Determinants of intestinal mucosal uptake of short- and medium-chain fatty acids and alcohols

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Abstract Uptake rates across the jejunal brush border have been measured for water-soluble fatty acids and alcohols and analyzed to determine the relative roles of the unstirred water layer and the lipid cell membrane as determinants of the intestinal absorptive process. Initial studies involving measurement of time courses of electrical transients developed across the intestine exposed to poorly permeant solute molecules showed no anomalous discrimination of probe molecules of different size or charge. This finding suggests that the diffusion barrier in the intestine can be considered as an unstirred water layer. Next, uptake rates of fatty acid were found to be linear with respect to concentration of the test solute, demonstrated no competitive inhibition or contralateral stimulation, had low temperature dependency, and were insensitive to metabolic inhibition, indicating that uptake proceeds by passive diffusion. Passive permeability coefficients, $*P$, varied from 22 ± 1.4 to 395 ± 9.2 nmoles·min^{-1}·100 mg^{-1}·mM^{-1} for the saturated fatty acids 2:0 through 12:0 and from 119 ± 3.3 to 581 ± 45.2 for the saturated alcohols 6:0 through 10:0. Vigorous stirring of the bulk buffer solution enhanced $*P$ values in direct proportion to chain length while the presence of bile acid micelles depressed apparent permeability coefficients in proportion to fatty acid chain length. These results demonstrate that uptake of short-chain fatty acid monomers is rate limited by the lipid cell membrane but diffusion through the unstirred water layer becomes increasingly rate limiting as the chain length increases. It is also possible to conclude from these data that diffusion through the unstirred water layer becomes totally rate limiting for uptake of long-chain fatty acid monomers of physiological importance.

Supplementary key words membrane permeation · unstirred water layer · diffusion coefficients · intestinal absorption

THE GENERAL FEATURES of fat absorption in the intestine are now well known. Dietary triglyceride is hydrolyzed in the intestinal lumen, after which the products of hydrolysis, principally fatty acids and β-monoglycerides, are solubilized by bile acids in mixed micelles. These products are then absorbed into the intestinal mucosal cell where reesterification of the fatty acids and β-monoglycerides takes place. After the addition of small amounts of a specific protein, cholesterol, cholesteryl ester, and phospholipid, the triglyceride is mobilized from the mucosal cell into the intestinal lymph in chylomicrons and very low density lipoproteins (1).

While there are considerable data on the intraluminal and intracellular events during fat absorption (1–4), there is still controversy concerning the actual process whereby fat is absorbed across the jejunal brush border. For example, passive diffusion and a carrier mechanism involving receptor sites in the cell membrane have both been suggested as mechanisms for fat uptake (5–8). Furthermore, the role of the mixed micelle in this transport process remains obscure; contradictory data have been published suggesting, on the one hand, that the intact mixed micelle crosses the luminal membrane of the absorptive cell and, on the other hand, that it does not (5, 9). Finally, recent data from this laboratory have emphasized the rate-limiting nature of the unstirred layers adjacent to the mucosal cell membrane to certain absorptive processes. Therefore, not only are

Abbreviations: $*P$, apparent permeability coefficient; P, true permeability coefficient corrected for unstirred layer effects; J_d, experimentally determined flux rate with the units nmoles·min^{-1}·100 mg^{-1}; J, flux rate corrected for surface area of the unstirred layer with the units nmoles·sec^{-1}·cm^{-2}; D, free diffusion coefficient; d, thickness of the unstirred layer; S, effective surface area of the unstirred layer in an amount of intestine equivalent to 100 mg dry weight of tissue with the units cm^2·100 mg^{-1}; C_1, concentration of solute in the bulk perfusate; C_2, concentration of solute at the aqueous–lipid interface of the intestinal mucosa; C_3, concentration of solute just inside the mucosal cell membrane; M, molecular weight.

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TABLE 1. Diffusion coefficients used for various calculations in these studies

Compound	D	Temperature	Reference
	$cm^2 \cdot sec^{-1} \times 10^6$	°C	
Urea	17.3[a]	37	13
Pentaerythritol	10.2[b]	37	14
Mannitol	9.29[a]	37	13
Sucrose	6.99[a]	37	13, 15
Raffinose	5.83[b]	37	14
NaCl	20.3[a]	35	16
Glycine	10.64[a]	25	17
β-Alanine	9.33[a]	25	17
Mannitol	6.82[a]	25	17
Fatty Acids[c]			
2:0	16.7[d]	37	18
3:0	13.9[d]	37	18
4:0	12.1[d]	37	18
5:0	10.9[d]	37	18
6:0	10.5[e]	37	18
7:0	10.2[f]	37	18
8:0	9.49[f]	37	18
9:0	8.91[f]	37	18
10:0	8.40[f]	37	18
12:0	7.59[f]	37	18
14:0	6.96[f]	37	18
16:0	6.46[f]	37	18
18:0	6.04[f]	37	18

These diffusion coefficients were obtained by several different means, as follows.

[a] Direct experimental determination.

[b] Experimentally determined values at lower temperatures were taken from the stated reference and plotted as an Arrhenius plot. The value at 37°C was then determined by extrapolation.

[c] Number of carbon atoms: number of double bonds.

[d] Experimental values at 27°C were found to fit a modified form of the semiempirical equation of the Wilke-Chang format (19).

[e] Predicted diffusion coefficients of methods *d* and *f* were averaged.

[f] Values were calculated directly from the Wilke-Chang semiempirical correlation equation (19).

the membrane transport processes operative in fat absorption poorly understood, but, in addition, it is not clear whether it is the unstirred water layer or the lipid cell membrane that is rate limiting to cell uptake.

Using a new technique that allows determination of essentially unidirectional uptake rates of both water- and fat-soluble compounds across the unstirred layer and brush border of the intestinal epithelium (10), we have recently investigated in detail the absorption of bile acids. These studies demonstrated that the rate of uptake of monomers of polar bile acids essentially is determined by the rate of penetration of the lipid cell membrane; for monomers of less polar bile acids, however, the unstirred water layer begins to exert significant resistance to absorption. This resistance was shown to be proportionately greater for absorption of bile acids from micellar solutions, where the rates of diffusion of the large micelles across the diffusion barrier necessarily are lower than for the corresponding monomers. Finally, on the basis of three separate sets of data we also have shown that once the bile acid micelle crosses the unstirred water layer and reaches the aqueous–lipid interface it does not cross the brush border membrane intact. Thus, the role of the bile acid micelle during fat absorption must be explained in other terms (9).

In order to clarify next the mechanisms of fatty acid absorption into the intestinal mucosa the present experiments were undertaken. These studies were performed using short- and medium-chain fatty acids that are water soluble so that membrane transport phenomena could be defined, and the relative resistance of the cell membrane and unstirred water layer could be determined without having to deal with the complex bulk phase interactions between fatty acid monomers and bile acid micelles. These studies demonstrate, first, that the membrane penetration step is a passive diffusion process; second, that the permeability coefficients for the various fatty acids increase with chain length; and third, that the unstirred water layer becomes progressively more rate limiting the higher the rate of passive permeation. On the basis of these data, it is possible to calculate the incremental free energy change for absorption across the microvillus brush border associated with the addition of each —CH_2— group to the fatty acid molecule and, most important, it is also possible to demonstrate that the unstirred water layer is absolutely rate limiting to the uptake of long-chain fatty acid monomers in the small intestine.

MATERIALS AND METHODS

Flux rate determinations

Measurements of unidirectional flux rates across the brush border of the rat jejunum were carried out using techniques developed in this laboratory and described in detail elsewhere (10). Briefly, the everted mucosa of the intestine was exposed to buffer containing both a radiolabeled test substance (^{14}C-labeled) and a nonpermeant molecule (^{3}H-labeled dextran) used as a marker of adherent mucosal volume. These incubations were carried out in test tubes having an inside diameter of 1.5 cm. At the bottom of each tube was a $^5/_8$ inch, round, Teflon-coated stirring bar. In studies designated "stirred," this bar was driven at 1800 rpm by a magnetic stirring device; the bar was kept stationary in the "unstirred" studies. After an incubation period, usually of 4 min, at 37°C the tissue was washed for 5 sec in cold buffer, blotted, and transferred to a tared counting vial. After determination of tissue dry weight, the tissue was hydrolyzed with NaOH and radioactivity was

measured; an external standardization technique was used to correct for quenching of the two isotopes. After correction for mass of test molecules carried over in the adherent mucosal fluid by means of the marker compound, flux rates (J_d) were calculated and have the units nmoles taken up into the mucosa per min per 100 mg dry tissue weight, i.e., nmoles·min^{-1}·100 mg^{-1}. Although J_d values were measured at concentrations of the test solutes from 0.15 to 1.0 mM, the data are normalized to a 1 mM concentration and, therefore, are presented as apparent permeability coefficients, $*P$, with the units nmoles·min^{-1}·100 mg^{-1}·mM^{-1}. The ^{3}H-labeled dextran, mol wt of 15,000–17,000, was obtained from New England Nuclear Corp., Boston, Mass., and was used as supplied. 1-^{14}C-labeled fatty acids and alcohols of various chain lengths along with the corresponding unlabeled compounds were synthesized, purified, and supplied by Applied Science Laboratories, Inc., State College, Pa. Most solutions were prepared with Krebs bicarbonate buffer, from which the calcium was omitted, and were oxygenated with 5% CO_2 in oxygen, giving a pH of 7.4 at 37°C. In specific experiments, solutions also were prepared with Tris–phosphate buffer having the following composition: Na^+, 140 meq/l; K^+, 5 meq/l; Cl^-, 135 meq/l; phosphate, 10 meq/l; and Tris, (2-amino-2-[hydroxymethyl]-1,3-propanediol), 10 mM. Solutions prepared with this buffer were adjusted to a pH of 6.0 at 37°C, using concentrated hydrochloric acid, and were gassed with 100% oxygen.

Several features of this incubation technique require emphasis. First, we have demonstrated in previous work that *1*) the incubation time is sufficient for the unstirred water layers to become uniformly labeled with the nonpermeant marker; *2*) the rate of uptake of the various probe molecules is linear with respect to time between 1 and 4 min and extrapolates to zero at 0 time; and *3*) the rate of tissue uptake is linear with respect to tissue dry weight (9, 10). Second, we also have shown that the vigorous stirring employed in some of these studies during the 4-min incubation does not alter either the histology or the transport characteristics of the membrane even when the stirring is carried out in the presence of surface active agents such as bile acids or bile acids and phospholipid (9, 10). Finally, we have characterized in detail the transport characteristics of a variety of substances in this system. Uptake of probe molecules that are passively absorbed manifests rates that are linear with respect to the concentration of the probe molecule in the bulk water phase, shows no competition among related compounds, is not altered by metabolic inhibition, and generally has low temperature coefficients. In contrast, uptake of probe molecules that are actively transported manifests saturation kinetics and competition among related compounds, is suppressed by metabolic inhibitors, and has generally high temperature coefficients (9–11).[3]

Determination of unstirred layer thickness

The thickness of the unstirred layer in the intestine was measured by the method of Diamond (12). This technique involves measurement of the half-time ($t_{1/2}$) for evolution of an electrokinetic or diffusion potential across a biological membrane. Knowing the diffusion coefficient (D) for the molecules used to induce the potential difference, one can calculate the thickness of the unstirred water layer (d) by means of the formula

$$d = \left(\frac{D \cdot t_{1/2}}{0.38}\right)^{1/2} \qquad \text{Eq. 1}$$

To measure these half-times, an everted segment of intestine similar in length to those used in the incubation experiments was tied over an open glass cannula at one end and fixed to a closed cannula at the other. The serosal compartment was filled with buffer through the glass cannula, and the potential difference across the intestinal wall was measured using salt bridges placed in the outer mucosal and inner serosal solutions. In these experiments, a change in potential difference was generated by adding urea, pentaerythritol, mannitol, sucrose, raffinose, glycine, or β-alanine to the mucosal buffer solution at a concentration of 100 mM or by decreasing the concentration of NaCl (replaced isosmotically with mannitol) in the mucosal perfusate.

Diffusion coefficients

Free diffusion coefficients, D, used in this study are listed in Table I. Since diffusion coefficients for only a few compounds actually have been determined at 37°C, most values listed in this table have been calculated using one of several techniques. Since experimentally determined D values at various temperatures give a linear relationship when using an Arrhenius plot, this method was used to obtain diffusion coefficients for several sugars at 37°C. The coefficients for fatty acids up to six carbons have been experimentally determined at 25°C by Bidstrup and Geankoplis (18) and have been shown to fit a modified form of the Wilke-Chang equation. This equation therefore has been used to calculate diffusion coefficients for these fatty acids at 37°C. The value in the data of Bidstrup and Geankoplis (18) for the fatty acid 6:0, however, actually was intermediate between the prediction of the modified Wilke-Chang equation and the standard Wilke-Chang equation, so that the value for fatty acid 6:0 was cal-

[3] Westergaard, H., B. E. Lukie, and J. M. Dietschy. Unpublished data from this laboratory.

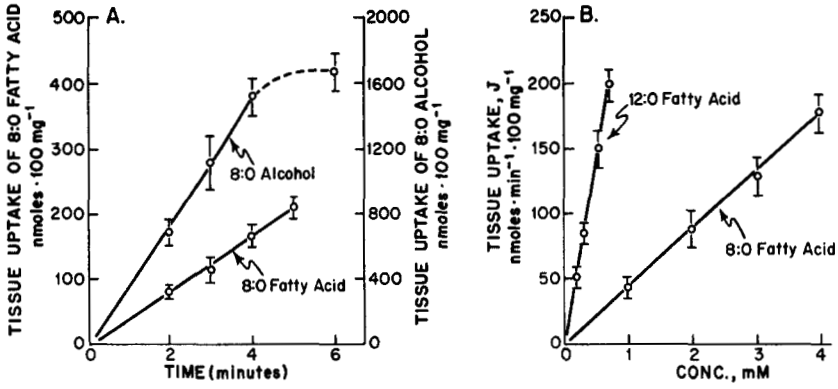

FIG. 1. Tissue uptake of the 8:0 alcohol and 8:0 and 12:0 fatty acids as a function of time and bulk phase concentration. Jejunal mucosa was incubated in Krebs bicarbonate buffer at a pH of 7.4 at 37°C. Panel *A* shows the uptake rates as a function of time for the 8:0 alcohol and 8:0 fatty acid; these values have been normalized to a bulk buffer phase concentration of 1 mM. Panel *B* illustrates the rates of uptake as a function of concentration of the 8:0 and 12:0 fatty acids in the bulk buffer phase. The values shown are means ± SEM for determinations in sacs from four to six animals.

culated as the average between the predictions of those two equations; values for fatty acids of longer chain length were calculated using the standard Wilke-Chang equation (19).

RESULTS

Nature of the diffusion barrier in the intestine

Although it is commonly assumed that the diffusion barrier against biological membranes is a series of water lamellae extending outward from the aqueous lipid interface until they blend imperceptibly into the bulk solution (20, 21), initial experiments were undertaken to determine if some anatomical structure in the intestine such as mucus or the glycocalyx might contribute to this barrier. We reasoned that if this were the case, the diffusion barrier might discriminate between probe molecules of varying size and charge differently than would a layer of water.

In order to examine this possibility, the half-times were measured of the electrical transients of the streaming potentials developed when the mucosa was exposed to hyperosmotic solutions. As shown in experiment A of Table 2, in the case of uncharged solutes the $t_{1/2}$

TABLE 2. Determination of the thickness of the unstirred layer in rat jejunum using probe molecules of different size and charge

Probe Molecule	Molecular Weight	$t_{1/2}$	$Dt_{1/2}$	d
		sec	$cm^2 \times 10^6$	
Experiment A				
Urea	60	7.3 ± 0.26 (15)	126.3 ± 4.5	182 ± 4
Pentaerythritol	136	12.5 ± 0.58 (8)	127.5 ± 5.9	183 ± 4
Mannitol	182	16.1 ± 1.09 (7)	149.6 ± 10.1	198 ± 7
Sucrose	342	18.5 ± 0.81 (10)	129.3 ± 5.7	184 ± 4
Raffinose	594	22.8 ± 1.22 (8)	132.9 ± 7.1	187 ± 5
Experiment B				
Sucrose	342	18.2 ± 0.79 (26)	127.3 ± 5.5	183 ± 4
NaCl	58	8.0 ± 0.71 (16)	162.4 ± 14.4	207 ± 9
Experiment C				
Glycine	75	13.2 ± 0.54 (4)	140.4 ± 5.7	192 ± 4
β-Alanine	89	15.0 ± 0.88 (4)	140.0 ± 8.2	192 ± 5
Mannitol	182	20.4 ± 0.71 (6)	139.1 ± 4.8	191 ± 4

All studies were performed using mid-jejunum. Experiments A and B were undertaken at 37°C; experiment C was performed at 25°C. All streaming potentials were induced using a concentration of 100 mM of the various impermeant solute molecules. The NaCl diffusion potentials were generated by exposing the mucosal surface to solutions with NaCl concentrations of 117, 86, and 26 mM, and all data were combined. These latter solutions were kept isosmotic with mannitol. Mean values ± SEM are shown for the number of determinations indicated in parentheses.

values varied from 7.3 ± 0.26 sec to 22.8 ± 1.22 sec as the molecular weight of the probe molecule was varied from 60 (urea) to 594 (raffinose). Similarly, there was significant variation in the $t_{1/2}$ values when diffusion and streaming potentials were generated with the charged substances NaCl, glycine, and β-alanine. However, when these values were used to calculate the thickness of the unstirred layer, d, essentially identical values, in the range of 182 to 207 μm, were obtained. This results from the fact that the experimentally determined $t_{1/2}$ values varied in an almost exact inverse fashion with D, so that the product $t_{1/2} \cdot D$ is constant. This is the result that would be anticipated if the diffusion barrier were water, so these experiments provided no evidence of an anatomical barrier that produces anomalous discrimination with respect to size or charge of the probe molecules. Hence, in the remainder of these studies the diffusion barrier in the intestine is treated as a layer of unstirred water.

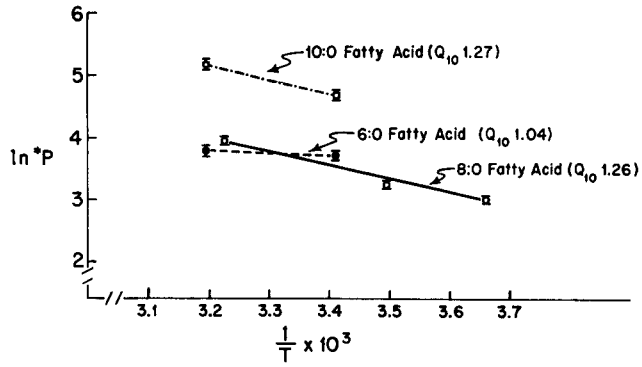

FIG. 2. The effect of temperature on uptake of the 6:0, 8:0, and 10:0 fatty acids. Jejunal sacs were preincubated at the various temperatures indicated in the figure then transferred to buffer solutions containing a 1 mM concentration of the different fatty acids at the same respective temperatures, and uptake rates were determined during a 4-min incubation. The natural logarithm of the apparent permeability coefficient, $*P$, is plotted against the reciprocal of absolute temperature. The data are mean values ± SEM for six to eight determinations.

Transport characteristics of short- and medium-chain fatty acid uptake in the intestine

In order to define the membrane transport process for the short- and medium-chain fatty acids, uptake of several representative acids was characterized with respect to kinetics, temperature dependency, and sensitivity to metabolic inhibition. As seen in Fig. 1, in the case of 8:0 fatty acid and alcohol, tissue uptake was linear with respect to time up to at least 4 min. This was true for various other compounds that we have tested in this system; therefore, all subsequent incubations were carried out for this time interval. As also shown by the examples in Fig. 1*B*, tissue uptake was linear with respect to bulk phase concentration; in no instance have we found saturation kinetics suggestive of a carrier-mediated transport system for short- and medium-chain fatty acids. Similarly, tissue uptake of these compounds did not manifest competition from related substances, e.g., the fatty acid 6:0 did not inhibit uptake of the fatty acid 8:0, and we were not able to show contralateral stimulation of unidirectional flux rates. For example, octanoate effluxed from preloaded tissue into the bulk buffer at rates of 21.0 ± 2.0 and 21.6 ± 1.1 nmoles·min^{-1}·100 mg^{-1} in the absence and presence, respectively, of 1 mM 8:0 fatty acid in the perfusate. Similarly, tissue uptake of this acid from 1 mM solutions occurred at a rate of 69.3 ± 5.0 nmoles·min^{-1}·100 mg^{-1} in the control situation and at a rate of 66.8 ± 4.3 nmoles·min^{-1}·100 mg^{-1} when the tissue had been preloaded with the 8:0 fatty acid.

As shown in Fig. 2, reduction in temperature of the incubation system had relatively little effect on tissue uptake of the 6:0, 8:0, and 10:0 fatty acids; the Q_{10} values for these three acids equaled only 1.04, 1.26, and 1.27, respectively. Finally, as shown in Table 3, uptake of octanoate also is unaffected by metabolic

TABLE 3. Effect of metabolic inhibitors on tissue uptake of the 8:0 fatty acid

	Apparent Permeability Coefficient, $*P$, for the 8:0 Fatty Acid	[2-^{14}C]Acetate Incorporation into:		
		CO_2	Fatty Acids	Cholesterol
	nmoles·min^{-1}·100 mg^{-1}·mM^{-1}		*nmoles·g^{-1}·hr^{-1}*	
Control	64.3 ± 6.6 (7)	7502 ± 371 (6)	1472 ± 58 (6)	43.0 ± 3.3 (6)
m-Cl-CCP	60.3 ± 6.3 (9)	1386 ± 102 (6)	105 ± 17 (6)	8.5 ± 0.4 (6)
Iodoacetamide	54.1 ± 4.0 (9)	296 ± 17 (6)	111 ± 13 (6)	0.3 ± 0.1 (6)

Everted jejunal segments were preincubated for 10 min at 37°C in Krebs bicarbonate buffer alone (control) or in buffer containing carbonyl cyanide *m*-chlorophenyl hydrazone (*m*-Cl-CCP) (1 × 10^{-6} M) (9) or iodoacetamide (1 × 10^{-3} M). The sacs were then used to determine rates of fatty acid uptake or rates of incorporation of [2-^{14}C]acetate into CO_2, long-chain fatty acids, and cholesterol. In the latter experiments the tissue was incubated for 30 min in Krebs-Ringer bicarbonate buffer containing 4.2 μmoles/ml of acetate as previously described (22). Mean values ± SEM are shown for the number of determinations indicated in parentheses.

TABLE 4. Apparent permeability coefficients, $*P$, for saturated fatty acids and alcohols of different chain lengths under stirred conditions

	$*P$	
	pH 7.4	pH 6.0
	$nmoles \cdot min^{-1} \cdot 700\ mg^{-1} \cdot mM^{-1}$	
Fatty Acids		
2:0	22 ± 1.4 (32)	112.9 ± 13.4 (6)
4:0	21 ± 2.4 (15)	110.9 ± 7.7 (6)
5:0	22 ± 3.8 (12)	124.6 ± 9.3 (6)
6:0		154.6 ± 13.3 (6)
7:0	28 ± 4.1 (10)	128.4 ± 14.6 (6)
8:0	48 ± 1.8 (82)	175.8 ± 5.4 (6)
9:0	85 ± 4.4 (6)	155.0 ± 12.0 (6)
10:0	117 ± 5.6 (51)	237.3 ± 9.3 (6)
12:0	395 ± 9.2 (32)	
Alcohols		
6:0	119 ± 3.3 (6)	
8:0	345 ± 16.4 (8)	
10:0	581 ± 45.2 (7)	

Everted jejunal sacs were incubated under stirred conditions for 4 min at 37°C in Krebs-Ringer bicarbonate buffer at pH 7.4 or in Tris–phosphate buffer at pH 6.0 containing various concentrations of fatty acids or alcohols. All uptake rates were then normalized to a concentration of 1 mM. Mean values ± SEM are shown for the number of determinations in parentheses.

inhibition. In these studies where the compounds carbonyl cyanide *m*-chlorophenyl hydrazone (*m*-Cl-CCP) and iodoacetamide caused as much as 99% inhibition of such intracellular metabolic processes as CO_2 production and fatty acid and sterol synthesis from acetate, there was essentially no inhibition of fatty acid absorption.

Apparent permeability coefficients of the short- and medium-chain fatty acids

Since the foregoing experiments indicated that short- and medium-chain fatty acid absorption into the intestinal cell occurred via a passive diffusion process, the relative rates of uptake of these various fatty acids can be appropriately described using apparent permeability coefficients, $*P$. These are shown in Table 4 for the homologous series of saturated fatty acids between 2:0 and 12:0 and for the 6:0 and 8:0 alcohols under vigorously stirred conditions. Since the pK_a for all these fatty acids is much lower than 7.4 (23), the $*P$ values listed in the first column may be considered essentially the apparent permeability coefficients for the fatty acid ions. In general, $*P$ increased with increases in chain length; however, when these mean data are plotted as shown in Fig. 3 as ln $*PM^{1/3}$, it is apparent that there is a linear relationship only between fatty acids 7:0 and 12:0, while apparent permeability coefficients for the shorter-chain fatty acids deviate upward from this relationship.

The effects of three specific experimental manipulations on the apparent permeability coefficients of these fatty acids were next investigated. First, when the pH of the bulk buffer solution was decreased to 6.0, $*P$ values increased, as shown in the second column of Table 4. Since we previously have shown that such an alteration in pH does not change the permeability characteristics of the cell membrane, this enhanced uptake presumably is due to the fact that shifting the pH downward results in a relatively higher concentration of protonated fatty acid, which permeates the lipid cell membrane at a higher rate than does the ionized species. Second, $*P$ values for the 7:0, 8:0, 10:0, and 12:0 ionized fatty acids also were measured under unstirred conditions. When plotted in Fig. 3, it is apparent that stirring, which reduces the thicknesss of the unstirred layer (11), has relatively little effect on uptake of the 7:0 and 8:0 fatty acids but has a much greater effect on the more permeant molecules.

Finally, $*P$ values for the various fatty acids were measured in the presence of a constant concentration of bile acid, 20 mM taurodeoxycholic acid. As shown in Fig. 4, tissue uptake of the 7:0 and 8:0 fatty acids was unaffected by the presence of the bile acid micelles, but as fatty acid chain length increased there was a progressive lowering of tissue uptake and, therefore, a progressive decrease in the apparent permeability coefficients for the fatty acids.

DISCUSSION

The experimental system used in these studies measures essentially instantaneous unidirectional flux rates across the brush border of the intestinal epithelial cell. With probe molecules that are absorbed by carrier-mediated, energy-linked mechanisms such as bile acid in the ileum (9) and sugars and amino acids throughout the small intestine (11),[3] this technique gives transport characteristics that show saturation kinetics, competitive phenomena among related compounds, high temperature coefficients, sensitivity to metabolic inhibition, and, commonly, dependency on sodium concentration in the bulk buffer. In contrast, with passively absorbed compounds, such as bile acids in the jejunum, mucosal uptake is linear with respect to the concentration of the probe molecule in the bulk phase, manifests no competition among related molecular species, has a low temperature dependency, and is insensitive to metabolic inhibition (9, 10). It is clear from the present studies that jejunal uptake of short- and medium-chain fatty acids in this system has all of the latter characteristics and, therefore, they presumably are absorbed by simple passive diffusion.

In this situation there are two potentially rate-limiting membranes that may regulate the rate of fatty

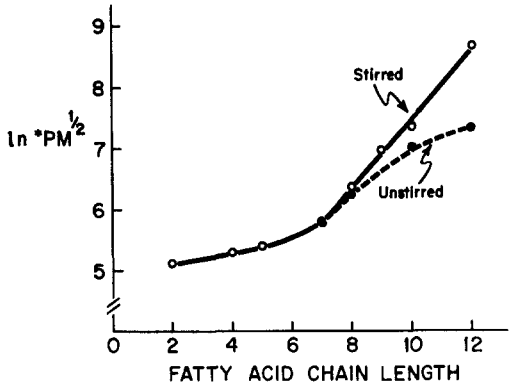

FIG. 3. Apparent permeability coefficients for fatty acids of different chain lengths under stirred and unstirred conditions. The $*P$ values listed in Table 4 have been multiplied by the square root of the molecular weight, $M^{1/2}$, and the natural logarithm of this product is plotted against fatty acid chain length. In addition to these values measured under stirred conditions, values of ln $*PM^{1/2}$ also are shown for four fatty acids measured under completely unstirred conditions. Values are means of the number of determinations given in Table 4.

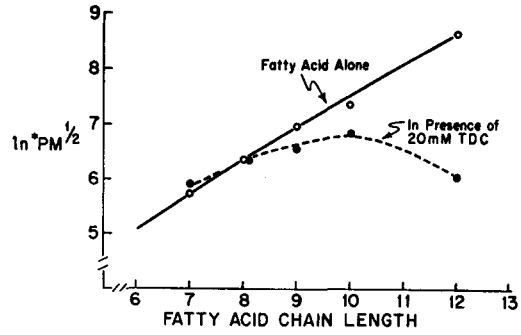

FIG. 4. Apparent permeability coefficients for the fatty acids 7:0 through 12:0 in the presence and absence of bile acid micelles. The quantity ln $*PM^{1/2}$ is plotted for the fatty acids of various chain lengths where $*P$ was determined in the absence and in the presence of 20 mM taurodeoxycholic acid. Mean values for 4–14 determinations are shown.

acid absorption: the diffusion barrier adjacent to the mucosa and the lipid membrane of the microvillus surface. Because of the prominence of anatomical structures such as mucus and glycocalyx external to the lipid cell membrane in the intestine, it is theoretically possible that such structures might play a role in the diffusion barrier. However, the initial observations in these studies that the quantity $t_{1/2} \cdot D$ is constant when electrical transients are generated by molecules of widely varying molecular weight (10-fold) and charge provide no support for this possibility. At least these findings demonstrate that these various molecules move through the diffusion barrier at the relative velocities predicted from their free diffusion coefficients in water. This observation, coupled with the fact that the mean thickness of the diffusion barrier, approximately 190μm, is compatible with values found in simpler membranes such as gallbladder (12) and even artificial membranes that have no mucus or glycocalyx (24), strongly supports the idea that the diffusion barrier in the intestine may be considered a simple unstirred water layer.

The flux (J) of fatty acids across the unstirred water layer is given by the expression

$$J = (C_1 - C_2)(D/d) \qquad \text{Eq. 2}$$

where C_1 and C_2 are the concentrations of the acid in the bulk phase and at the aqueous–lipid interface, respectively. The flux rate across the lipid cell membrane, in turn, equals

$$J = (C_2 - C_3)(P) \qquad \text{Eq. 3}$$

where C_3 equals the concentration of the fatty acid just inside the microvillus membrane and P is the true permeability coefficient of the lipid cell membrane. When movement of fatty acids across the unstirred water layer and cell membrane reaches a steady state it follows that

$$J = (C_1 - C_2)(D/d) = (C_2 - C_3)(P) \qquad \text{Eq. 4}$$

In this formulation, in effect, D/d equals the permeability coefficient of the unstirred water "membrane" while P is the permeability coefficient for the lipid membrane—both have the units cm·sec^{-1}. In extreme situations either the unstirred water layer or the cell membrane may be completely rate limiting to uptake in the cell, i.e., D/d may be very much larger than P or vice versa.

Two lines of evidence from the present study strongly suggest that the cell membrane primarily is rate limiting for short- and medium-chain fatty acid uptake. First, it can be calculated that the rate of permeation of the water membrane (D/d) is much greater than for the lipid membrane (P).[4] Thus, for example, D/d for the 2:0, 4:0, and 8:0 fatty acids equals 3.34 ×

[4] Although the mean value of d in these studies was 190 μm, we have shown in separate studies that the thickness of the unstirred layer over the sites of absorption in the upper villus under conditions of stirring employed in these studies is approximately 50 μm. In these unpublished observations (H. Westergaard and J. M. Dietschy), a new chamber has been utilized in which the thickness of two components of the unstirred water layer can be measured independently: these two components consist of a layer of water that overlies the surface of the villus tips and the unstirred layers that interdigitate between the villi. In addition, we have shown that most absorption, both passive and active, occurs in the upper villus tips and, therefore, the value of d that is most appropriate for calculation of unstirred layer effects is the thickness of the superficial component of the total unstirred water layer. The value of 50 μm, therefore, has been utilized in these studies to calculate D/d. For comparative purposes, $*P$ values also must be changed from the units nmoles·min^{-1}·100 mg^{-1}·mM^{-1} to cm·sec^{-1}. This was accomplished by dividing by S, the effective surface area of the unstirred water layer per 100 mg dry weight of jejunum (4.17 cm^2·100 mg^{-1}) (Ref. 11) times 60 sec·min^{-1}.

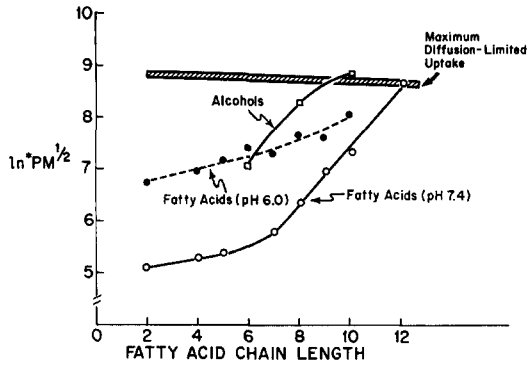

FIG. 5. Apparent permeability coefficients for fatty acids at different pH values and for alcohols. *P values were determined for the fatty acids 2:0 to 12:0 at bulk buffer pH values of 7.4 and 6.0 and for the alcohols 6:0, 8:0, and 10:0 at a pH of 7.4. These various values are plotted as ln *$PM^{1/2}$ against chain length. The shaded line is the solution to the equation $J_d = (D)\ (8.34 \times 10^5$ nmoles·cm^{-2}·100 mg^{-1}), which gives the limit for uptake, J_d, from a 1 mM solution of the various probe molecules when the unstirred water layer becomes absolutely rate limiting. For the purposes of this calculation, d equaled 0.5×10^{-2} cm and S equaled 4.17 cm^2·100 mg^{-1} (Ref. 11).

10^{-3}, 2.42×10^{-3}, and 1.90×10^{-3} cm·sec^{-1}, respectively, while *P for these same three acids equals only 8.9×10^{-5}, 8.4×10^{-5}, and 1.9×10^{-4} cm·sec^{-1}.

Second, if the unstirred water layer were rate limiting, then the quantity *$PM^{1/2}$ would be essentially constant and independent of chain length because for compounds of lower molecular weight D essentially is inversely proportional to $M^{1/2}$ (25). Clearly this is not the case, as shown in Fig. 3, since the quantity ln *$PM^{1/2}$ progressively increases with chain length. Indeed, for the fatty acids 7:0 to 12:0 there is an essentially linear relationship between these two variables. This also would be anticipated where the primary resistance to absorption is the lipid membrane. In this situation, flux into the cell primarily is determined by the permeability coefficient of the membrane; this term, in turn, is proportional to $e^{-\Delta F/RT}$, where ΔF is the partial molar free energy change associated with movement of the fatty acid from the aqueous phase to the lipid phase of the membrane. Since ΔF becomes progressively more negative with the addition of each —CH_2— group to the fatty acid molecule, it follows that the quantity ln *$PM^{1/2}$ should increase linearly with increases in chain length.

However, while these two sets of data clearly establish that the major resistance to the passive absorption of these compounds is the brush border membrane, it is also apparent that unstirred layer resistance can be detected. As is evident in Fig. 3, in the unstirred condition, where the diffusion barrier is much thicker, the *P values are consistently lower than those determined in the stirred situation. The difference between the apparent permeability coefficients in the stirred and unstirred situations becomes greater the larger the absolute value of *P. Hence, fatty acid absorption has characteristics similar to those we have described for bile acid monomer uptake (9). The lipid membrane is completely rate limiting for the slowly permeant, polar members of the series; however, for the less polar, more rapidly permeant molecules the unstirred layer begins to contribute an increasingly important proportion of the resistance to overall uptake.

Indeed, on the basis of these and other data published from this laboratory (11) it is now possible to calculate the maximum rate of passive mucosal uptake that can be achieved for any molecule in the intestine under the conditions of stirring used in these experiments. This is the extreme situation where diffusion across the unstirred water layer becomes absolutely rate limiting, and the concentration of the probe molecule approaches zero at the aqueous–lipid interface, i.e., at C_2. In this limiting situation the maximum rate of uptake (J_d) that can be achieved from a 1 mM solution in the rat jejunum is given by the equation[5]

$$(J_d) = (D)(8.34 \times 10^5 \text{ nmoles} \cdot \text{cm}^{-2} \cdot 100 \text{ mg}^{-1}) \quad \text{Eq. 5}$$

This equation has been solved for the various short- and medium-chain fatty acids and is shown as the shaded line in Fig. 5.

The apparent permeability coefficients for the ionized fatty acids under stirred conditions are also plotted in Fig. 5. Also shown are the data for fatty acid absorption where the pH has been decreased to 6.0 to enhance nonionic absorption and the three alcohols. It is apparent that in no instance was it possible to obtain a rate of absorption that significantly exceeded the theoretical maximum, although the 12:0 fatty acid and 10:0 alcohol essentially achieved their respective limiting values; thus, these data provide experimental support for the values calculated from equation 5. On the basis of these considerations a very important conclusion can be reached concerning long-chain fatty acid monomer absorption, i.e., the unstirred water layer is absolutely rate limiting to the mucosal uptake of these long-chain compounds. Equation 5, for example, would give a maximum rate of uptake of the 16:0 fatty acid from a 1 mM solution of only 324 nmoles·min^{-1}·100 mg^{-1}, yet, as can be extrapolated from the data in Fig. 3, the permeability coefficient of the lipid membrane for this compound is many times greater than this value.

Two other observations in these experiments warrant emphasis. First, as shown in Fig. 3, the *P values for

[5] In this special circumstance, equation 2 reduces to $J = C_1 D/d$. Substituting J_d/S for J gives $J = S \cdot C_1 \cdot D/d$, where S equals 4.17 cm^2·100 mg^{-1} for the jejunum (11).

the short-chain fatty acids 2:0 to 5:0 have higher values than would be predicted from a linear extrapolation of the line connecting the values for fatty acids 7:0 to 12:0. This finding is similar to that described by Diamond and Wright (26), who also reported that very polar, low molecular weight compounds consistently have higher rates of penetration across epithelial surfaces such as intestine and gallbladder than would be predicted from permeation rates of larger, less polar compounds. This finding implies that in addition to passive absorption of molecules across the lipid membrane of the mucosal cell there also is movement of small molecules through a more polar diffusion pathway in the epithelial surface.

Second, it is apparent from the data in Fig. 4 that when total fatty acid concentration is kept constant, the presence of bile acid micelles inhibits mucosal uptake. Since we have shown that at this concentration of bile acid and with this degree of stirring of the bulk phase the critical micellar concentration is achieved against the aqueous–lipid interface (9), this effect cannot be the result of the diffusion barrier limiting movement of the mixed micelle. Rather, this effect must be attributable to a decrease in fatty acid monomer activity as fatty acid is partitioned into the bile acid micelle: the longer the fatty acid chain length the greater the partitioning into the micelle and the lower the fatty acid monomer concentration at C_2. Again, this situation is identical to that which we have described in the case of bile acid uptake (9). The rate of passive absorption of these compounds appears to be determined only by the concentration of monomer in solution at the aqueous–lipid interface: fatty acid or bile acid in micelles does not contribute to the chemical gradient driving absorption.

Finally, these experimental results also yield valuable information concerning the nature of the diffusion pathway in the intestinal mucosa. After correcting the $*P$ values of the 7:0 to 12:0 fatty acids for the unstirred layer resistance, the true permeability coefficients, P, increase by an average factor of 1.78 for each —CH_2— group added to the fatty acid chain. This corresponds to an overall incremental free energy change, $\delta\Delta F_{W\rightarrow 1}$, of −1489 joules·mole^{-1} (−356 calories·mole^{-1}) and to an incremental free energy change of solution in the lipid membrane, $\delta\Delta F_1$, of only −820 joules·mole^{-1} (−196 calories·mole^{-1}).[6] This latter value for $\delta\Delta F_1$ is considerably smaller than those reported by Diamond and Wright (26) for the membrane of *Nitella* (−1882 joules·mole^{-1}), olive oil (−2092 joules·mole^{-1}), ether (−2133 joules·mole^{-1}), and isobutanol (−1548 joules·mole^{-1}) and is consistent with our previous results with bile acids (9) that indicate that the diffusion pathway for these compounds occurs through a cell membrane (or region of a cell membrane) that is relatively very polar.

This work was supported by U.S. Public Health Service research grants AM 16386 and HL 09610 and training grant AM 05490 and by a grant from the John and Mary Markle Foundation.

Manuscript received 5 September 1972 and in revised form 5 February 1973; accepted 19 March 1973.

[6] Joules = calories × 4.184.

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