). Apparent monomer activity of saturated fatty acids in micellar bile salt solutions measured by a polyethylene partitioning system. *Journal of Lipid Research* **15**, 56–64.

SALLEE, V. L. (1978). Fatty acid determinants of fatty acid and alcohol monomers activities in mixed micellar solutions. *Journal of Lipid Research* **19**, 207–224.

SALLEE, V. L. (1979). Permeation of long-chain fatty acids and alcohols in rat intestine. *American Journal of Physiology* **236**, E721–727.

SALLEE, V. L., WILSON, F. A. & DIETSCHY, J. M. (1972). Determination of unidirectional uptake rates for lipids across the intestinal brush border. *Journal of Lipid Research* **13**, 184–192.

SHIAU, Y-F. (1981). Mechanism of fat absorption. *American Journal of Physiology* **240**, G1–9.

SHIAU, Y-F. (1987). Lipid digestion and absorption. In *Physiology of the Gastrointestinal Tract*, 2nd edn, ed. JOHNSON, L. R., pp. 1527–1556. Raven Press, New York.

SHIAU, Y-F., FERNANDEZ, P., JACKSON, M. J. & MCMONAGLE, S. (1985). Mechanisms maintaining a low pH microclimate in the intestine. *American Journal of Physiology* **248**, G608–619.

SHIAU, Y-F. & LEVINE, G. M. (1980). pH dependence of micellar diffusion and dissociation. *American Journal of Physiology* **239**, G177–182.

THOMSON, A. B. R. & DIETSCHY, J. M. (1981). Intestinal lipid absorption: major extracellular and intracellular events. In *Physiology of the Gastrointestinal Tract*, ed. JOHNSON, L. R., pp. 1147–1220. Raven Press, New York.

WESTERGAARD, H. & DIETSCHY, J. M. (1974). Delineation of the dimensions and permeability characteristics of the two major diffusion barriers to passive mucosal uptake in the rabbit intestine. *Journal of Clinical Investigation* **174**, 718–732.

WESTERGAARD, H. & DIETSCHY, J. M. (1976). The mechanism whereby bile acid micelles increase the rate of fatty acid and cholesterol uptake into the intestinal mucosal cell. *Journal of Clinical Investigation* **58**, 97–108.

WILSON, F. A. & DIETSCHY, J. M. (1972). Characterization of bile acid absorption across the unstirred water layer and brush border of the rat jejunum. *Journal of Clinical Investigation* **51**, 3015–3025.

WILSON, F. A., SALLEE, V. L. & DIETSCHY, J. M. (1971). Unstirred water layer in the intestine: rate determinant for fatty acid absorption from micellar solutions. *Science* **174**, 1031–1033.