The Mechanism Whereby Bile Acid Micelles Increase the Rate of Fatty Acid and Cholesterol Uptake into the Intestinal Mucosal Cell

HENRIK WESTERGAARD and JOHN M. DIETSCHY

From the Gastrointestinal-Liver Section of the Department of Medicine, The University of Texas Health Science Center at Dallas, Dallas, Texas 75235

ABSTRACT Studies were undertaken to define the mechanism whereby bile acid facilitates fatty acid and cholesterol uptake into the intestinal mucosal cell. Initial studies showed that the rate of uptake (J_d) of several fatty acids and cholesterol was a linear function of the concentration of these molecules in the bulk phase if the concentration of bile acid was kept constant. In contrast, J_d decreased markedly when the concentration of bile acid was increased relative to that of the probe molecule but remained essentially constant when the concentration of both the bile acid and probe molecule was increased in parallel. In other studies J_d for lauric acid measured from solutions containing either 0 or 20 mM taurodeoxycholate and saturated with the fatty acid equaled 79.8±5.2 and 120.8±9.4 nmol·min^{-1}·100 mg^{-1}, respectively: after correction for unstirred layer resistance, however, the former value equaled 113.5±7.1 nmol·min^{-1}·100 mg^{-1}. Maximum values of J_d for the saturated fatty acids with 12, 16, and 18 carbons equaled 120.8±9.4, 24.1±3.2, and 13.6±1.1 nmol·min^{-1}·100 mg^{-1}, respectively. These values essentially equaled those derived by multiplying the maximum solubility times the passive permeability coefficients appropriate for each of these compounds. The theoretical equations were then derived that define the expected behavior of J_d for the various lipids under these different experimental circumstances where the mechanism of absorption was assumed to occur either by uptake of the whole micelle, during interaction of the micelle with an infinite number of sites on the microvillus membrane or through a monomer phase of lipid molecules in equilibrium with the micelle. The experimental results were consistent both qualitatively and quantitatively with the third model indicating that the principle role of the micelle in facilitating lipid absorption is to overcome unstirred layer resistance while the actual process of fatty acid and cholesterol absorption occurs through a monomer phase in equilibrium with the micelle.

INTRODUCTION

In various studies carried out in animals as well as in man it has been demonstrated that bile acids play an important role in facilitating the absorption of various dietary fats by the gastrointestinal tract. There are quantitative differences, however, in the dependency of various lipids on the presence of these micelle-forming compounds. Fatty acids of medium chain length, for example, are absorbed nearly as well in the absence as in the presence of bile acid micelles. As the chain length is increased the fatty acids become progressively more dependent upon the presence of the bile acid micelle for efficient uptake into the mucosal cell, and, in the case of very nonpolar compounds such as cholesterol, essentially no absorption occurs in the absence of bile acids (1–5). Despite considerable work in this area there is currently little information dealing with the exact mechanism(s) whereby the bile acid micelle actually enhances the rate of lipid uptake into the intestinal mucosal cell. However, the recent observation that an unstirred water layer adjacent to the luminal cell surface, and not the microvillus membrane, is actually rate limiting to the uptake of long chain fatty acids and steroids (6, 7) raises the possibility that the major physiological function of the bile acid micelle is to overcome this resistance and so augment lipid absorption.

Dr. Westergaard was a postdoctoral research fellow in gastroenterology while these studies were undertaken. His current address is Medical Department P, University Hospital, DK-2100 Copenhagen Ø, Denmark.

Received for publication 19 August 1975 and in revised form 2 March 1976.

It is equally unclear how the individual lipid molecules carried in the micelle are actually transferred into the mucosal cell. Earlier workers postulated that the entire micellar particle might be taken up into the intestinal cell intact (8, 9). However, more recent work from several laboratories has shown that the various constituent molecules carried in the micelle are absorbed at apparently independent rates suggesting that uptake must occur by some other mechanism (10–12). The two most likely alternative possibilities are that molecular transfer occurs *via* an intermediate, obligatory monomer phase or that transfer takes place directly into the cell membrane during "collision" or interaction between the micelle and the lipid-protein phase of the microvillus membrane.

The purpose of the present study, therefore, is twofold. First, experiments are described that define in both qualitative and quantitative terms the manner in which the bile acid micelle facilitates lipid absorption. Second, other studies are presented that allow differentiation as to which mechanism is operative during the actual transfer of lipid molecules from the bile acid micelle into the intestinal mucosal cell.

METHODS

Chemicals. Unlabeled and [1-^{14}C] long chain fatty acids, unlabeled and [4-^{14}C] cholesterol and unlabeled β-sitosterol were all > 99% pure as supplied by Applied Science Labs, Inc., State College, Pa. Unlabeled taurodeoxycholic acid was from Sigma Chemical Co., St. Louis, Mo. and [24-^{14}C] sodium taurodeoxycholate was from Mallinckrodt Inc., St. Louis, Mo. The purity of these compounds was > 98% as checked by thin layer chromatography (13, 14). Glucose was obtained from J. T. Baker Chemical Co., Phillipsburg, N. J. and iodoacetamide from Nutritional Biochemicals Corp., Cleveland, Ohio. [G-^{3}H] dextran with an approximate mol wt of 20,000 from New England Nuclear, Boston, Mass. was used as a nonpermeant marker of adherent mucosal volume. All other compounds were of reagent grade.

Preparation of micellar incubation solutions. Using sterile technique and solutions, an appropriate amount of both a [^{14}C] and unlabeled probe molecule was dissolved in an exact volume of chloroform : methanol (2 : 1, vol : vol) in an incubation beaker, and aliquots were taken in triplicate to determine the specific activity of the molecule under study. The chloroform : methanol phase was then evaporated under nitrogen following which the beaker was placed under vacuum for an additional 1 h to insure complete removal of the organic solvents. 55 ml of a 40 mM taurodeoxycholate solution in Krebs-bicarbonate buffer (with Ca^{++} omitted) was added to the beaker and the solution was stirred with a magnetic bar for 2 h. The solution was then further diluted by the addition of 55 ml of Krebs-bicarbonate buffer to give a final volume of 110 ml and a final taurodeoxycholate concentration of 20 mM. The beaker was then gassed with 95% O_2:5% CO_2, sealed, and shaken in a metabolic incubator at 37°C overnight. The next morning the solution was again gassed with 95% O_2:5% CO_2 for 2 h at 37°C and, if necessary, the pH was readjusted to 7.40. A trace amount of the radiolabeled volume marker, [G-^{3}H]dextran, was then added and the solution was ready to be used for determination of tissue uptake rates. The distribution of C^{14} on thin-layer chromatography of the fatty acids was the same before and after these preparations.

Measurement of tissue uptake. Tissue uptake rates of fatty acids, cholesterol, and taurodeoxycholate were determined with a technique which has been described in detail (15). Briefly, unfasted albino New Zealand rabbits weighing 2–2.5 kg were killed by decapitation, and a 15–20-cm portion of the proximal jejunum was removed. The intestine was rinsed, cut in circular pieces, and mounted in chambers that expose 0.78 cm^2 of the mucosal surface to the incubation solutions. The mounted tissue preparations were kept at 4°C in oxygenated Krebs-bicarbonate buffer until used in the various studies. At the start of an experiment the chambers were transferred to preincubation beakers with oxygenated Krebs-bicarbonate buffer at 37°C to equilibrate the tissue for 8 min at this temperature. The chambers were then transferred to the incubation beakers containing the [^{14}C] probe molecule and [^{3}H] dextran and incubated for the desired length of time. The preincubation and incubation buffers were all maintained at pH 7.40, and solutions were stirred at identical stirring rates with circular magnetic bars. In all studies the stirring rate was kept constant at 600 rpm using a strobe light (7, 15). At the end of the incubation the chambers were removed from the solutions, and the tissue was briefly rinsed in cold 0.9% NaCl solution. The exposed tissue was then cut out, divided into two pieces, and placed in tared scintillation vials. The tissue was dried overnight to determine dry weight and was then saponified with NaOH. Scintillation fluid was added and radioactivity was determined by means of an external standardization technique to correct for variable quenching of the two isotopes (16). The rate of uptake, (J_d),[1] was calculated after the total [^{14}C] radioactivity had been corrected for the amount of the probe molecule present in the adherent mucosal fluid volume. These rates are expressed as the nanomoles of the probe molecule taken up into the mucosal cells per minute per 100 mg of dry tissue weight (nmol·min^{-1}·100 mg^{-1}).

Determination of maximum solubility of fatty acids in Krebs-bicarbonate buffer. 50-ml solutions of octanoate, decanoate, dodecanoate, palmitate, and stearate were made up in Krebs-bicarbonate buffer as described above except that taurodeoxycholate was omitted. The [^{14}C] fatty acids were added in amounts to give high specific activities and the unlabeled fatty acids were present in amounts to give a final concentration of octanoate, decanoate, dodecanoate, palmitate, and stearate equal to 22, 4, 1.5, 0.1, and 0.1 mM, respectively. In each case the final solution was grossly turbid and had visible microcrystals or oil droplets at the air-water interface. Four small dialysis bags containing 1.0–1.5 ml of Krebs-bicarbonate buffer were added to each solution and the beakers were sealed and placed in a metabolic shaker at 37°C. At 24-h intervals one dialysis bag was removed from each solution and triplicate 100 μl samples were obtained from each bag. The bags and pipettes were maintained at 37°C during sampling. Each 100 μl sample was transferred to a scintillation vial and the pipette was rinsed 10 times with chloroform : methanol (2 : 1) and the rinses also were added to the vial. The contents of the vial were taken to dryness under nitrogen, a triton-toluene scintillation fluid was added, and the amount of radioactivity

[1] *Abbreviations used in this paper:* C_1, concentration of a solute molecule in the bulk buffer solution; C_2, concentration of a solute molecule at the aqueous membrane interface; FA, fatty acid; J_d, rate of uptake.

was assayed (16). The concentration of each fatty acid in the Krebs-bicarbonate buffer in the dialysis bag was then calculated from the total radioactivity in solution divided by the appropriate specific activity.

RESULTS

Before beginning experiments designed specifically to elucidate the role of the bile acid micelle in facilitating lipid absorption, initial studies were undertaken to evaluate three technical aspects of the incubation procedure utilized in these studies. First, it was necessary to be certain that an adequate concentration of taurodeoxycholate, the bile acid used in these experiments, be present in the bulk perfusate so that the critical micelle concentration was clearly exceeded at the aqueous-microvillus interface. To evaluate this important point, uptake of taurodeoxycholate into the jejunal mucosa was measured as a function of the concentration of the bile acid in the bulk buffer solution (C_1), as shown in Fig. 1. As is apparent, J_d achieved an essentially constant value above a bulk phase concentration of 20 mM. These data, therefore, are consistent with the view that micelles are present throughout the unstirred water layer and, most importantly, at the aqueous-membrane interface: the constant value of J_d above a concentration of 20 mM presumably reflects an essentially constant concentration of bile acid monomer against the microvillus surface. It should be emphasized that this relatively high concentration of bile acid was used to be certain that the critical micelle concentration was exceeded at the aqueous-membrane interface so that appropriate mathematical treatment of the data could be carried out. Under physiological circumstances the concentration of bile acid in the gut contents is lower so that the micelle might conceivably disintegrate before completely traversing the unstirred water layer. This does not alter the general principles put forth in this paper since the

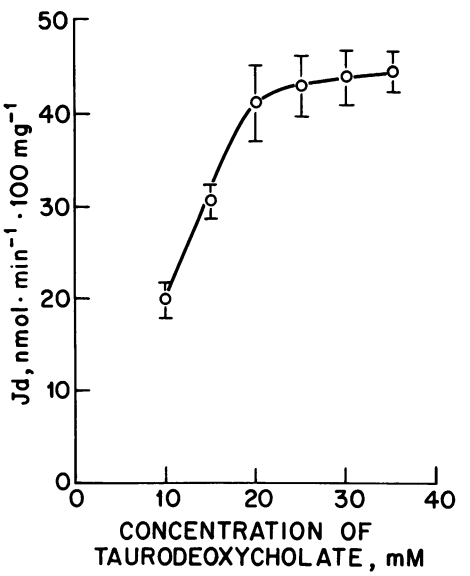

FIGURE 1 The uptake of taurodeoxycholate into the intestinal mucosa as a function of the bile acid concentration in the bulk mucosal solution. Specimens of rabbit jejunum were incubated in Krebs-bicarbonate buffer stirred at 600 rpm for 8 min and containing taurodeoxycholate at concentrations varying from 10 to 35 mM. J_d, shown in this figure represent mean values±1 SE for determinations in specimens from five animals.

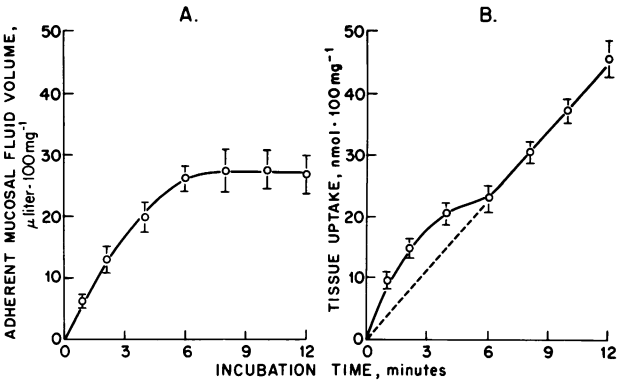

FIGURE 2 Characteristics of labeling of the adherent mucosal fluid volume as a function of time in the presence of bile acid using [G-^{3}H]-dextran as the volume marker. Panel A illustrates the manner in which the volume of the adherent mucosal fluid varied with the time of incubation while panel B shows the calculated rates of mucosal uptake of palmitate from Krebs-bicarbonate buffer containing taurodeoxycholate (20 mM) and the FA 16:0 (0.25 mM). Mean values±1 SE for determinations in specimens from five animals are shown in this diagram.

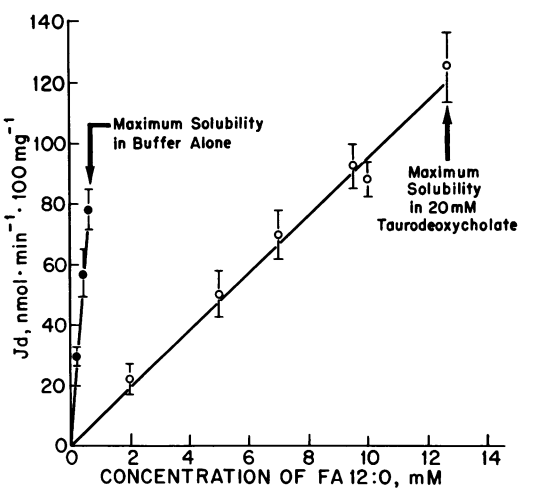

FIGURE 3 Comparison of the J_d of laurate in the presence and absence of bile acid micelles. In this study J_d was measured at varying concentrations of the FA 12:0 in the mucosal solution up to the limits of solubility of this FA in either Krebs-bicarbonate buffer alone (solid circles) or in buffer containing 20 mM taurodeoxycholate (open circles). Each point represents the mean±1 SE for determinations in five to nine animals.

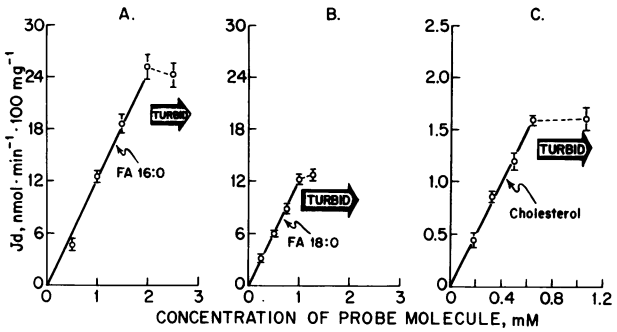

FIGURE 4 The effect of increasing the concentration of the probe molecules relative to that of the bile acid on the mucosal uptake of two long-chain FA and cholesterol. All of these studies were carried out using Krebs-bicarbonate buffer containing 20 mM taurodeoxycholate. Varying amounts, up to the limits of solubility, of palmitate (FA 16:0), stearate (FA 18:0), and cholesterol were dissolved in the incubation solutions over a prolonged period of time, as described in the Methods section, to give clear micellar solutions. In addition, an additional solution of each of the probe molecules was prepared in which the limits of solubility were exceeded so that the solutions were grossly turbid and contained a crystal phase. Each point represents the mean±1 SE for determinations in five to nine animals.

resistance of the diffusion barrier is reduced under any circumstance to the extent that the micelle does penetrate the unstirred water layer, but the mathematical treatment of such a circumstance becomes more complex. Second, since the results of this initial experiment indicated that subsequent studies must be undertaken using a bile acid concentration of 20 mM, it was essential to show that this concentration did not alter membrane permeability under the conditions of these studies. Data on this point have already been published (15) and show that there is no demonstrable effect. Third, since we have also previously shown that the presence of 20 mM taurodeoxycholate does change the volume of the adherent mucosal fluid (15), it was also important to establish that this fluid layer was fully equilibrated with the volume marker. As shown in panel A of Fig. 2, in the presence of 20 mM taurodeoxycholate, the adherent mucosal fluid volume required fully 6 min to become uniformly labeled and it reached the relatively high value of approximately 25 $\mu l \cdot 100\ mg^{-1}$. As seen in panel B, in this representative experiment the uptake of fatty acid (FA) 16:0 was linear with respect to time beyond 6 min of incubation and this relationship extrapolated to zero at 0 time. As anticipated, J_d obtained from mucosal specimens incubated for less than 6 min gave calculated values of J_d that were artifactually high because of incomplete equilibration of the volume marker at these incubation times. Identical results were obtained using cholesterol, FA 8:0, FA 10:0, FA 12:0, and FA 18:0 as the probe molecules. Thus, all subsequent experiments were carried out using an 8-min incubation period, a taurodeoxycholate concentration of 20 mM, and a stirring rate of 600 rpm.

While all previous work has suggested that FA and steroid absorption in the intestine occurs by passive mechanisms (2–4, 6, 10, 11), initial studies were undertaken to reevaluate this uptake step under the precisely controlled conditions utilized in these studies where essentially unidirectional J_d could be measured. For example, there was no evidence of competition for uptake between FA 16:0, FA 18:0, and cholesterol and structurally-related compounds suggesting that a finite number of transport sites was not involved in the absorptive process. The Q_{10} for the uptake of both the FA and cholesterol varied from 1.1 to 1.3, a finding that again suggested passive transport. However, temperature changes might affect micelle structure as well as partitioning of the probe molecules between the water phase and the micelle so that these Q_{10} values do not necessarily reflect the characteristics of the mucosal uptake step. Finally, preincubation of the tissue with either a metabolic inhibitor, iodoacetamide, or with an actively transported sugar, glucose, also did not alter the J_d of the FA or cholesterol. All of these findings, then, are consistent with previous data indicating that the absorption of lipids, in contrast to sugars and amino acids, takes place by passive diffusion.

Accepting then, that uptake of FA and cholesterol into the intestinal mucosa occurs by passive means, four

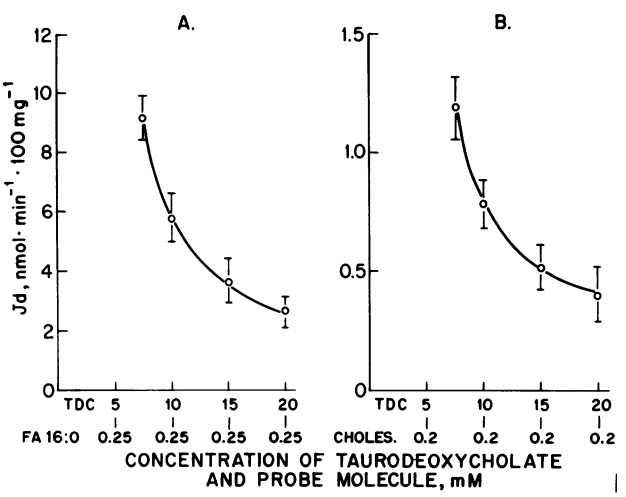

FIGURE 5 The effect of increasing the concentration of bile acid relative to that of the probe molecules on the mucosal uptake of a FA and cholesterol. In these experiments the uptake of palmitate (FA 16:0) and cholesterol was measured under circumstances where the concentration of each of the probe molecules was kept constant while the concentration of the taurodeoxycholate in the solutions was increased from 5 to 20 mM. Each point represents the mean ±1 SE for determinations in five animals.

general types of studies were next undertaken to provide the necessary data from which an analysis of the role of the bile acid micelle in this process could be made. In the first study, shown in Fig. 3, advantage was taken of the fact that FA 12:0 has sufficient solubility that its absorption can be studied from Krebs-bicarbonate buffer alone as well as from buffer containing bile acid. As is seen in this figure, in the absence of taurodeoxycholate the J_d was a linear function of the concentration of the FA in the perfusate: J_d achieved a maximum value of 79.8±5.2 nmol·min^{-1}·100 mg^{-1} at the maximum concentration of FA 12:0 that could be achieved in the Krebs-bicarbonate buffer (0.72 mM). With the addition of 20 mM taurodeoxycholate the maximum solubility of the FA was increased nearly 20-fold but the J_d increased only about 36% to 120.8±9.4 nmol·min^{-1}·100 mg^{-1}. As an aside, it should also be recognized that this study again illustrates that at any total concentration of FA in the perfusate the presence of bile acid micelles actually inhibits uptake (6): for example, at a FA 12:0 concentration of 0.5 mM, J_d equaled approximately 4 nmol·min^{-1}·100 mg^{-1} in the presence of taurodeoxycholate but was 16-fold higher in the absence of bile acid micelles.

In a second group of studies, the J_d of two long-chain FA and cholesterol, as seen in Fig. 4, was measured under circumstances where the concentration of the three probe molecules was varied up to their maximum solubilities in Krebs-bicarbonate buffer containing

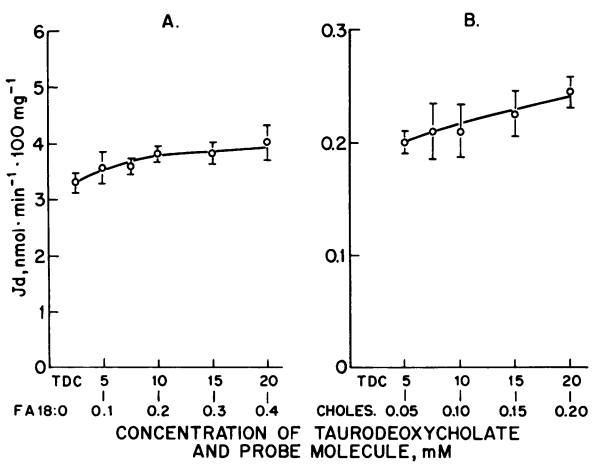

FIGURE 6 The effect of increasing the concentration of both the bile acid and the probe molecules on the mucosal uptake of a FA and cholesterol. In these experiments the uptake of stearate (FA 18:0) and cholesterol was measured under circumstances where the concentration of each of the probe molecules and the concentration of the taurodeoxycholate was varied in such a manner as to maintain a constant molar ratio in the mucosal solution. This molar ratio of bile acid to stearate equaled 50:1 and of bile acid to cholesterol was 100:1. Each point represents the mean±1 SE for determinations in four or five animals.

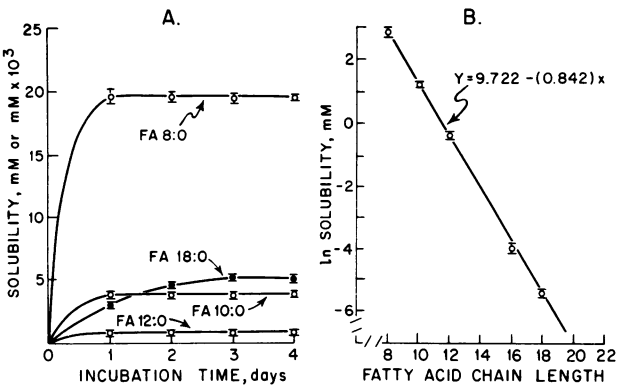

FIGURE 7 Determination of the maximal solubilities of various FA in Krebs-bicarbonate buffer at 37°C. Panel A shows the concentrations achieved by four different FA in the buffer solution inside the dialysis bags as a function of the time of incubation at 37°C. The units of concentration on the vertical scale equal mM for the first three FA and mM × 10^3 for FA 18:0. In panel B the natural logarithms of the maximum solubility of FA 8:0, 10:0, 12:0, 16:0, and 18:0 are plotted as a function of chain length. Each point represents the mean±1 SE for three to six separate determinations.

taurodeoxycholate at a constant concentration of 20 mM. Two important points regarding these studies are evident. First, the J_d of the FA 16:0 and FA 18:0 and of cholesterol is a linear function of the concentration of the three probe molecules in the micellar perfusate up to the limits of their respective solubilities. Second, once maximum solubility was achieved, J_d plateaued at a constant value.

The results of a third group of studies is illustrated in Fig. 5 where the uptake of FA 16:0 and cholesterol was measured under circumstances where the concentration of the probe molecules was kept constant but the concentration of taurodeoxycholate was increased so that the ratio of the number of micelles to the mass of each probe molecule increased. As is apparent, in both instances J_d decreased strikingly as the concentration of taurodeoxycholate was increased.

In a fourth group of studies, illustrated in Fig. 6, the J_d of FA 18:0 and cholesterol was measured under circumstances where the concentration of both the probe molecule and taurodeoxycholate was increased in parallel so that the ratio of the two remained constant. Under these experimental conditions J_d increased only slightly as the concentration of the two respective probe molecules was increased over a four- to eightfold range.

Finally, to be able to properly interpret these data in quantitative terms, it was necessary to have values for the maximum solubility of the various FA at 37°C in the Krebs-bicarbonate buffer utilized in these various studies. As seen in panel A of Fig. 7, the medium chain-length FA 8:0, FA 10:0, and FA 12:0 achieved maxi-

mum solubility in the buffer within 24 h and this concentration remained constant for up to 4 days. FA 18:0 (and FA 16:0, not shown in this figure) required 2–3 days to achieve maximum aqueous solubility. In this study the maximum solubility of the saturated FA containing 8, 10, 12, 16, and 18 carbons was found to equal 19.8, 3.80, 0.700, 0.0189, and 0.0051 mM, respectively. In panel B, these values are plotted semilogarithmically against the number of carbons in the FA chain. The best fit linear regression curve derived from these data has the formula $y = 9.722 - 0.842x$ so that the solubility of the FA in Krebs-bicarbonate buffer at 37°C decreased by a factor of 2.32 for each CH_2 group added to the chain.

DISCUSSION

In general terms, the unidirectional movement of solutes into various cells is determined by at least three factors: the concentration that the solute can attain in the bulk fluid perfusing the cell, the resistance encountered as the solute penetrates the unstirred layers around the cell, and the resistance encountered during molecular translocation across the cell membrane (17, 18). Lipids, in general, commonly have low solubility in the bulk solution and, because of the very low free energy changes associated with movement of these solutes from the aqueous to the membrane phase, usually can passively penetrate cell membranes at very high rates. Indeed, for such compounds the resistance encountered in crossing the unstirred layers outside the cell may exceed that encountered in crossing the cell membrane itself so that this diffusion barrier becomes absolutely rate limiting to cell uptake. Clearly this appears to be the situation in the intestine where the uptake of virtually all medium and long chain length FA and steroids has been shown to be diffusion-limited (6, 7).[2] Thus, it follows that the function of the bile acid micelle in augmenting lipid absorption must be explained in terms of reducing this resistance. Furthermore, any formulation of the exact mechanism by which this occurs must also explain the quantitative differences that exist in the dependency of different lipids on the presence of bile acids in the intestinal lumen for efficient absorption.

The experiments reported here provide data along several different lines that allow analysis of this problem in both qualitative and quantitative terms. In the presence of adequate amounts of pancreatic enzymes, long chain FA and free cholesterol are generated from dietary lipids and partition into bile acid micelles also present in the bulk intestinal contents. These mixed micelles then diffuse towards the microvillus membrane at a rate determined by the unstirred layer resistance which is proportional to $d/D \cdot S_w$ where D is the appropriate free diffusion coefficient for the mixed micelle, S_w is the effective surface area of the unstirred water layer through which diffusion takes place, and d is the effective thickness of this barrier. Once the micelle reaches the vicinity of the aqueous-membrane interface the constituent lipid molecules carried in the micelle could be absorbed into the mucosal cell by at least three different mechanisms: (*1*) the micelle might be taken up into the cell intact, (*2*) the constituent lipids might partition into the cell membrane during a direct interaction or "collision" between the micelle and the cell membrane or, (*3*) absorption might occur only through the monomer phase of lipid molecules present in the aqueous environment in equilibrium with the lipids in the micelle. There are adequate data in the literature to exclude the first possibility since the various constituent molecules in the mixed micelle are absorbed at essentially independent rates and, therefore, there is no evidence that the uptake step occurs through a process akin to pinocytosis (10–12).

In the second postulated mechanism it is assumed that the micelle and membrane interact in such a way that water is excluded from the interface between the two structures following which there is direct transfer of FA and cholesterol from the micelle to the microvillus membrane. If the number of such interactions is proportional to the concentration of micelles at the aqueous-membrane interface, then the observed J_d of a particular lipid molecule should equal

$$J_d = (P_a)(C_L)(V_L) \qquad (1)$$

where P_a is the passive permeability coefficient for the movement of the lipid from the micellar into the cytosol of the mucosal cell, C_L is the concentration of the lipid in the micelle, and V_L is the volume of the micellar phase. In this formulation the micelle is considered to be a homogeneous phase separate from the aqueous phase so that V_L can be considered to equal essentially the concentration of bile acid present as micelles in the aqueous phase times the partial specific volume of the bile acid: thus V_L is directly proportional to the concentration of bile acid in the perfusate. Assuming that in any experimental situation the aggregation number for the micelle remains essentially constant, it follows that the number of micelles and, therefore, the number of interactions with the membrane, is proportional to V_L so that J_d also is a direct function of V_L. Equation one may be rewritten substituting M_L/V_L for C_L where M_L equals the mass

[2] In unpublished studies we have shown that the J_d of members of a homologous series of saturated FA in perfused intestinal segments in vivo are essentially proportional to the free diffusion coefficient for each FA rather than to its passive permeability coefficient. This strongly suggests that under these in vivo experimental conditions, uptake is diffusion-limited.

of the lipid in the micellar phase: thus

$$J_d = (P_a)\left(\frac{M_L}{V_L}\right)(V_L) \quad (2)$$

For a given bile acid and lipid, P_a is constant and the V_L term cancels so that the observed velocity of uptake of the lipid molecule would be proportional to the mass of the lipid in the micellar phase, i.e., if this model correctly describes events during lipid absorption into the intestinal mucosal cell then

$$J_d \approx M_L \quad (3)$$

In the third possible model to explain lipid absorption, the assumptions are made that there is no direct interaction between the micelle and the microvillus membrane, that uptake occurs only by way of the lipid molecules present in aqueous solution at the interface, that as lipid molecules are absorbed they are replaced by movement of other molecules from the bile acid micelle into the aqueous phase and that the partitioning of lipid molecules from the micelle to the aqueous phase occurs much more rapidly than the uptake of these solutes into the intestinal mucosal cell. Under these circumstances, the observed rate of lipid uptake equals

$$J_d = (P_b)(C_W) \quad (4)$$

where P_b is the passive permeability coefficient for the uptake of the lipid from the aqueous phase of the perfusate into the cytosol and C_W is the concentration of the lipid in the aqueous phase at the membrane interface. This equation may be rewritten

$$J_d = (P_b)\left(\frac{M_W}{V_W}\right) \quad (5)$$

where M_W is the mass of the lipid in the aqueous phase and V_W is the volume of the aqueous phase. In a given experimental situation P_b will be constant and, for practical purposes, V_W also will not change so that the observed J_d of the probe molecule will be proportional to the mass of the lipid in the aqueous phase, i.e., if this model is correct then

$$J_d \approx M_W \quad (6)$$

It should be emphasized that while P_a and P_b are both passive permeability coefficients they will have very different values since P_b will be largely determined by the free energy change necessary to break all bonds between the solute molecule and the aqueous phase while P_a will be largely influenced by the energy necessary to disrupt all bonds between the lipid molecule and the micelle.

From these considerations it is apparent that the second and third models described above can be differentiated experimentally by examining whether the observed J_d of a particular lipid molecule varies in a predictable manner with either M_L or M_W. The relationship between the concentration of the lipid in the bile acid micelle and in the adjacent aqueous phase is given by the expression

$$KC_W = C_M \quad (7)$$

where K equals the partitioning coefficient for the lipid between the micellar and aqueous phases. This may be rewritten as

$$(K)\left(\frac{M_W}{V_W}\right) = \left(\frac{M_L}{V_L}\right) \quad (8)$$

If the mechanism of lipid absorption involves direct interaction between the micelle and the membrane then J_d should be proportional to M_L: equation 8 may be rewritten to yield this term by substituting $(M_T - M_L)$ for M_W where M_T represents the total mass of the lipid in both the aqueous and micellar phase: thus

$$M_L = \frac{KM_T}{\left(\frac{V_W}{V_L}\right) + K} \quad (9)$$

Similarly, if the third model is correct, then J_d should be proportional to M_W: by a similar manipulation equation 8 may be rewritten

$$M_W = \frac{V_W M_T}{KV_L + V_W} \quad (10)$$

Equations 9 and 10 have been plotted graphically in Fig. 8 using appropriate values for the concentration and partial specific volume of taurodeoxycholate and for the concentration of the probe molecule. Since accurate partitioning coefficients, K, for the various lipids between the micellar and aqueous phases are currently unknown, these curves have been calculated using two values, 1×10^2 and 1×10^3: we have indirect evidence that the K values for the long chain FA and cholesterol are either in this range or are higher. Panel A illustrates the manner in which M_W or M_L change as the concentration of the probe molecule is increased in the presence of a constant concentration of bile acid, while the curves in the other two panels show the same data under circumstances where the concentration of the probe molecule is constant while that of the bile acid is increased (panel B) and where the concentrations of both constituents are increased in parallel (panel C).

On the basis of these theoretical considerations and the various experimental results presented in this paper, it is now possible to present several lines of evidence that differentiate which of these two possible models correctly describes the mechanism of FA transfer from the micelle to the intestinal mucosal cell. First, as shown in

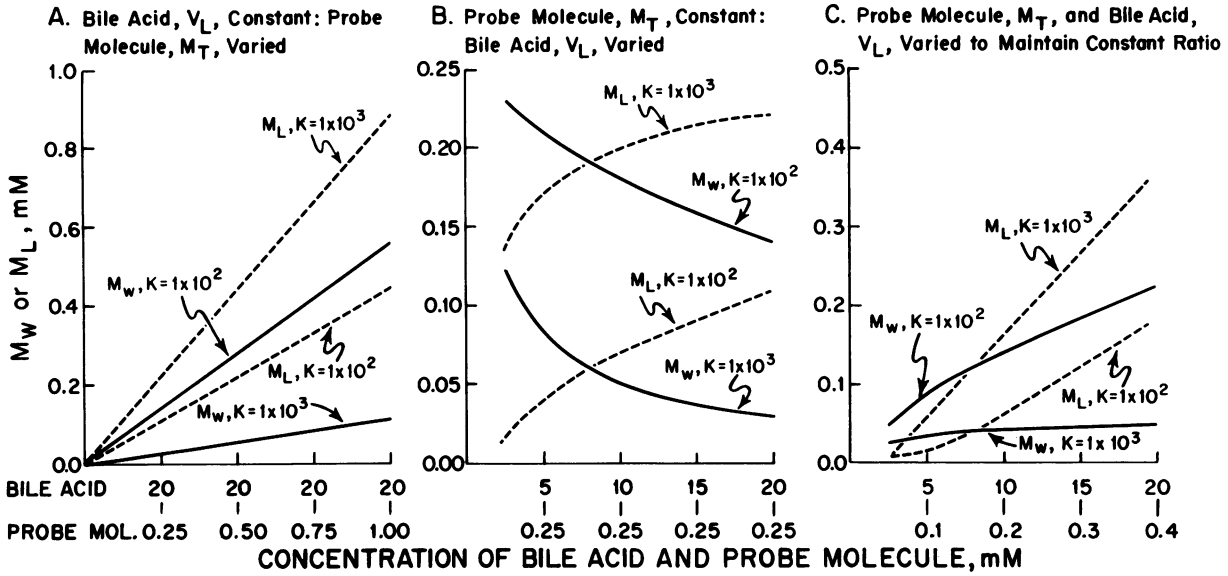

FIGURE 8 Theoretical curves illustrating the manner in which the mass of a probe molecule in the water phase (M_W) or in the micellar phase (M_L) varies under different experimental circumstances. The derivation of these curves is given in the text.

panel A of Fig. 8 both M_W and M_L, and therefore J_d, should increase linearly when the concentration of the probe molecule is increased while the concentration of the bile acid is kept constant. This was found to be the case of FA 12:0, FA 16:0, and FA 18:0 (Figs. 3 and 4): however, since this result was predicted for both models, these data do not discriminate between the two. Second, when the concentration of the bile acid is increased but that of the probe molecule is kept constant, there should be a curvilinear increase in M_L but a reciprocal decrease in M_W (panel B, Fig. 8). The experimentally determined values of J_d for FA 16:0 clearly follow the behavior predicted for M_W (panel A, Fig. 5). Third, when the concentration of both the bile acid and the probe molecule is increased in parallel, particularly when the probe molecule is very nonpolar and has a high partitioning coefficient, M_L should increase in a nearly linear fashion while M_W should show only a slight initial rise and then remain essentially constant: again, as seen in panel A of Fig. 6 the observed values of J_d for FA 18:0 follow the behavior predicted for M_W.

These experimental results, then, provide qualitative data to support the conclusion that FA absorption occurs through a monomer phase. Two other sets of data based upon quantitative considerations also support this conclusion. First, as seen in Fig. 3, J_d for FA 12:0 equaled 79.8±5.2 nmol·min^{-1}·100 mg^{-1} and 120.8±9.4 nmol·min^{-1}·100 mg^{-1}, respectively, when uptake was measured from solutions containing maximum concentrations of this FA in either buffer alone (0.72 mM) or buffer containing 20 mM taurodeoxycholate (13.6 mM). In the absence of the bile acid micelle the observed maximum J_d is determined by the maximum concentration of FA 12:0 achieved in the bulk perfusate (C_1) and the resistance encountered as the FA crosses both the unstirred water layer and the microvillus membrane. In this situation the concentration of FA at the aqueous membrane interface (C_2) can be calculated from the relationship (7)

$$C_2 = C_1 - \frac{(J_d)(d)}{(D)(S_w)} \qquad (11)$$

where d is the effective thickness of the unstirred water layer (1.37×10^{-2} cm), D is the free diffusion coefficient for the FA (7.59×10^{-6} cm^2·sec^{-1}) and S_w is the effective surface area of the unstirred water layer (11.7 cm^2·100 mg^{-1}) (7). From this calculation C_2 equals 0.51 mM: using this value to factor out unstirred water layer resistance, the observed J_d in the absence of bile acid can be corrected from the value of 79.8±5.2 nmol·min^{-1}·100 mg to 113.5±7.1 nmol·min^{-1}·100 mg. Thus, in this unique situation, even though the total concentration of FA in solution is increased nearly 20-fold by the presence of bile acid, the J_d is no greater than would be predicted if the only function of the micelle was to overcome unstirred layer resistance and maintain a maximum monomer concentration at C_2.

Second, while accurate coefficients are not available for the partitioning of various long chain FA into the taurodeoxycholate micelle, it is reasonable to assume in the special circumstances where a micellar solution is fully saturated with a particular long chain FA that the

concentration of the FA in aqueous solution in equilibrium with the micelle equals the maximum solubility of that FA in buffer alone. If this is the case and if absorption takes place only from the FA in the aqueous phase, then the maximum rate of FA uptake from saturated micellar solutions should equal the product of the passive permeability coefficient and the maximum aqueous solubility for a particular FA. In the case of FA 12:0, FA 16:0, and FA 18:0, the J_d predicted from this calculation would equal 131, 22.1, and 14.9 nmol·min^{-1}·100 mg^{-1}, respectively (7): as seen in Figs. 3 and 4, these predicted values coincide almost exactly with the experimentally determined values of maximum uptake which equaled 120.8±9.4, 24.1±3.2 and 13.6±1.1 nmol·min^{-1}·100 mg^{-1}, respectively.

Thus, taken together, these various lines of evidence provide strong support for the concept that during FA absorption there is no direct interaction between the micelle and the microvillus membrane: rather, uptake occurs only from the pool of FA in aqueous solution in equilibrium with the micelle. Furthermore, the same mechanism appears to apply in the case of cholesterol absorption since, as shown in Figs. 4–6, the uptake of cholesterol into the mucosal cell also behaves as if it occurred through an intermediate soluble phase. Since an experimentally determined passive permeability coefficient is currently not available for cholesterol it is not possible, however, to provide additional quantitative data to support this conclusion.

In general terms, then, the process of lipid uptake into the intestinal mucosal cell takes place as follows. FA and free cholesterol are generated by enzymatic activity in the bulk intestinal contents and partition into the bile acid micelle. These mixed micelles then diffuse down the existing concentration gradient through the unstirred water layer surrounding the villi and reach the immediate vicinity of the aqueous-microvillus interface. Uptake of the lipid molecules then occurs at a rate determined by the product of the passive permeability coefficient appropriate for each species, and the aqueous concentration of the molecule present at the microvillus surface. The concentration of the various lipids just inside the mucosal cell is presumably maintained at very low levels because of rapid esterification (2) while the concentration of these molecules outside the cell membrane is maintained at a relatively high value because of constant partitioning of FA and cholesterol out of the mixed micelle as dictated by the partitioning coefficient appropriate for each lipid species. The net effect of these various processes is the rapid movement of various lipids and steroids into the mucosal cell. There is also a finite concentration of bile acid monomers in solution in equilibrium with the bile acid molecules present in the micelle so that there is also passive uptake of these substances (12): the rate of such uptake is low, however, because of the very low passive permeability coefficients for the various bile acids. Since the critical micelle concentration should increase at the aqueous-membrane interface as lipids are absorbed from the mixed micelle (19, 20), the concentration of bile acid monomers is presumably higher at the aqueous-membrane interface than in the bulk phase of the intestinal contents: consequently, there is a gradient down which bile acid molecules can back-diffuse into the bulk phase of the intestinal contents to be reutilized in the creation of new mixed micelles as additional amounts of free cholesterol and FA are generated from dietary lipids. In a very real sense, the bile acid micelle acts as a shuttle between the bulk intestinal contents and the aqueous-microvillus interface.

In addition to this general description, sufficient data also are now available to define the system in more quantitative terms, as shown by the data in Table I, from which several additional points of major physiological importance can be derived. First, if there were no unstirred water layer in the intestine then the maximum J_d that could be achieved for any lipid would necessarily equal the maximum aqueous solubility of that lipid (column B) times its passive permeability coefficient (column C). Since, in the case of the FA series, maximum solubility decreases by a factor of 2.32 for each —CH_2— group added to the hydrocarbon chain while the passive permeability coefficient increases by a factor of only 1.58, it follows that the product of these two values, i.e., the maximum J_d, decreases by a factor of 1.47 for each carbon atom added to the FA chain. Thus, the more hydrophobic a molecule, the lower will be its maximum rate of movement into the mucosal cell. Conversely, the conversion of a lipid to a relatively more polar form, e.g., the hydrolysis of cholesterol esters to free cholesterol, or of triglyceride to β-monoglyceride and free FA, will significantly increase the maximum rate of membrane translocation that can be achieved. It should be emphasized that this situation occurs because the microvillus membrane in the intestine, as well as in other tissues that have been examined (6, 7, 12, 21), has been shown to be a relatively polar structure: the incremental free energy change, for example, associated with the addition of a substituent group like the —CH_2— moiety, equals only − 258 cal·mol^{-1} for the rabbit intestine (7). If this value were greater than − 517 cal·mol^{-1} then the opposite situation would prevail and the more hydrophobic members of the series would attain higher absolute rates of mucosal uptake.

Second, in the presence of a significant unstirred water layer this maximum J_d is reduced to the extent that the diffusion barrier exerts an additional resistance to the molecular uptake. Under circumstances where the

TABLE I

Effect of Bile Acid Micelles on Increasing the Maximum Rates of Fatty Acid and Cholesterol Uptake into the Intestinal Mucosal Cell

(A) Probe molecule	(B) Maximum solubility in bulk Krebs' buffer, C_1	(C) Passive permeability coefficient, P	(D) $\frac{C_2}{C_1}$	(E) Maximum uptake, J_d, in absence of bile acid micelles	(F) Maximum uptake, J_d, in presence of bile acid micelle at microvillus interface	(G) $\frac{F}{E}$
	mM	*nmol·min⁻¹·100 mg⁻¹·mM⁻¹*		*nmol·min⁻¹ ·100 mg⁻¹*	*nmol·min⁻¹ ·100 mg⁻¹*	
FA 4:0	575	4.7	1.00	2,703	2,703	1.00
6:0	107	11.9	0.99	1,261	1,273	1.01
8:0	19.8	29.9	0.95	562	592	1.05
10:0	3.68	74.8	0.86	237	275	1.16
12:0	0.682	187	0.68	86.7	128	1.48
14:0	0.127	468	0.43	25.6	59.4	2.32
16:0	0.0235	1,170	∼.00	7.78	27.5	3.53
18:0	0.00437	2,930	∼.00	1.35	12.8	9.48
20:0	0.00081	7,330	∼.00	0.23	5.9	25.83
22:0	0.00015	18,300	∼.00	0.04	2.7	68.75
Cholesterol	0.00004	40,000	∼.00	0.01	1.6	145.5

The data in column B were derived from the experimental values for maximum solubility shown in Fig. 7. The natural logarithms of these values were first plotted against FA chain length and gave a linear regression curve with the formula $y = 9.722 - 0.842x$: the values for the maximum solubility of each FA shown in column B were then calculated using this expression. Similarly, the data in column C were derived from the experimentally determined permeability coefficients given in Table V of reference 7: the natural logarithm of the P values for FA 8:0 through FA 12:0 plotted against chain length gave a linear regression curve with the formula $y = -0.271 + 0.459x$. The individual values given in column C were then calculated from this relationship assuming that the *P* values for even the shorter chain-length members of the FA series fell on this regression curve. Column D represents the fractional drop in the concentration of each probe molecule between the bulk perfusion solution, C_1, and the solution present at the aqueous-membrane interface, C_2, calculated to occur under the conditions used in these studies. At chain-lengths of greater than 14 carbon atoms the unstirred water layer becomes totally rate limiting to uptake so that C_2 essentially equals zero. The maximal J_d values for FA 4:0 through FA 14:0 shown in column E were calculated as the product of the appropriate values in columns B, C, and D. For those FA with chain lengths greater than 14 carbons where C_2 essentially equals zero, the maximum J_d in the absence of a bile acid micelle was calculated from the relationship $J_d = \frac{(S_w)(D)(C_1)}{d}$ where C_1 is taken as the maximum solubility value given in column B, D is the appropriate free diffusion coefficient for each fatty acid (6), and S_w and d have the values of 11.7 $cm^2 \cdot 100\ mg^{-1}$ and 137 μm, respectively (7). The data in column F were calculated as the product of the appropriate values in columns B and C. The data in column G illustrate the relative effect of bile acid micelles in facilitating uptake of each FA. No experimentally determined permeability coefficient was available for cholesterol: however, the maximum solubility of monomers (column B) was taken as 40 nM from reference 26 and the maximum J_d in the presence of bile acid micelles (column F) was experimentally determined in this study (Fig. 4). Assuming that the aqueous phase against the microvillus membrane was saturated with cholesterol molecules, *P* was then calculated by dividing 1.60 $nmol \cdot min^{-1} \cdot 100\ mg^{-1}$ by 40 nM (column C). The maximum J_d in the absence of bile acid micelles (column E) was then calculated as described above.

effective thickness and surface area of the diffusion barrier are constant, the magnitude of this resistance varies as a function of the passive permeability and free diffusion coefficients for a particular probe molecule. Even under the highly stirred conditions utilized in these studies where the mean thickness of the unstirred water layer was only 1.37×10^{-2} cm, as shown in column D, this barrier begins to exert a major resistance to uptake for FA 10:0 and becomes totally rate limiting for FA with 16 carbon atoms or greater. As a consequence, the maximum J_d observed in the presence of the unstirred water layer (column E) is considerably less for these more hydrophobic members of the series than would be predicted if no unstirred water layer were present in the intestine (column F). In vivo we have other data to suggest that the diffusion barrier is much thicker so that this effect is even more pronounced.

Third, if as formulated in this paper the function of the bile acid micelle is solely to overcome unstirred water layer resistance and so maintain the maximum monomer concentration possible at the aqueous-membrane interface, then the relative effect of bile acid in facilitating uptake of a particular lipid is directly related to the magnitude of the resistance encountered by

that molecule in crossing the unstirred water layer. Under the conditions of these studies the magnitude of this effect can be quantitated by taking the ratio of J_a in the presence of bile acid (column F) to J_a in the absence of the micelle (column E). As shown in column G, the presence of bile acid has little effect on the uptake of FA 4:0 to FA 10:0, has a moderate effect on the FA of medium chain length and has a more marked effect on the long chain FA and cholesterol. Stated in a different way, in the absence of bile acid, short and medium chain length FA are absorbed at essentially normal rates, there is a moderate degree of malabsorption of the longer chain length FA and there is essentially no absorption of very nonpolar compounds like cholesterol. These findings correlated well with observations made in intact animals and in man (1, 3, 4, *22, 23*).

Finally, there are two areas of uncertainty in these studies that should be emphasized. First, it is possible that bile acids might also affect other steps in the overall process of fat absorption such as the rate of chylomicron formation or delivery into the lymph: however, while the rate of delivery of lipids into the circulation with respect to time may be determined by the rate of chylomicron formation and movement out of the intestinal mucosal cell, other data suggest that the mass of lipids ultimately reaching the circulation is determined by the translocation step in the microvillus border. Second, the mathematical formulations presented in this paper and the data derived in Table I are predicated upon the assumption that the various probe molecules exist in aqueous solution predominantly in monomer form. This point of view is supported by the studies of Simpson and associates (24), and of Sallee (25), and by the data presented in panel A of Fig. 4. Nevertheless, there is still controversy as to whether significant association of the longer chain length FA does occur under physiological conditions and, furthermore, there is little data on the nature of the chemical species of cholesterol that may exist in aqueous solution. If significant complexing does occur, however, it would not alter the conclusions derived from analysis of equations 9 and 10 since such association should accentuate the differences in behavior of the J term in the two models rather than obliterate these differences.

ACKNOWLEDGMENTS

These studies were supported by U. S. Public Health Service Research grants 1 R01 AM 16386 and 5 R01 HL 09610 and by U. S. Public Health Service Training grant 5 TO1 AM 05490. Dr. Westergaard was supported by the U. S. Public Health Service Fogarty International Research Fellowship 5 F05 TW 01808.

REFERENCES

1. Siperstein, M. D., I. L. Chaikoff, and W. O. Reinhardt. 1952. C^{14}-cholesterol. V. Obligatory function of bile in intestinal absorption of cholesterol. *J. Biol. Chem.* **198**: 111–114.
2. Johnston, J. M. 1968. Mechanism of fat absorption. Handb. Physiol. Section 6. Alimentary Canal. **III**: 1353–1375.
3. Treadwell, C. R., and G. V. Vahouny. 1968. Cholesterol absorption. Handb. Physiol. Section 6. Alimentary Canal. **III**: 1407–1438.
4. Holt, P. R. 1971. Medium chain triglycerides. *Dis.-a-Mon.* June: 3–30.
5. Wilson, F. A., and J. M. Dietschy. 1971. Differential diagnostic approach to clinical problems of malabsorption. *Gastroenterology.* **61**: 911–931.
6. Sallee, V. L., and J. M. Dietschy. 1973. Determinants of intestinal mucosal uptake of short- and medium-chain fatty acids and alcohols. *J. Lipid Res.* **14**: 475–484.
7. Westergaard, H., and J. M. Dietschy. 1974. Delineation of dimensions and permeability characteristics of the two major diffusion barriers to passive mucosal uptake in the rabbit intestine. *J. Clin. Invest.* **54**: 718–732.
8. Sanders, E., and C. T. Ashworth. 1961. A study of particulate intestinal absorption and hepatocellular uptake. Use of polystyrene latex particles. *Exp. Cell Res.* **22**: 137–145.
9. Frazer, A. C. 1962. Fate of dietary fat in the body. *Nature (Lond.).* **194**: 908–909.
10. Hoffman, N. E. 1970. The relationship between uptake *in vitro* of oleic acid and micellar solubilization. *Biochim. Biophys. Acta.* **196**: 193–203.
11. Simmonds, W. J. 1972. The role of micellar solubilization in lipid absorption. *Aust. J. Exp. Biol. Med. Sci.* **50**: 403–421.
12. Wilson, F. A., and J. M. Dietschy. 1972. Characterization of bile acid absorption across the unstirred water layer and brush border of the rat jejunum. *J. Clin. Invest.* **51**: 3015–3025.
13. Hofmann, A. F. 1962. Thin-layer adsorption chromatography of free and conjugated bile acids on silicic acid. *J. Lipid Res.* **3**: 127–128.
14. Gregg, J. A. 1966. New solvent systems for thin-layer chromatography of bile acids. *J. Lipid Res.* **7**: 579–581.
15. Lukie, B. E., H. Westergaard, and J. M. Dietschy. 1974. Validation of a chamber that allows measurement of both tissue uptake rates and unstirred layer thicknesses in the intestine under conditions of controlled stirring. *Gastroenterology.* **67**: 652–661.
16. Sallee, V. L., F. A. Wilson, and J. M. Dietschy. 1972. Determination of unidirectional uptake rates for lipids across the intestinal brush border. *J. Lipid Res.* **13**: 184–192.
17. Diamond, J. M., and E. M. Wright. 1969. Biological membranes: The physical basis of ion and nonelectrolyte selectivity. *Annu. Rev. Physiol.* **31**: 581–646.
18. Dietschy, J. M., and H. Westergaard. 1975. The effect of unstirred water layers on various transport processes in the intestine. *In* Intestinal Absorption and Malabsorption, T. Z. Csaky, editor. Raven Press, New York. 197–207.
19. Carey, M. C., and D. M. Small. 1969. Micellar properties of dihydroxy and trihydroxy bile salts: Effects of counterion and temperature. *J. Colloid Interface Sci.* **31**: 382–396.

20. Small, D. M. 1968. Size and structure of bile salt micelles. Influence of structure, concentration, counterion concentration, pH, and temperature. *Adv. Chem. Ser.* **84**: 31–52.
21. Sherrill, B. C., and J. M. Dietschy. 1975. Permeability characteristics of the adipocyte cell membrane and partitioning characteristics of the adipocyte triglyceride core. *J. Membr. Biol.* **23**: 367–383.
22. Porter, H. P., D. R. Saunders, G. Tytgat, O. Brunser, and C. E. Rubin. 1971. Fat absorption in bile fistula man. A morphological and biochemical study. *Gastroenterology.* **60**: 1008–1019.
23. Fernandes, J., J. H. van de Kamer, and H. A. Weijers. 1962. Differences in absorption of the various fatty acids studied in children with steatorrhea. *J. Clin. Invest.* **41**: 488–494.
24. Simpson, R. B., J. D. Ashbrook, E. C. Santos, and A. A. Spector. 1974. Partition of fatty acids. *J. Lipid Res.* **15**: 415–422.
25. Sallee, V. L. 1974. Apparent monomer activity of saturated fatty acids in micellar bile salt solutions measured by a polyethylene partitioning system. *J. Lipid Res.* **15**: 56–64.
26. Haberland, M. E., and J. A. Reynolds. 1973. Self-association of cholesterol in aqueous solution. *Proc. Natl. Acad. Sci. U. S. A.* **70**: 2313–2316.