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The passive permeability properties of in vivo perfused rat jejunum

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The polarity of the microvillus membrane of rat and rabbit jejunum, determined in vitro by the incremental free energy changes associated with the addition of -CH_2 groups to fatty acids or -OH groups to bile acids, has been found to be more polar than other biological membranes. The reason for the difference in polarity is unexplained but could be due to artifacts caused by the in vitro conditions. In the current studies the apparent permeability coefficients were determined for a homologous series of fatty acids and bile acids in in vivo perfused rat jejunum. The true permeability coefficients were derived by correction for diffusion barrier resistance. The incremental free energy changes associated with the addition of a -CH_2 group to a fatty acid and a -OH group to a bile acid were −619 and +2069 cal/mol, respectively. These values correspond to values determined in other biological membranes such as erythrocytes and adipocytes. Thus, the rat microvillus membrane is more nonpolar than previously observed in vitro. The reason for the discrepancy between the in vivo and in vitro results is most likely due to an underestimation of the permeability coefficients in the in vitro studies.

Introduction

The intestinal uptake of fatty acids occurs by passive permeation through the lipid moiety of the microvillus membrane. The lipid composition of the membrane in turn determines the polarity of this structure and hence the permeability properties. The polarity of the rat and rabbit jejunal microvillus membrane determined in vitro was observed to be a more polar structure than other biological membranes, despite minor differences in lipid composition [1–4]. The discrepancy could be due to a real difference in polarity among various biological membranes or could be due to an underestimation of the passive permeability coefficients in the in vitro studies which would result in an erroneous estimate of the polarity of the intestinal microvillus membrane.

The current studies were undertaken to reassess the passive permeability properties of the rat jejunum in an in vivo perfused model with a homologous series of fatty acids and bile acids as permeability probes. The polarity of the rat microvillus membrane was determined from the incremental free energy changes associated with the addition of a methylene group to a fatty acid or a hydroxyl group to a bile acid [5,6]. The derived estimates of the incremental free energy changes demonstrate that the rat microvillus membrane is more nonpolar than previously observed and corresponds in polarity to other biological membranes.

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Methods

Chemicals

The unlabeled saturated fatty acids and the unconjugated bile acids were supplied by Sigma (St. Louis, MO). The ^{14}C-labeled fatty acids were obtained from Pathfinder Laboratories (St. Louis, MO), the ^{14}C-labeled cholic acid, chenodoxycholic acid and ^{3}H-labeled poly(ethylene glycol) (M_r 4000) were from New England Nuclear (Boston, MA). The ^{14}C-labeled lithoholic acid was obtained from Amersham (Arlington Heights, IL). All other compounds were reagent grade.

Measurement of intestinal uptake

Female Sprague-Dawley rats weighing 200–225 g and maintained on a normal rat chow (Wayne Lab Blox) with free access to water were used in these studies. The rats were light-cycled (light phase 3 p.m. to 3 a.m., dark phase 3 a.m. to 3 p.m.), and the uptake studies were performed in the dark phase of the cycle on unfasted rats. The in vivo perfusion technique has been described in detail elsewhere [7]. Briefly, the rats were anesthesized with diethyl ether and nembutal, and the abdomen was opened through a midline incision. A 15 cm loop of the proximal jejunum was isolated, cannulated with tubing and replaced in the abdominal cavity. The tubings were connected with a perfusion chamber in a water bath (37 °C), and the perfusate, a modified Krebs bicarbonate buffer, was recirculated through the isolated loop by means of a peristaltic pump at a perfusion rate of 5 ml/min. The composition of the modified Krebs bicarbonate buffer was 125 mM NaCl/5 mM KCl/25 mM $NaHCO_3$. The perfusate was gassed with 95% O_2/5% CO_2 to maintain pH at 7.40. The loop was first perfused for 30 min with buffer alone, after which the perfusate was exchanged with the experimental solution, i.e., buffer with the unlabeled and labeled probe molecules at appropriate concentrations. ^{3}H-labeled poly(ethylene glycol) was added in trace quantities as a volume marker to correct for net water movement. The perfusate was recirculated for 60 min and duplicate 50 μl samples were removed at 10 min intervals and added to 15 ml scintillation solution. The intestinal loop was removed at the end of the experiment, and the length was measured. The ^{3}H and ^{14}C radioactivity was counted in a Beckman LS6800 with automatic quench correction. The ^{14}C dpm/ml of the probe molecules, corrected for water movement by ^{3}H-labeled poly(ethylene glycol), was plotted against time and the best fit of the slope was obtained by linear regression analysis. The negative slope is the disappearance rate of the probe molecules and the rate of uptake was calculated from the disappearance rate (in dpm/ml per min) divided by the specific activity of the probe molecules and the length of the perfused loop. The results are expressed in nmol solute absorbed per min per cm length of the intestine (nmol/min per cm).

Results

Fatty acids and bile acids are weak acids, and the rate of intestinal uptake is critically dependent on the perfusate pH and the buffer capacity of the perfusate. Initial studies were performed to validate the use of a modified Krebs bicarbonate buffer as the perfusate buffer in intestinal perfusion studies. The rate of uptake of 2 mM octanoic acid (8 : 0) at pH 7.40 was measured with two different perfusate buffers, the modified Krebs bicarbonate buffer and a Hepes (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer (50 mM Hepes/125 mM NaCl). The Hepes buffer was gassed with nitrogen, and the pH remained at 7.40 $\pm$ 0.20 in both buffers during the perfusions. The rate of uptake of octanoic acid was 27.5 $\pm$ 1.2 nmol/min per cm from the Krebs bicarbonate buffer and 25.9 $\pm$ 2.9 nmol/min per cm from the Hepes buffer. The difference between the two values is not significantly different. Thus, the modified Krebs bicarbonate buffer has been used as the perfusate buffer in the subsequent studies.

Next, the rate of uptake of a homologous series of saturated fatty acids (chain length: 4 : 0, 6 : 0, 8 : 0, 9 : 0, 10 : 0 and 12 : 0) was measured as a function of increasing solute concentration in the perfusate (Fig. 1). The concentrations of the individual fatty acids were kept within their aqueous solubility limits at pH 7.40 and 37 °C. The rate of uptake (J_d) was linear with concentration for all six fatty acids and the slopes intercepted at the origin as expected for passively permeable compounds. The slope for 12 : 0 is not depicted in Fig.

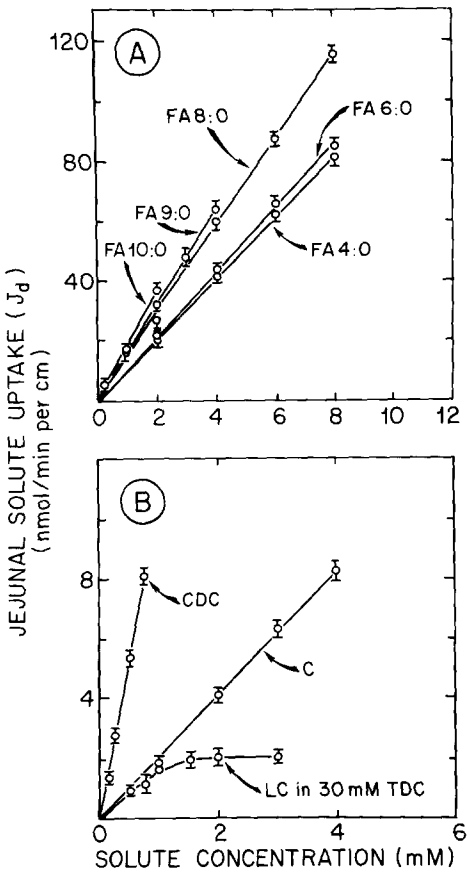

Fig. 1. Panel A. The rates of uptake (J_d) of 4:0, 6:0, 8:0, 9:0 and 10:0 as a function of solute concentration in the perfusate. The curve for 12:0 is not depicted as it overlaps 10:0. All experiments were performed at a perfusion rate of 5 ml/min. The points are the mean ± S.E. for four to eight rats. Panel B. The rates of uptake of cholic (C), chenodeoxycholic (CDC) and lithocholic (LC) acid as a function of perfusate concentration. Lithocholate uptake was measured in a micellar solution of 30 mM taurodeoxycholate (TDC) due to the low aqueous solubility of lithocholic acid.

1 because it overlaps with the slope of 10:0. The slopes of the lines were determined by linear regression analysis and the slope constants correspond to the apparent permeability coefficients ($*P$) in nmol/min per cm per mM. The calculated apparent permeability coefficients for the six fatty acids are listed in Table I. The permeability of 4:0 and 6:0 is of the same magnitude but further elongation of the fatty acid chain length by the addition of $-CH_2$ groups results in progressively steeper slopes (Fig. 1) because each additional $-CH_2$ group increases the membrane permeability by a constant factor [5,6]. However, the apparent permeability coefficient of 12:0 is identical to that of 10:0 despite the addition of two $-CH_2$- groups. The lack of further increase in the rate of uptake of 12:0 indicates that this fatty acid is diffusion limited by the diffusion barrier resistance in the perfused rat jejunum at a perfusion rate of 5 ml/min. The apparent permeability coefficients are uncorrected for this resistance, and thus the rate of uptake of diffusion limited compounds will reach a constant value [3,7].

Finally, the effect of addition of -OH groups was studied with the three unconjugated bile acids: cholic, chenodeoxycholic and lithocholic acid which differ only in the number of hydroxyl groups. The rate of uptake of each bile acid was again measured as a function of perfusate concentration as shown in Fig. 1. The concentration range for the individual bile acids was kept below the critical micellar concentration and the pH was maintained at 7.40 ± 0.20 in order to avoid bile acid precipitation. Lithocholic acid has a very low aqueous solubility estimated at 1 μM [8]. Thus, it is not possible to measure lithocholic acid uptake as a function of concentration from an aqueous solution. The lithocholic acid uptake studies were therefore performed with a micellar solution where lithocholate in increasing concentrations was dissolved in a 30 mM taurodeoxycholic acid solution. The solutions with the highest lithocholate concentrations (2.0 and 3.0 mM) were supersaturated with visible crystals at the air/water interface. The rates of uptake of cholic and chenodeoxycholic acid are both linear with concentration (Fig. 1), and it is apparent that the slope of chenodeoxycholic acid uptake is considerably steeper than the slope of cholic acid uptake. The rate of uptake of lithocholic acid from a micellar solution, however, is not linear but displays pseudosaturation kinetics. The plateau in the rate of uptake is presumed to reflect that a maximal aqueous concentration has been achieved at the microvillus membrane interface, and hence, the rate of uptake remains constant [9]. The apparent permeability coefficients for cholic and chenodeoxycholic acid are listed in Table I, whereas that of lithocholic acid cannot be determined because the aqueous concentration in the perfusate is unknown.

TABLE I

THE APPARENT AND TRUE PASSIVE PERMEABILITY COEFFICIENTS

The apparent permeability coefficients (*P) for the fatty acids and bile acids were determined from the slope of the concentration curves (Fig. 1) by linear regression analysis except for lithocholic acid as explained in the Results section. The values are the mean ± S.E. The true permeability coefficients (P) were obtained by correcting the apparent permeability coefficient for diffusion barrier resistance. The units for both sets of permeability coefficients are nmol/min per cm per mM.

	*P	P
Butyric (4:0)	10.3 ± 0.6	18.7
Hexanoic (6:0)	10.8 ± 0.4	22.1
Octanoic (8:0)	14.6 ± 0.5	70.0
Nanonoic (9:0)	15.9 ± 0.8	194.7
Decanoic (10:0)	16.0 ± 0.7	523.1
Dodecanoic (12:0)	15.1 ± 0.9	-
Cholic acid	2.1 ± 0.3	2.6
Chenodeoxycholic acid	10.8 ± 0.5	72.9
Lithocholic acid	-	2070

Discussion

The purpose of the current studies was to characterize the passive permeability properties of the rat jejunal microvillus membrane during in vivo perfusion. The results of previous in vitro studies of rat and rabbit microvillus membrane permeability from this laboratory led to the conclusion that the rat and rabbit microvillus membrane were more polar than other biological membranes [1–3]. However, the determined values of the apparent permeability coefficients in these studies may be underestimated for the following reasons. First, the Krebs bicarbonate buffer utilized in these studies has a low buffering capacity as compared to synthetic buffers. Second, there may be tissue accumulation of the probe molecules during in vitro incubation and hence dissipation of the concentration gradient. Both of these factors will result in an erroneously low rate of uptake of the probe molecules and thus cause an underestimation of the apparent permeability coefficients [10].

The initial studies were conducted in order to validate the use of a modified Krebs bicarbonate buffer in intestinal perfusion experiments. The rate of uptake of 2 mM octanoic acid from a Krebs bicarbonate buffer or from a Hepes buffer at pH 7.40 was not significantly different. Thus, both buffers appear to maintain similar pH gradients across the diffusion barriers. It has been claimed that the reactions between CO_2 and bicarbonate in the Krebs bicarbonate buffer are too slow to maintain a constant pH across the diffusion barriers [10]. If this is the case, then the rate of uptake of octanoic acid would be lower from a Krebs bicarbonate buffer as compared to a Hepes buffer, and the apparent permeability coefficient would be underestimated. However, the fact that the rate of octanoic acid uptake was almost identical in both buffers indicates that a similar pH gradient was maintained with both buffers up to the microvillus membrane interface. The interface region appears to constitute an acid microclimate with a constant pH of about 6.0–6.5 which is maintained despite large variations in bulk perfusate pH [11].

These preliminary studies allow the conclusion that the utilization of a Krebs bicarbonate buffer in intestinal fatty acid uptake studies does not result in an underestimation of apparent permeability coefficients. Furthermore, the use of a Krebs-bicarbonate buffer in the previous in vitro studies cannot explain the observed difference in polarity between microvillus membranes and other biological membranes.

Next, the rate of uptake of a homologous series of saturated fatty acids and three unconjugated bile acids was measured as function of concentration at a constant perfusion rate and at a constant pH. The apparent permeability coefficients for the

fatty acids and bile acids were determined from the slopes of the concentration curves except for lithocholic acid as explained in the Results section (Table I). The apparent permeability coefficients ($*P$) are uncorrected for diffusion barrier resistance and are, therefore, underestimates of the true membrane permeability coefficients. The permeability properties of the rat microvillus membrane can only be defined by means of the true permeability coefficients which can be obtained by correction of the apparent permeability coefficients for diffusion barrier resistance. The diffusion barrier resistance was determined by first identifying diffusion limited compounds at a perfusion rate of 5 ml/min. The rate of uptake of diffusion limited compounds is essentially determined by the rate of diffusion across the diffusion barriers and the apparent permeability coefficient divided by the diffusion coefficient (D) will reach a constant value for these compounds [7]. The ln $*P/D$ for 12:0 was 3.50 which is identical to the value for 10:0 (3.46) and defines 12:0 as a diffusion-limited compound.

It is assumed that the concentration of probe molecules at the membrane interface (C_2) approaches zero for diffusion limited compounds and the rate of uptake (J_d) is determined by the equation [7]:

$$J_d = C_1 \frac{D}{R} \tag{1}$$

where C_1 is the perfusate concentration of the probe molecules, D is the aqueous diffusion coefficient and R is the resistance of the diffusion barriers. The resistance term (R) is determined by the thickness (d) and surface area (S_w) of the barriers overlying the villus tips and the magnitude of the resistance depends mainly on the perfusion rate [7]. The apparent permeability coefficient ($*P$) equals J_d/C_1, and thus:

$$*P = \frac{D}{R} \tag{2}$$

Thus, after identifying diffusion limited compounds the value of the resistance term can easily be calculated by rearranging Eqn. 2:

$$R = \frac{D}{*P} \tag{3}$$

The calculated resistance of the diffusion barriers in the perfused rat jejunum determined with the apparent permeability coefficient of 12:0 was found to be 0.031 ± 0.001 ($n = 26$) at 5 ml/min. The apparent permeability coefficients of the homologous series of fatty acids were corrected for diffusion barrier resistance in order to obtain the true permeability coefficients (P) by means of the equation [12]:

$$\frac{1}{P} = \frac{1}{*P} - \frac{R}{D} \tag{4}$$

The values of the true permeability coefficients for 4:0 to 10:0 are listed in Table I. Since 12:0 is diffusion limited, the true permeability coefficient cannot be determined. The natural logarithm of the true permeability coefficients of the fatty acids have been plotted against chain length in Fig. 2. The plot should demonstrate a log-linear relationship if all fatty acids permeated only through the lipid moiety of the microvillus membrane. A linear relationship is observed only for 8:0 through 10:0 with a slope constant of 2.75, i.e., the addition of a $-CH_2$ group to a fatty acid increases the permeability 2.75-fold in in vivo perfused rat jejunum. The linear regression line has been extended (dashed line) to illustrate two points. First, 4:0 and 6:0 are more permeable than predicted from the linear relationship which has also been observed in other biological membranes [13]. The deviation from linearity is thought to reflect that these smaller molecules also permeate through more polar pathways in the membrane. The anomalous behavior of 4:0 and 6:0 is not observed in erythrocytes or pure phospholipid membranes where the linear relationship is maintained down to 2:0 [10,14]. Second, the extension of the linear slope beyond 10:0 (dashed line) permits the derivation of the true permeability coefficients for 12:0 and the longer chain fatty acids.

The polarity of a biological membrane can be defined by determining the incremental free energy change associated with the addition of substituent groups to the probe molecules as described by Diamond and Wright [5]. The incremental free energy change ($\delta\Delta F_{w\rightarrow l}$) associated with the addition of a $-CH_2$ group (or removal of a -OH group) was calculated from the relationship

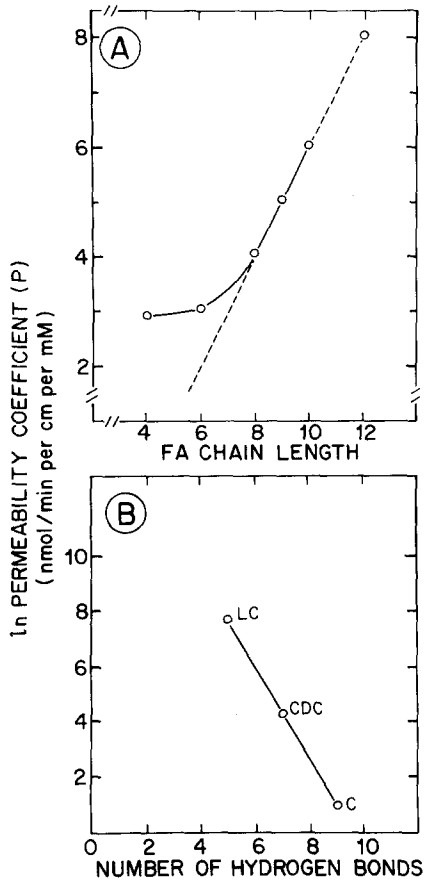

Fig. 2. Panel A. The natural logarithm of the true permeability coefficients of a homologous series of fatty acids (FA) (Table I) plotted as a function of chain length. The dashed line is extrapolated from the linear part of the slope. Panel B. The natural logarithm of the true permeability coefficients of three unconjugated bile acids: cholic (C), chenodeoxycholic (CDC) and lithocholic acid (LC) plotted as a function of the number of hydrogen bonds each bile acid can form.

[5,6]:

$$\delta\Delta F_{w\to l} = -RT \ln \frac{P_i}{P_o} \qquad (5)$$

where P_i and P_o are the true permeability coefficients for two fatty acids that differ only by one $-CH_2$ group. The incremental free energy change calculated from the linear slope of Fig. 2 corresponds to -619 cal/mol which is considerably more negative than previously found in rat jejunum (-356 cal/mol) or rabbit jejunum (-258 cal/mol) in vitro in our laboratory [1,3]. In three more recent in vitro studies where the permeability characteristics of the rat jejunal microvillus membrane were determined with different techniques, the incremental free energy change associated with the addition of a $-CH_2$ group ranged from -545 to -695 cal/mol [15–17]. The most negative value was obtained in studies of long-chain fatty acid uptake into everted jejunal sacs from saturated micellar solutions where the bile acid micelles serve to overcome diffusion barrier resistance similar to the lithocholic acid uptake studies (Fig. 1). The $\delta\Delta F_{w\to l}$ of -619 cal/mol determined in in vivo perfused rat jejunum is in perfect agreement with these in vitro studies which allows the following conclusions. First, the microvillus membrane of the rat jejunum is more hydrophobic than previously claimed. Second, the less negative $\delta\Delta F_{w\to l}$ value previously determined in vitro in our laboratory must be an underestimate. Pure phospholipid membranes are even more hydrophobic with $\delta\Delta F_{w\to l}$ values in the range from -800 to -900 cal/mol [10]. The difference in incremental free energy values between biological membranes and phospholipid membranes may be due to several factors. First, biological membranes are more complex in regard to composition than pure phospholipid membranes. In general, the lipid components of the plasma membrane are various phospholipids, cholesterol and sphingolipids with a cholesterol/phospholipid ratio of 0.7 to 0.8 [4]. The presence of cholesterol in the membrane in a relative high ratio imposes a more ordered (less fluid) membrane and, hence, less permeable than a pure phospholipid membrane. Second, the linear slope of the relationship between ln P and chain length is determined from only three points (8 : 0, 9 : 0 and 10 : 0) because of the relatively large diffusion barrier resistance in perfused rat jejunum. The steepness of the slope is sensitive to an accurate estimate of the diffusion barrier resistance which determines the calculated value of the true permeability coefficients. An underestimation of diffusion barrier resistance will result in a more shallow slope and hence a less negative value for incremental free energy change. However, the general agreement between the values for incremental free energy change in rat jejunum determined with different techniques argues for a reasonable accurate estimate of the resistance term in the current studies.

Next, the effect of the removal of a hydroxyl group on membrane permeability was investigated with the three unconjugated bile acids: cholic, chenodeoxycholic and lithocholic acid, which are tri-, di- and monohydroxy bile acids, respectively. The apparent permeability coefficients of cholic and chenodeoxycholic acid were determined from the slopes of the concentration curves (Fig. 1) and are listed in Table I. It is apparent that the removal of one hydroxyl group (cholic → chenodeoxycholic acid) results in a considerable increase in the rate of uptake (Fig. 1). The apparent permeability coefficient for lithocholic acid could not be determined due to the very low aqueous solubility, and the true permeability coefficient was determined from the plateau phase of the rate of uptake (2.07 ± 0.09 nmol/min per cm) divided by its maximal aqueous solubility (1 μM) and equals 2070 ± 90 nmol/min per cm per mM. The true permeability coefficients for cholic and chenodeoxycholic acid were determined by means of Eqn. 4 and are listed in Table I. The natural logarithm of the true permeability coefficients of the three bile acids have been plotted against the number of hydrogen bonds each bile acid can form (Fig. 2) [2]. The bile acid permeability coefficients are log-linearly related to the degree of hydrogen bonding with a steep negative slope ($k = -28.5$). Thus, the addition of one hydroxyl group to a bile acid decreases membrane permeability by a factor of 28.5 which corresponds to an incremental free energy change of +2069 cal/mol. This value is also at variance with previous observations using everted rat jejunum sacs where the addition of a hydroxyl group to a bile acid only reduced permeation by a factor of 4.1 ($\delta\Delta F_{w\to l}$: +874 cal/mol) [2].

The incremental free energy changes associated with the addition of a methylene or a hydroxyl group have now been determined in several biological tissues and solvents as listed in Table II. The $\delta\Delta F_{w\to l}$ values determined in rat jejunum in four different studies are within the same range and correspond to values determined in rat adipocytes, rat myocytes, human erythrocytes and Nitella mucronata. Excluding the first two lower values, the mean $\delta\Delta F_{w\to l}$ value for $-CH_2$ groups from the last eight studies averages -606 ± 18 cal/mol. The $\delta\Delta F_{w\to l}$ of −547 cal/mol for rat adipocytes is probably an underestimate due to the anomalous high permeability of the shorter chain fatty acids (2 : 0 to 6 : 0). When 5 : 0 and 6 : 0 are excluded from the regression analysis the calculated $\delta\Delta F_{w\to l}$ is −638 cal/mol for rat adipocytes. These observations allow several important conclusions. First, biological membranes with widely different functions such as erythrocytes, myocytes, adipocytes and enterocytes have very similar lipid permeability properties which may reflect an inherent similar lipid composition of the plasma membrane. The close correlation among $\delta\Delta F_{w\to l}$ values for the $-CH_2$ groups in various cell types indicates that these membranes have the same selectivity for fatty acid chain length and is indicative of a simple membrane diffusion process. The $\delta\Delta F_{w\to l}$ reflects the energy necessary to transfer a fatty acid molecule from the aqueous interface into the lipid cell membrane. The rate-limiting step in the transfer process may be the transition from an aqueous to a lipid phase or membrane diffusion, but the current studies cannot discriminate between the two possibilities.

TABLE II

INCREMENTAL FREE ENERGY VALUES FOR METHYLENE AND HYDROXYL GROUPS IN MEMBRANES AND SOLVENTS

The $\delta\Delta F_{w\to l}$ values for methylene and hydroxyl groups in various membranes and solvents were obtained from the cited references.

	$\delta\Delta F_{w\to l}$ (cal/mol)		
	$-CH_2-$	-OH	Ref.
Membranes			
rabbit jejunum	−258	+564	3
rat jejunum	−356	+874	1
rat jejunum	−545	–	15
rat jejunum	−589	–	16
rat jejunum	−619	+2069	This study
rat jejunum	−695	–	17
rat adipocyte	−547	+1225	13
erythrocytes	−593	–	14
rat myocytes	−650	–	18
Nitella mucronata	−610	+3600	5
Solvents			
adipocyte triacylglycerol	−830	+2070	13
ether	−670	+2100	5
olive oil	−660	+2800	5

Second, the microvillus membrane of rat jejunum is a more nonpolar structure than previously found with lipid solubility properties that corresponds to partitioning into an organic solvent like diethyl ether (Table II).

The degree of membrane hydrophobicity will determine how the addition of a $-CH_2$ group will influence the maximal rate of uptake of a fatty acid which is the product of the maximal aqueous solubility and the true permeability coefficient of a particular fatty acid. The ln maximal aqueous solubility and the ln permeability coefficients for a homologous series of fatty acids are illustrated in Fig. 3, panel A. The maximal aqueous solubility of a series of saturated fatty acids was determined in a previous study [9], whereas the true permeability coefficients are from the current study and derived from the linear slope of Fig. 2, panel A. The anomalous high permeability coefficients of the shorter chain 4 : 0 and 6 : 0 have been ignored. The solubility decreases by a factor of 2.32 per added $-CH_2$ group, whereas the permeability increases by 2.75. The maximal rate of uptake, shown by the full line in Fig. 3, panel B, increases with chain length, since the addition of a $-CH_2$ group has a greater effect on membrane permeability than on aqueous solubility, contrary to previous observation in rabbit jejunum [19]. The incremental free energy change for a $-CH_2$ group in rabbit jejunum was only -258 cal/mol which probably is an underestimate, since the lipid composition of the rabbit microvillus membrane is almost identical to the rat microvillus membrane [4]. The true permeability coefficients are corrected for diffusion barrier resistance which means that the log-linear increase in the maximal rate of uptake of a homologous series of fatty acids will only be observed in the absence of a diffusion barrier resistance. In the in vivo perfused rat jejunum, which has an appreciable diffusion barrier resistance at a perfusion rate of 5 ml/min, 12 : 0 and longer fatty acids are diffusion limited. In the presence of the determined diffusion barrier resistance at this perfusion rate the maximal rate of uptake of fatty acids as a function of chain length describes a rather steep negative slope (dashed line in panel B, Fig. 3), where the addition of a $-CH_2$ group decreases the maximal rate of uptake by a factor of 2.44. It should also be noted that the two lines

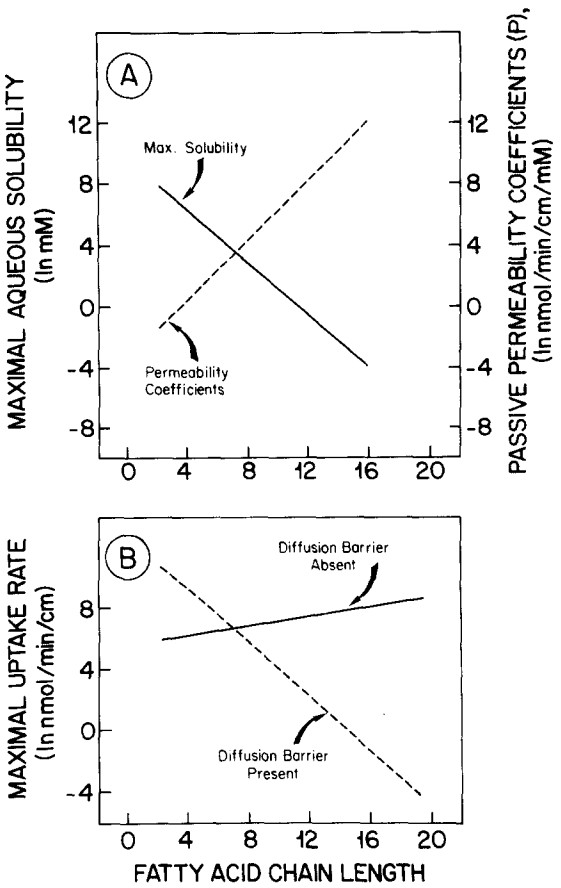

Fig. 3. Panel A. The solid descending line is the natural logarithm of the maximal aqueous solubility (left y-axis) of a homologous series of fatty acids plotted as a function of chain length. The line is defined by ln $y = 9.72 - 0.84\ x$ and the slope constant equals $2.32/CH_2$ group. The dashed ascending line is the natural logarithm of the true permeability coefficients for the same series of fatty acids and corresponds to the linear part of the slope in Fig. 2. The line is defined by ln $y = -3.76 + 1.01\ x$, and the slope constant equals $2.75/CH_2$ group. Panel B. The solid line illustrates the maximal rate of uptake of fatty acids as a function of chain length in the absence of diffusion barriers. The maximal rate of uptake is defined as the product of the maximal solubility and the true permeability coefficient for each fatty acid as derived from the linear regression equations in panel A. The dashed line illustrates the maximal uptake rate in the presence of the determined diffusion barrier resistance in perfused rat jejunum. These values were determined from the product of the apparent permeability coefficients and the maximal aqueous solubility for each fatty acid.

in Fig. 3, panel B intersects at 7 : 0 and deviates progressively as fatty acid chain length is lengthened. For example, in the presence of diffusion barriers the maximal uptake rate of 16 : 0 is

only 0.26 nmol/min per cm but removal of the diffusion barrier increases the maximal uptake rate to 4272 nmol/min per cm. The paradoxical finding that the short-chain fatty acids are more permeable in the presence of diffusion barriers than in their absence is due to the fact that these fatty acids permeate both through the lipid membrane and through more polar pathways. The solid line in Fig. 3 panel B only reflects permeation through the lipid moiety of the microvillus membrane. Finally, it should be emphasized that the rat jejunum is bathed in a micellar solution of bile acids during lipid digestion and absorption. The primary function of the bile acid micelles is to solubilize the water insoluble long-chain fatty acids released by lipolysis and transport the fatty acid across the diffusion barriers up to the microvillus membrane and thus maintain a certain aqueous concentration at the membrane interface. In the presence of increasing concentrations of bile acid micelles in the perfusate, the dashed line in Fig. 3, panel B, will approach the solid line and reach the line when a maximal aqueous concentration of a fatty acid is maintained at the interface. Thus, another important function of bile acid micelles is to overcome the diffusion barrier resistance in the intestine.

The effect of the addition of hydroxyl groups is less explored in biological membranes. The two previously determined values for incremental free energy change for -OH groups in rat jejunum and rat adipocytes in vitro were +854 and +1225 cal/mol, respectively (Table II). In contrast, the estimate from the current in vivo studies is much larger (+2069 cal/mol) and is of the same magnitude as the estimates for partitioning into the triacylglycerol core of adipocytes or into an ether solvent phase.

The discrepancy between the $\delta\Delta F_{w\rightarrow l}$ values for the $-CH_2$ and -OH group obtained in the previous in vitro studies in our laboratory and the results obtained in the current in vivo perfusion studies of rat jejunum may be due to several factors. First, the diffusion barrier resistance determined in vitro may be too low, which will result in underestimation of the true permeability coefficients and thus a more shallow slope in the relationship between ln P and fatty acid chain length. The determined diffusion barrier resistance in the in vitro studies, however, agrees with the resistances determined with similar preparations in other studies (Table II, Ref. 20). Second, the apparent permeability coefficients determined in vitro may be underestimated because of tissue accumulation of the probe molecules during the incubation with dissipation of the concentration gradient. An underestimation of the apparent permeability coefficients will also cause an underestimation of the calculated true permeability coefficients and hence the determined incremental free energy changes will be too low. In preliminary experiments the rate of uptake of octanoic acid was compared in parallel studies in vivo and in dead rats to simulate the in vitro condition. The rate of uptake in the dead rats was only one-third of the measured rate in the in vivo perfused rats and uptake ceased after 20 min perfusion. These observations substantiate the argument that octanoic acid accumulates rapidly during in vitro perfusion which results in significantly lower uptake rates as compared to in vivo perfusion even during short perfusion periods.

The important conclusion from these studies of the permeability properties of the in vivo perfused rat jejunum is that the microvillus membrane is considerably less polar than previously claimed, and in fact, is of the same polarity as other nonepithelial cell membranes. The physiological implications of a more nonpolar cell membrane is that lipid absorption may occur at a faster rate and that the maximal rate of uptake of fatty acids increases with chain length in the presence of bile acid micelles. Lastly, the discrepancy between the in vitro and the in vivo results is of concern and warrants a reappraisal of the in vitro studies.

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