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Lipid permeability of rat jejunum and ileum: correlation with physical properties of the microvillus membrane

Jonathan B. Meddings

Gastrointestinal Research Group, Department of Internal Medicine, University of Calgary, Calgary, Alberta (Canada)

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It is now generally accepted that dietary lipids permeate through the cholesterol-phospholipid bilayer of the intestinal microvillus membrane during the process of intestinal absorption. Therefore, it has been assumed that rates of lipid permeation depend upon the physical properties of the microvillus membrane. In this study the lipid permeability properties of the microvillus membrane were compared in two regions of the intestine, jejunum and ileum. Since the jejunum is exposed to the majority of dietary lipid it would be reasonable to suppose that it would be more efficient at lipid absorption. The ileum was found to be less permeable to all fatty acids examined and this could be correlated with increased rigidity of ileal microvillus membrane vesicles measured with multiple fluorescent probes. Differences in membrane fluidity were found in both the outer third and central regions of the bilayer. When measurements of membrane fluidity were performed either in the presence or the absence of fatty acids, it could be demonstrated that these acids perturb the physical properties of the outer region or the membrane. Therefore, this suggests that the rate-limiting step in fatty acid permeation may be localized to the outer third of the bilayer. Pharmacologic or dietary manipulations attempting to alter rates of lipid permeability should, therefore, be directed towards altering the physical properties of this region of the microvillus membrane.

Introduction

It has become clear that in order for lipids to enter the intestinal epithelial cell two barriers must be crossed. First, the aqueous diffusion barrier that lies external to the microvillus membrane and secondly, the microvillus membrane itself [1,2]. Depending upon experimental conditions either barrier may become rate limiting for intestinal lipid absorption. The majority of lipids cross cell membranes by a process of simple diffusion without the aid of a protein carrier. The most important points suggesting this are: (a) rates of lipid absorption are a linear function of concentration in most studies, (b) competition between lipids for absorptive sites has not been convincingly demonstrated and (c) for any given membrane, the membrane permeability coefficient for a series of lipid probes predictably increases with the hydrophobicity of the probe [1,3–7]. Although lipid binding proteins do exist in the enterocyte [8] it is difficult to reconcile the above observations with the suggestion that these proteins mediate the transmembrane movement of lipids, especially for fatty acids and cholesterol. Therefore, the rate of lipid movement across the

Correspondence: J.B. Meddings, Gastrointestinal Research Group, Department of Internal Medicine, University of Calgary, 1403-29 Street N.W., Calgary, Alberta, Canada T2N 2T9.

microvillus membrane should be dictated by the physical properties of the cholesterol-phospholipid bilayer which represents a significant fraction of the membrane. During the normal process of digestion the jejunum absorbs the majority of dietary lipid and would, therefore, be expected to be more efficient at lipid absorption than the ileum. In this study rates of jejunal and ileal fatty acid absorption are quantitated in the rat and correlated with the physicochemical properties of microvillus membrane isolated from these intestinal regions. It is demonstrated, in agreement with other studies, that the physical properties of the microvillus membrane differ between these regions of the intestine. Furthermore, when fatty acids permeate through microvillus membrane vesicles, in vitro, it is shown that they perturb the physical properties of a specific region of the membrane.

Materials and Methods

Chemicals. Unlabelled fatty acids were obtained from either Sigma (St. Louis, MO) or Nu Chek (Elysian, MN) and their purity established by gas-liquid chromatography (GLC). Any lots found to be less than 99% pure were discarded. [^{3}H]Polyethylene glycol and [^{14}C]dodecanoic acid (12:0) were obtained from New England Nuclear (Boston, MA) and used as supplied. The fluorescence probes 1,6-diphenyl-1,3,5-hexatriene (DPH) and seven isomers of anthroyloxy stearic acid were purchased from Molecular Probes (Junction City, OR) and also used as supplied. All other reagents and chemicals were of the highest grade available.

Perfusion studies. Rates of fatty acid absorption were measured by a recently established technique, based upon a previous perfusion system [12]. Briefly, following anesthesia with pentobarbital (65 mg/kg intraperitoneally) a 10–15 cm loop of intestine was fashioned either just distal to the ligament of Treitz or just proximal to the ileocecal valve in female Sprague-Dawley rats (200–225 g). The ends of the loop were cannulated with teflon tubing and returned to the abdominal cavity. During an equilibration period of 20 minutes, the loop was perfused in a closed reperfusion system with buffer alone, (mM) NaCl (150), KCl (5), Hepes (50), pH 7.5. The infusate was then changed to a solution identical to the buffer but containing eight fatty acids (μM), 5:0 (800), 7:0 (400), 8:0 (400), 9:0 (200), 10:0 (180), 11:0 (100), 13:0 (60), and trace quantities of [^{14}C]12:0. The perfusion continued for 48 min at a rate of 5 ml/min and aliquots of the perfusate were regularly obtained for the determination of fatty acid concentrations as well as of the nonabsorbable marker, [^{3}H]polyethylene glycol. Rates of fatty acid absorption for each probe were calculated from the linear fall in concentration of each fatty acid during the course of the perfusion. Absorption rates were normalized to both the length of the perfused loop and the driving concentration gradient, assumed to be the geometric mean concentration of the probe during the perfusion, as described by Winne and Markgraf [9]. The resultant value is defined as the apparent permeability coefficient, P^*, and represents the nmoles of probe absorbed per min per cm length of intestine per mM concentration gradient.

Diffusion barrier resistance was measured by the diffusion limited probe method [10–12]. This value, established in each animal, allowed derivation of the true membrane permeability coefficient, P, for fatty acid probes 5:0 through 10:0 which were then used to characterize the lipid permeability properties of the microvillus membrane. The interfacial concentration, C_2, of each probe during the course of the perfusion was calculated and assumed to approximate the transmembrane concentration gradient [12,13].

$$C_2 = C_1 - \frac{Jd}{DS_w} \tag{1}$$

In this expression C_1 represents the concentration of probe in the bulk phase, J represents the absorption rate of the probe, D its aqueous diffusion coefficient, d the thickness of the diffusion barrier and S_w its surface area. The ratio d/S_w is a measure of the resistance of this barrier. The concentration of unlabelled fatty acids were determined by gas-liquid chromatography (GLC) while [^{14}C]12:0 and [^{3}H]polyethylene glycol concentrations were quantitated by standard liquid scintillation techniques. Free fatty acids were extracted into chloroform from aliquots of perfusate following acidification with hydrochloric acid and

saturation of the aqueous phase with sodium sulfate. Extraction rates varied between 95–100% with this technique and the inclusion of two internal standards, fatty acids 6:0 and 12:0, allowed determination of all fatty acid concentrations with a precision of ±2% over the concentration range used (data not shown). Furthermore, rates of absorption for all fatty acids were identical whether determined from this perfusate or from a perfusate containing a single fatty acid, thereby excluding significant interactions between these substrates either at the level of the membrane or within the perfusate. All GLC analyses were performed using a Hewlett-Packard 5890 GLC system (Hewlett-Packard, Avondale PN) and quantitated with a Hewlett-Packard 3390 integrater coupled to a FID detector. Free fatty acids from the perfusion were separated on a packed column containing GP 10% SP-216-PS (Supelco, Bellafonte PA). Two internal standards, fatty acids 6:0 and 12:0, were used to correct for extraction variability.

Besides quantitating the permeability properties of the microvillus membrane to fatty acids these data can also be used to calculate the relative hydrophobicity of the microvillus membrane. There exists a log-linear relationship between the membrane permeability coefficients of a series of lipid probes and the hydrophobicity of the probe, i.e. chain length for a series of fatty acids [3,11–13]. This relationship holds true for the longer chain length members of the fatty acid series. However, in most studies the shorter chain length members appear to be anomalously permeable suggesting the presence of accessory transmembrane pathways. In the present system it has previously been shown that fatty acid probes 9:0 and 10:0 are not anomalously permeable so that the permeability coefficients for these probes can be used to define the change in incremental free energy that occurs when a methylene group partitions from the aqueous luminal environment into the membrane, $\delta\Delta F_{w\rightarrow l}$ [11,12].

$$\delta\Delta F_{w\rightarrow l} = -RT \ln\left(\frac{P_+}{P_-}\right) \quad (2)$$

In this equation R represents the gas constant, T the absolute temperature, and P_+ or P_- the permeability of the probe with and without the additional methylene group, respectively. The units become cal/mole and a more hydrophobic membrane, i.e. one having a steeper relationship between permeability and chain length, would have a more negative value for this term.

Membrane isolation. Microvillus membrane vesicles were prepared from both jejunal and ileal mucosal scrapings using a previously published method [14]. Purification was assessed by the concentration of sucrase specific activity [15] and for all preparations was similar, ranging from 15- to 20-fold. Basolateral contamination, assessed by the marker enzyme Na^+/K^+-ATPase was not detected. Compositional analysis was normalized to the protein content of the vesicles determined by the method of Bradford [16]. Membrane lipids were extracted by the method of Folch. Cholesterol content was quantitated by GLC using a Supelco SP-2250 packed column with stigmasterol as the internal standard. Membrane lipids were saponified prior to extraction to facilitate analysis of total cholesterol. Total phospholipids were assayed by the method of Ames and Dubin [17]. Brush-border membrane fatty acid methyl esters were prepared from lipid extracts of the vesicle preparation by the method of Morrison and Smith [18]. These were separated and quantitated by GLC on a Supelcowax 10 capillary column using a temperature programmed run from 170°C to 240°C. Under these conditions fatty acid methyl esters identified from standard mixtures ranged from 14:0 to 22:0. Authentic standards were used to identify all compounds quantified by GLC.

Fluorescence polarization studies. Eight fluorescent probes were used; 1,6-diphenyl-1,3,5-hexatriene (DPH), and seven isomers of stearic acid labelled with 9-anthroyloxy groups at either carbon numbers 2, 3, 6, 7, 9, 10 or 12. Steady-state polarization measurements were performed using a SLM polarization spectrofluorometer (SLM-Aminco, Urbana, IL). Previously published methods [19] were used to load the vesicles with the fluorescent probe. The final molar probe:lipid ratio approximated 1:1000. Corrections for light scattering and intrinsic fluorescence were routinely made by subtracting the signal obtained from identical but unlabelled samples and the fluorescence of the buffer plus label alone. These

never exceeded 5% for DPH or 13% for the anthroyloxy probes. Furthermore, 2:1 dilutions of all samples were obtained and the anisotropy of these solutions were identical with the originals. The absorbance of vesicle plus buffer at the excitation wavelength (360 nm) varied from 0.020 to 0.030. Although not measured, fluorescent lifetimes were assumed not to have changed since the total fluorescence between samples did not vary. Data are reported as the steady-state anisotropy parameter, r_s. It has been shown by Van Blitterswijk et al. [20] that the limiting hindered anisotropy, r_∞, can be calculated from this measurement using an empirical relationship. For DPH this value is relatively high suggesting that membrane order plays a prominent role in determining the motional freedom of DPH. The order parameter can be calculated as:

$$S = \left(\frac{r_\infty}{r_0}\right)^{1/2} \quad (3)$$

where r_0 represents the maximal hindered anisotropy of the probe, taken as 0.362 for DPH [19].

Fluorescent probes with relatively low values of r_∞, such as the anthroyloxy derivatives of stearic acid, are less influenced by membrane order and the anisotropy parameter for these probes reflects primarily their rotational movement, i.e.; a 'dynamic component' of membrane fluidity [21]. Since this varies as a function of depth in the bilayer this parameter was determined with seven isomers of anthroyloxy stearic acid that differed in the carbon labelled with the fluorescent group. The use of *n*-(9-anthroyloxystearic) fatty acids to measure fluidity gradients in membranes has been extensively reviewed [22,23]. These probes orient themselves in a vertical direction in the bilayer with the labelled group placed progressively deeper in the membrane in a graded fashion. It is important to note that fluorescent lifetime and relative quantum yield increase as the fluorescent group is moved further into the membrane interior [24]. Furthermore, the rotational movement of these probes in liquids of well characterized viscosity are not identical [23] and, therefore, the interpretation of data obtained from such experiments must be made with caution. However, these probes are useful in determining relative fluidity gradients in a variety of membrane preparations [22–26].

Statistical analysis. Unless stated otherwise all data are presented as the mean ± S.E. Comparisons between groups were assessed by the Man-Whitney test with the level of significance set at $p < 0.05$.

Results

Fatty acid absorption rates

Table I illustrates fatty acid absorption rates obtained in the ileum and jejunum with these fatty acid probes. The data are presented first as apparent permeability coefficients. P^*, the form in which the measurement is originally obtained. In this format the data are uncorrected for diffusion barrier effects. Therefore, the differences observed may reflect changes in either diffusion barrier resistance or intrinsic differences in the permeability properties of the microvillus membrane that line these two areas of the intestine. Since these fatty acid probes represent a homologous series and the membrane permeability, P, for each probe increases with chain length [11,12] it would be expected that a diffusion limited situation will arise as chain length increases beyond a certain point. This is illustrated in the second column where the data is now corrected for the ability of each probe to diffuse in an aqueous environment by dividing P^* for each probe by its diffusion coefficient, D. For both ileum and jejunum a constant value for P^*/D is achieved representing the diffusion limited state. It can be seen that this value is greater in the ileum than the jejunum signifying that the ileum has a lower value for diffusion barrier resistance than the jejunum. Diffusion barrier resistance, d/S_w, calculated from all diffusion limited probes ($n = 18$ for ileum, $n = 26$ for jejunum), is significantly lower in the ileum than the jejunum (0.036 ± 0.001 vs. 0.039 ± 0.001).

An accurate value for diffusion barrier resistance in each group allows calculation of the interfacial concentration, C_2, for each probe. These are presented in Table I with the assumption that the bulk phase concentration of each probe was 1 mM for the sake of convenience. Since intracellu-

TABLE I

FATTY ACID TRANSPORT DATA

Data are presented as the mean ± S.E. from ten separate perfusions of either jejunum or ileum. Absorption rates for individual fatty acids are given as the apparent permeability coefficients. The derivation of subsequent data is discussed in the text. It should be noted that true membrane permeability coefficients, P, cannot be calculated for probes that are diffusion limited (12:0 and 13:0 for ileum, 11:0, 12:0, and 13:0 for jejunum).

Source	Probe	Apparent permeability coefficient P^* (nmol/min per cm per mM)	P^*/D	Interfacial probe concentration C_2 (mM)	Membrane permeability coefficient P (nmol/min per cm per mM)
Ileum					
($n=10$)	5:0	6.82 ± 0.15 [a]	10.38 ± 1.02 [a]	0.62	11.00 ± 0.40 [a]
	7:0	8.10 ± 0.47 [a]	13.60 ± 1.06 [a]	0.51	15.88 ± 1.92 [a]
	8:0	9.38 ± 0.41	16.45 ± 1.04	0.40	23.45 ± 2.74 [a]
	9:0	10.12 ± 0.49	18.92 ± 1.05	0.31	32.65 ± 4.20 [a]
	10:0	10.66 ± 0.49	21.12 ± 1.05	0.23	46.35 ± 9.80 [a]
	11:0	11.83 ± 0.52	24.53 ± 1.05	0.11	107.55 ± 8.22
	12:0	12.90 ± 0.50 [a]	27.94 ± 1.04 [a]	–	–
	13:0	12.00 ± 0.58	28.22 ± 1.05	–	–
Jejunum					
($n=10$)	5:0	8.43 ± 0.50	12.94 ± 1.06	0.49	17.20 ± 2.17
	7:0	9.20 ± 0.36	15.49 ± 1.04	0.39	23.59 ± 2.43
	8:0	10.24 ± 0.33	17.99 ± 1.03	0.30	34.13 ± 4.00
	9:0	10.69 ± 0.20	20.09 ± 1.01	0.21	50.91 ± 4.52
	10:0	11.32 ± 0.24	22.42 ± 1.02	0.12	94.33 ± 12.45
	11:0	11.87 ± 0.33	25.43 ± 1.03	–	–
	12:0	11.76 ± 0.47	25.53 ± 1.10	–	–
	13:0	11.20 ± 0.49	25.31 ± 1.04	–	–

[a] $p < 0.05$ ileum vs. jejunum.

lar concentrations of these probes appear to approach zero [12,13] this value approximates the transmembrane concentration gradient and allows calculation of the membrane permeability coefficient, P, for each probe.

$$P = \frac{P^*}{C_2} \tag{4}$$

It is apparent that the ileal microvillus membrane is much less permeable than jejunal microvillus membrane to all fatty acid probes. The differences seen here are much greater than first appreciated by examining the apparent permeability coefficients of the fatty acid probes. This is simply because diffusion barrier resistance is higher in the jejunum and therefore the apparent lipid permeability of the jejunum underestimates the true lipid permeability of the jejunal microvillus membrane to a greater extent than in the ileum.

There are two potential explanations for the differences in membrane lipid permeability of these different regions of the intestine. Firstly, since the data are normalized to the length of perfused intestine, the ileum may have less membrane surface area per cm available for fatty acid permeation. Secondly, the physical properties of ileal microvillus membrane may differ from those of jejunum. Either or both explanations may be true. That membrane physical properties do differ between these intestinal regions is evidenced by the calculated hydrophobicity of the microvillus membrane obtained from these data. The jejunal microvillus membrane is significantly more hydrophobic than ileal microvillus membrane, implying a steeper relationship between increasing membrane permeability and increasing hydrophobicity of the probe molecule [11,13]. For a methylene group $\delta\Delta F_{w\to i}$ approximates -215 ± 58 cal/mol in the ileum vs. -463 ± 50 in the jejunum ($p < 0.05$).

TABLE II

LIPID COMPOSITION OF MICROVILLUS MEMBRANE

Data are presented as the mean ± S.E. from either four or five membrane preparations. Each membrane preparation was obtained by pooling mucosal scapings from four rats. Ileum was defined as the distal half of the small intestine and the remainder was considered jejunum.

		Microvillus membrane source	
		ileum ($n = 4$)	jejunum ($n = 5$)
Cholesterol (nmol/mg protein)		383 ± 16 [a]	289 ± 25
Phospholipid (nmol/mg protein)		802 ± 34 [a]	563 ± 59
Cholesterol: phospholipid (mol : mol)		0.48 ± 0.3	0.52 ± 0.09
Total phospholipid fatty acids (mol%)	14:0	1.0 ± 0.1	0.9 ± 0.3
	16:0	28.4 ± 0.7	24.1 ± 4.3
	16:1	0.9 ± 0.1	1.5 ± 0.3
	18:0	27.4 ± 1.7 [a]	18.3 ± 0.5
	18:1	14.9 ± 0.9 [a]	20.6 ± 2.3
	18:2	16.7 ± 1.2 [a]	27.7 ± 2.6
	20:0	0.6 ± 0.1	0.6 ± 0.1
	20:4	10.1 ± 1.1 [a]	6.3 ± 0.2

[a] $p < 0.05$ ileum vs. jejunum.

Membrane composition

The lipid composition of microvillus membrane isolated from both intestinal regions is presented in Table II. Ileal microvillus membrane has a greater concentration of both cholesterol and phospholipid when expressed per mg of membrane protein. Alternatively, the protein content of ileal microvillus membrane may be less than that of the jejunum. The cholesterol : phospholipid ratio is not significantly different.

Membrane composition in terms of total phospholipid fatty acids are quite different between these regions of the intestine. These differences are most pronounced in the relative amounts of the eighteen and twenty carbon families. In the jejunum linoleic acid predominates with a corresponding decrease in arachidonic acid whereas the ileum contained greater amounts of stearic acid and proportionally more arachidonate and less linoleate.

Fluorescence polarization studies

Physical properties of the microvillus membrane extracted from these two regions of the intestine were evaluated by steady-state polarization properties for two classes of fluorescent probes. The first of these, of which DPH is an example, are probes whose motion in the membrane is largely determined by the degree of membrane order, and from which the membrane order parameter, S, can be calculated. When assessed with DPH ileal microvillus membrane is significantly more rigid than microvillus membrane isolated from the jejunum (Table III). This translates into a higher value for S, calculated by the method of van Blitterswijk [20], 0.872 ± 0.008 vs. 0.841 ± 0.007 ($p < 0.05$). In addition probes such as the anthroyloxy labelled stearic acid derivatives, which measure the 'dynamic component' of membrane fluidity, also find ileal microvillus membrane more rigid than the jejunum. However, this component of membrane fluidity can be measured as a function of depth in the bilayer and, as illustrated in Table III, the differences between ileum and jejunum are not constant as the labelled group is placed deeper and deeper within the bilayer. With the fluorescent group placed in a superficial position in the bilayer, differences between the two groups are maximal. By the time the label reaches the tenth carbon of stearic acid there is no difference in the lipid environment experienced by the probe. At the twelfth carbon differences once again become evident with the ileum being more rigid than the jejunum.

It is unclear whether these differences relate in any way to the permeation of lipids across the bilayer and therefore a final experiment was performed. Ileal microvillus membrane labelled with the same probes underwent steady state polarization determinations in the presence or absence of 8 mM octanoic acid in an attempt to see which portion of the bilayer was perturbed by movement of this lipid across the membrane. During these studies microvillus membrane vesicles were incubated with the fatty acid for 2 hours and, therefore, it is unlikely that net movement of the fatty acid occurred but equivalent bidirectional movement was almost certainly present. Incubation of the microvillus membrane with octanoic acid resulted in an increase of the anisotropy parameter

TABLE III

FLUORESCENCE POLARIZATION STUDIES OF MICROVILLUS MEMBRANES AT 25°C

Data are presented as the mean ± S.E. obtained from the same membrane preparations used in Table II. At least seven individual measurements were obtained for every probe in each membrane preparation. The anthroyloxy stearic acid probes are denoted 'AS' with the preceeding number representing the carbon to which the label is attached. The higher the number the deeper within the bilayer the label is located.

Probe	Microvillus membrane	Anisotropy r_s	Limiting hindered anisotropy r_∞	Order parameter S
DPH	ileum	0.281 ± 0.004 [a]	0.275 ± 0.005 [a]	0.872 ± 0.008 [a]
	jejunum	0.267 ± 0.003	0.256 ± 0.004	0.841 ± 0.007
2-AS	ileum	0.158 ± 0.002 [a]	–	–
	jejunum	0.146 ± 0.003	–	–
3-AS	ileum	0.153 ± 0.004 [a]	–	–
	jejunum	0.144 ± 0.003	–	–
6-AS	ileum	0.150 ± 0.005 [a]	–	–
	jejunum	0.141 ± 0.002	–	–
7-AS	ileum	0.154 ± 0.004 [a]	–	–
	jejunum	0.147 ± 0.002	–	–
9-AS	ileum	0.129 ± 0.004	–	–
	jejunum	0.121 ± 0.004	–	–
10-AS	ileum	0.078 ± 0.007	–	–
	jejunum	0.077 ± 0.006	–	–
12-AS	ileum	0.112 ± 0.005 [a]	–	–
	jejunum	0.099 ± 0.006	–	–

[a] $p < 0.05$ ileum vs. jejunum.

for probes with the fluorescent group located in the superficial portion of the bilayer (Table IV), a pattern similar to the differences between jejunal and ileal microvillus membrane presented in Table III.

TABLE IV

FLUORESCENCE POLARIZATION STUDIES OF ILEAL MICROVILLUS MEMBRANES AT 25°C WITH AND WITHOUT ADDED OCTANOIC ACID (8 mM)

The data represent the mean ± S.E. for five separate determinations performed in two membrane preparations different from those reported in Table III. Membranes were incubated with five separate aliquots of octanoic acid and studied separately.

Probe	r_s, added fatty acid	
	none	8:0
2-AS	0.150 ± 0.004	0.152 ± 0.003
3-AS	0.143 ± 0.003	0.153 ± 0.004 [a]
6-AS	0.142 ± 0.002	0.153 ± 0.002 [a]
7-AS	0.144 ± 0.004	0.148 ± 0.003
9-AS	0.118 ± 0.002	0.121 ± 0.005
10-AS	0.075 ± 0.003	0.068 ± 0.005
12-AS	0.104 ± 0.002	0.108 ± 0.004

[a] $p < 0.05$ ileum vs. jejunum.

Discussion

Following a meal the majority of dietary triacylglycerol is absorbed in the jejunum; under normal circumstances the ileum is rarely exposed to large quantities of fat. Therefore, it would not be surprising if jejunal microvillus membrane was more lipid permeable than ileal microvillus membrane. During the normal process of lipid absorption dietary lipid must diffuse across both an aqueous diffusion barrier that lies external to the microvillus membrane and the microvillus membrane itself. Therefore, lipid transport rates are determined by the resistance of two barriers aligned in series. Several conclusions can be reached from data obtained in this study. First, diffusion barrier resistance measured in the ileum and jejunum, under identical perfusion conditions, is significantly lower in the ileum. Secondly, lipid

permeability coefficients for ileal microvillus membrane, determined in vivo, were much lower than those found in the jejunum. Thirdly, decreased lipid permeability of the ileal microvillus membrane correlated with a more rigid lipid environment in this membrane, as assessed by numerous fluorescent probes. The increased lipid rigidity of ileal microvillus membrane can be at least partially localized to the superficial region of the bilayer (i.e. proximal to the tenth carbon of stearic acid). Finally, when fatty acids are allowed to permeate through microvillus membrane vesicles in vitro, they affect the lipid fluidity of the membrane in regions similar to those that exhibit the greatest differences between jejunum and ileum. This suggests that the increased rigidity found in superficial regions of ileal microvillus membrane compared to jejunal microvillus membrane may be important in determining the lower lipid permeability properties of this membrane.

The purpose of the present study was to evaluate rates of transmembrane lipid absorption across two membranes where these rates could reasonably be expected to differ. Permeability coefficients for all fatty acids were much lower across ileal microvillus membrane than jejunal microvillus membrane. There are two possible explanations for this observation. Firstly, since absorption rates are normalized to the length of the perfused loop rather than the unknown quantity of membrane surface area, it is possible that the ileum had less membrane surface area per cm of intestine than jejunum. It is important to recognize that functional membrane surface area available for lipid permeation may have little relationship to that measured microscopically. Secondly, even if functional membrane surface area were equivalent in the two regions of intestine, ileal microvillus membrane may be intrinsically less permeable to lipids than jejunal microvillus membrane. From the data presented in this study it appears that both explanations are correct. Ileal microvillus membrane appears intrinsically less permeable than jejunal microvillus membrane since the measured hydrophobicity, $\delta\Delta F_{w\to l}$, of the ileum is less than that found for jejunal microvillus membranes. This means that the increment in membrane permeability for a unit increase in the hydrophobicity of the probe molecule is less in the ileum than in the jejunum. Given that membrane permeability for short chain fatty acids is lower in the ileum (Table I) ileal membrane lipid permeability, for lipids where this relationship holds, must always be less than found in the jejunum. Since this value is independent of membrane surface area [11,13] it implies a fundamental difference in the physical properties of these two membranes. However, it also appears that functional membrane surface area available for fatty acid absorption may be less in the ileum than the jejunum. The evidence for this comes from the permeability coefficients of those lipid probes that are anomalously permeable, in this system fatty acids 5:0 and 7:0. The relationship between membrane permeability and probe hydrophobicity does not hold for these compounds and this has led some authors to suggest that these molecules cross the membrane both by permeating through the lipid bilayer and by an accessory route [11–13]. To the extent that this accessory route reflects membrane surface area it would appear that the functional membrane surface area available for fatty acid transport is less in ileum than jejunum. This data is in agreement with previous work demonstrating that total mucosal weight per unit of serosal surface area is less in the ileum than jejunum [27]. However, as stated above, total membrane surface area may not be directly related to the functional membrane surface area involved in lipid absorption.

Since lipid permeability reflects one aspect of the physical properties of the microvillus membrane it was of interest to correlate these measurements with more conventional methods that assess the physical properties of a membrane. It has been recognized for some time that ileal microvillus membranes are less 'fluid' than their jejunal counterparts [19,28,29]. This has been demonstrated using probes that measure both the 'static' and 'dynamic' component of membrane fluidity [19]. Results similar to previously published values were obtained in this study. However, since a large number of fluorescent probes that measure the dynamic component of fluidity were used in this study it was possible to better characterize lipid fluidity as a function of depth within the bilayer. Analysis of the present data reveal that lipid fluidity remains almost constant for labels attached to stearic acid carbons two through seven both in

ileal and jejunal microvillus membrane. As the label is placed on carbons nine or ten its motional freedom greatly increases suggesting a more fluid environment in this region of the bilayer. This environment is similar in both jejunal and ileal membranes. Upon reaching the twelfth carbon fluidity once again decreases but not to the extent seen in the superficial regions of the bilayer (i.e. proximal to carbon seven).

Correlating this change in fluidity with the chemical composition of the membrane is difficult since these probes may localize in 'microdomains' of the membrane where the lipid composition may be different from that of the membrane as a whole [21]. Despite this problem though, several general rules appear to hold. Increasing the molar ratio of cholesterol, sphingomyelin, protein, or saturated fatty acids generally decreases lipid fluidity as assessed by these probes [20,21]. In this study three of these four parameters were measured. No difference in the cholesterol: phospholipid molar ratio was detected and protein content of the ileal membranes was less than that found in jejunal membranes, a difference that would tend to increase fluidity rather than rigidity of the ileal microvillus membrane. Individual phospholipids were not quantitated in this study however their fatty acid content was examined. Major differences exist in this parameter between these intestinal regions. The ileum had a much higher content of saturated stearic acid and a lower content of the doubly unsaturated linoleic acid. The content of arachidonate varied inversely with the linoleate composition, an observation that has been previously reported [30]. It is difficult to quantify the effect that these compositional changes may have on the physical properties of the membrane but several methods have been proposed. A saturation index for membranes has been used [31] that represents the number of saturated acyl chains divided by the sum of each unsaturated chain multiplied by the number of double bonds. From the data in Table II this has a value of 0.64 ± 0.006 in the ileum vs. 0.43 ± 0.04 in the jejunum, a difference that is statistically significant ($p < 0.05$). This suggests that the fatty acids of ileal microvillus membrane are, in general, more saturated than their counterparts in the jejunum. The converse of this measurement is double bond index which represents the sum of each unsaturated chain multiplied by the number of double bonds and divided by 100. The double bond index is 0.89 ± 0.05 vs. 1.03 ± 0.06 ($p < 0.05$), in ileal and jejunal membrane, respectively. Therefore, on the basis of these measurements it would be expected that ileal microvillus membrane would be less fluid than jejunal microvillus membrane as found in this study.

The final problem is whether these altered physical properties of ileal microvillus membrane have anything to do with the decreased lipid permeability of this membrane. On purely theoretical grounds it has been suggested by Stein [32] that the rate-limiting step for lipid permeation across a bilayer would be found in the highly ordered outer third of the bilayer, proximal to the first double bonds in the acyl chains. This is indeed where major differences in fluidity were found in this study but this may be fortuitous. Although the anthroyloxy labelled stearic acid derivatives are derived from a fatty acid there is no proof that they localize in a biological membrane within the 'microdomain' through which other fatty acids permeate. If fatty acids permeate across a specific domain of the membrane it would be reasonable to assume that they would affect the fluidity properties of that portion of the membrane as they crossed. In the final experiment reported here, lipid fluidity was assessed by all stearic acid probes both in the presence and absence of 8 mM octanoic acid. Under these conditions of bidirectional octanoic acid fluxes it was apparent that the local fluidity properties experienced by some of the probes were altered. Those affected were stearic acid probes (Table IV) labelled on carbons likely to be in the outer third of the bilayer. Conditions deep within the membrane were identical either in the presence or absence of octanoic acid.

The data derived from the present study would support the hypothesis that the physical properties of the outer third of the bilayer determine rates of lipid permeation across the bilayer. It should be mentioned that similar studies have been recently performed examining the physical effects of longer chain fatty acids on the platelet plasma membrane [33]. In these studies, myristic acid (14:0) had no effect on the observed lipid fluidity either superficially or deep in the bilayer. However, this study

differs from the present work in that a low concentration, 30 μM, or myristic acid was used.

If the hypothesis presented here is correct, then attempts to change the fatty acyl groups of the phospholipids (e.g. by dietary manipulations) may not be the most efficient means of altering the lipid permeability properties of the microvillus membrane. Although these have a demonstrable influence on lipid fluidity and permeability, as shown in the present study, it is the sterol ring structure that would be expected to have the greatest influence on the physical properties of this region of the membrane. Drugs are now available that can alter the structure of this region of the bilayer and studies examining the effect of this manipulation both on lipid permeability and lipid fluidity are underway.

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