A rapid method to assess the hydrophobicity of the intestinal microvillus membrane in vivo

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Summary Absorption rates for many biologically important compounds are determined by the relative hydrophobicity of the jejunal microvillus membrane. An estimate of this parameter may be obtained by measuring the incremental change in free energy that occurs when a methylene group partitions into the bilayer from an external aqueous solution. Although sensitive, this measurement has been difficult to quantitate in vivo; therefore, these studies have historically been performed in vitro. We describe a rapid, simple technique to measure this parameter in vivo. Furthermore, this method directly quantitates the resistance of aqueous unstirred layers that lie external to the microvillus membrane. —**Meddings, J. B., and J. M. Dietschy.** A rapid method to assess the hydrophobicity of the intestinal microvillus membrane in vivo. *J. Lipid Res.* 1989. **30:** 1289–1296.

Supplementary key words lipid permeability • intestinal perfusion

There are two kinetically distinct mechanisms for intestinal absorption termed the receptor-dependent or receptor-independent pathways. As a general rule, the former route is predominantly involved in the transport of relatively small, polar compounds with low lipid solubility, while the latter is utilized by hydrophobic molecules that are often very lipid-soluble (1). These hydrophobic molecules constitute an important part of our dietary intake and include compounds such as cholesterol, fatty acids, and the fat-soluble vitamins. It has become increasingly clear over the last several years that the intestine is a dynamic organ and is able to adapt to a variety of different stimuli. These adaptations appear to include the in vivo regulation of these various transport mechanisms (2, 3). For the receptor-dependent pathway, in vivo regulation can be quantitated by changes in receptor number (as assessed by the maximal transport velocity, J_m) or by changes in receptor affinity (as assessed by the Michaelis constant, K_m). However, quantitating changes in the receptor-independent pathway in vivo is a more difficult problem. An increased rate of receptor-independent absorption may be caused by either an increase in the available surface area for absorption or an absolute increase in the permeability properties of a constant membrane surface area. It is now clear that the permeability properties of the microvillus membrane (MVM) are dependent upon its chemical composition and these, in turn, may be influenced by dietary intake and disease states (2, 4). Therefore, in order to understand the changes that occur in the rates of receptor-independent absorption with either disease or dietary manipulations, a method is needed to accurately assess both the permeability properties of the microvillus membrane and its functional surface area. We describe herein a method that accurately provides these measurements in vivo and is sufficiently rapid that it can be used in single subjects, either in animal models or human volunteers, before and after any experimental intervention.

METHODS

Background

For molecules absorbed by receptor-independent mechanisms, the rate of intestinal absorption (J_d) is defined by the following relationship:

$$J_d = PC_2 \qquad \textit{Eq. 1)}$$

where C_2 is the concentration of the molecule at the microvillus membrane and P represents the membrane permeability coefficient for that molecule. It has been recognized for some time that, for any given membrane, plotting the permeability coefficients of a series of compounds of increasing hydrophobicity results in the pattern illustrated in **Fig. 1.** As the hydrophobicity of the probe molecule increases, there is a log-linear increase in its membrane permeability coefficient. This pattern of membrane permeability suggests that the MVM, like other biological membranes, discriminates on the basis of the hydrophobicity or the lipid solubility of the probe molecule. It is also apparent that there is a group of smaller molecules whose permeability coefficients are higher than would be predicted simply on the basis of their hydrophobicity alone, suggesting that they have an alternate pathway for absorption. These molecules, which have anomalously high permeabilities, are represented in Fig. 1 by fatty acids 4:0 to 6:0. In a homologous series of fatty acids or alcohols, the only difference between two consecutive members of the series is a single methylene

Abbreviations: MVM, microvillus membrane; PEG, polyethylene glycol; GLC, gas–liquid chromatography; HEPES, 4-(1-hydroxyethyl)-1-piperazinepropanesulfonic acid.

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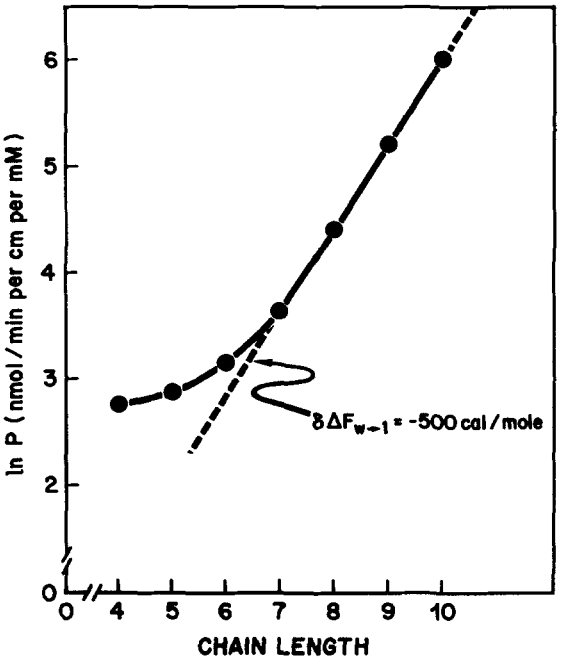

Fig. 1. The relationship between membrane permeability coefficients for a homologous series of fatty acids and their hydrophobicity. The slope of the linear portion of this curve is directly related to the incremental change in free energy that accompanies the partitioning of a methylene group from the aqueous phase into the interior of the membrane ($\delta\Delta F_{w\to l}$). This is explained more completely in the text.

group. Therefore, the difference in membrane permeability coefficients between these two probes is that produced by the increased hydrophobicity associated with an additional methylene group. This difference in permeability can be translated into a thermodynamic parameter associated with that particular membrane; the incremental free energy change associated with the partitioning of a single methylene group from the aqueous phase into the membrane, measured in calories per mole (5):

$$\delta\Delta F_{w\to l} = -RT\ln\left(\frac{P^+}{P^0}\right) \qquad Eq.\ 2)$$

In the expression R represents the gas constant, T the absolute temperate, P^+ and P^0 the permeability coefficients of the probe with and without the additional methylene group, respectively.

This represents an important parameter of membrane function and in one sense estimates a particular membrane's hydrophobicity. For most biological membranes, where adequate data are available, the value for the incremental change in free energy associated with the partitioning of a methylene group from the aqueous phase into the membrane is on the order of − 500 cal/mol. A similar figure based upon fatty acid partitioning between water and triglyceride is close to − 800 cal/mol (6). These data suggest that biological membranes are relatively more polar than pure triglyceride. Changes in the structure or composition of the cholesterol-phospholipid bilayer that affect its relative hydrophobicity would be expected to change the value of this parameter; increasing the hydrophobicity of the membrane resulting in a more negative value while a more polar membrane would have a value more positive. Therefore, this represents a convenient measurement of the relative hydrophobicity of the MVM and can be obtained from the permeability coefficients of a homologous series of fatty acids or alcohols.

This technique has been used in the past to assess the polarity of the MVM under into vitro conditions (2, 3). However, there have been several problems that have prevented more widespread acceptance of this method and its adaptation to in vivo studies. The first of these is the problem of diffusion barriers that lie external to the MVM. The presence of these barriers results in a progressive fall in the concentration molecule from the bulk phase of the intestinal contents to the MVM such that the concentration of probe at the MVM (C_2) may be much less than the concentration measured in the bulk phase (C_1). It can be seen from equation 1 that in order to calculate P an accurate value for C_2 must be obtained. This can be calculated from the bulk phase concentration (C_1) provided the rate of uptake of the probe molecule (J_d) and the resistance of the diffusion barrier (d/S_w) are known (7, 8):

$$C_2 = C_1 - \frac{J_d d}{DS_w} \qquad Eq.\ 3)$$

In this formulation d represents the average thickness of the unstirred layer, S_w its surface area, and D the free aqueous diffusion coefficient of the probe molecule. Recently, techniques have become available to accurately quantitate in vivo diffusion barrier resistance (d/S_w), and therefore, C_2 may be calculated for any nondiffusion-limited probe molecule (8).

The second major problem has been that the method, as used in the past, is relatively impractical. In order to assess the hydrophobicity of the MVM the uptake rates of at least four fatty acids must be measured. Two uptake rates are needed to calculate the value for D/S_w while the remaining two, if only partially diffusion-limited, may be used to estimate the slope of the linear relationship between lnP and chain length and therefore assess the hydrophobicity of the MVM. In order to quantitate the uptake rate of any probe molecule, an accurate means of measuring its aqueous concentration must be available. In the past this has traditionally been performed using radioisotopic methods and, therefore, has limited the number of probe molecules that may be used at any one

time in each animal or man. Thus, in order to obtain these permeability coefficients, multiple perfusions had to be performed. This problem is a limitation of the traditional method, rather than the technique itself, since the rate of uptake of several fatty acids from the same solution would be expected to be entirely independent of one another. Therefore, if the concentration of multiple fatty acids could be accurately determined from the same perfusate, then in a single perfusion study, in either man or an experimental animal, several important measurements could be made. These include the resistance of the diffusion barrier (d/S_w), the absolute permeability coefficients of multiple fatty acids (P), and an estimate of the relative hydrophobicity of the MVM ($\delta\Delta F_{w,l}$ for a methylene group). However, for this technique to be practical, several problems must be overcome. First, a rapid and reproducible method must be developed to measure the aqueous concentrations of multiple free fatty acids. Since the change in concentration for any fatty acid during the course of a perfusion study is of the order of 20%, the technique must be accurate over this concentration range. Secondly, it must be shown that for the purposes of intestinal absorption a solution containing multiple fatty acids is equivalent to a solution containing a single fatty acid. In other words, the uptake of each fatty acid must be completely independent of the remaining fatty acids. Finally, in order to measure diffusion barrier resistance, it must be shown that the rate of absorption for at least one fatty acid contained in the perfusate is diffusion-limited.

Materials

A homologous series of saturated fatty acids (5:0 to 13:0) was obtained from Sigma (St. Louis, MO). The purity of each fatty acid was checked by gas–liquid chromatography (GLC) and in each case was greater than 98%. The radioisotopes used in this study were obtained from New England Nuclear (Boston, MA) and included [^{3}H]PEG (polyethylene glycol, mol wt 4000) as a volume marker and [^{14}C]dodecanoic acid (12:0). Both were used as supplied. The remaining reagents and buffers were obtained from Sigma and were all of the highest grade available.

Perfusion studies

Female Sprague-Dawley rats of 125–150 g or female Golden Syrian hamsters (Charles River, Wilmington, MA) weighing 75–100 g were used in all experiments. Prior to the studies these animals were maintained on a pelleted rodent diet (Allied Mills, Chicago, IL) and allowed free access to water. The animals were anesthetized with pentobarbital and an in vivo perfusion loop was fashioned using a modification of a previously described method (8). Briefly, a 10–15 cm loop of jejunum was isolated just distal to the ligament of Treitz. Keeping its blood supply intact, the loop was cannulated at the proximal and distal ends with Teflon catheters that were brought through the abdominal wall and connected to a perfusion pump. The loop was perfused for 30 min with buffer alone to allow for equilibration. The composition of the buffer (mM), was Na(150), K(5), Cl(155), and HEPES(50) titrated to a pH of 7.5. Following the equilibration period the buffer was replaced with an identical test solution containing seven fatty acids at the following concentrations (mM) 5:0(0.8), 7:0(0.4), 8:0(0.4), 9:0(0.3), 10:0(0.25), and 11:0(0.1), and [^{14}C]12:0 was added in tracer quantities. This solution also contained [^{3}H]PEG as a volume marker. The test solution was perfused through the loop at a rate of 5 ml/min for a total of 48 min. Samples were taken every 8 min and the concentration of each fatty acid was determined by gas–liquid chromatography (GLC) techniques. In addition, the concentration of PEG and fatty acid 12:0 were ascertained by standard liquid scintillation methods (8).

Fatty acid concentrations

The concentrations of all fatty acids, except 12:0, were determined by GLC using a Hewlett Packard 5890A GLC system and a Supelco (Bellefonte, PA) packed column containing GP 10% SP-216-PS. Fatty acids were extracted from the aqueous sample into chloroform (as described in the Results section) and injected directly onto the column. Separation was achieved using a temperature gradient technique and the concentration of each fatty acid was assessed using dual internal standards for reference purposes. The sample was injected with an inlet temperature of 280°C at a flow rate of 30 ml/min using nitrogen as the carrier gas. During the run the oven temperature was increased at 5°C/min from 90°C to 170°C and the eluted fatty acids were quantitated using a flame ionization detector maintained at 350°C.

Calculations

The rates of uptake for each fatty acid were determined from the rate of decline in concentration of each fatty acid with time and normalized to intestinal length. The driving concentration was assumed to be the geometric mean concentration between the beginning and end of the perfusion as described by Winne and Markgraf (9). Rates of uptake (J_d) were then normalized to those that would occur at a standard 1 mM concentration of probe in the bulk phase by dividing the observed rate of uptake by the driving concentration. This value was defined as the apparent permeability coefficient($^*P$).

$$^*P = \frac{J_d}{C_1} \qquad \textit{Eq. 4)}$$

The rate of uptake for fatty acid 12:0 was determined from the rate of disappearance of ^{14}C dpm. This can be

expressed in several forms, the first being a clearance term (Cl) that reflects the volume of perfusate completely cleared of fatty acid 12:0 per unit time per cm length of intestine.

$$Cl = \frac{\Delta dpm}{\Delta t} \times \frac{1}{SL} \qquad Eq.\ 5)$$

where S represents the geometric mean concentration of fatty acid 12:0 in dpm/ml (9) and L represents the length of the loop.

Since the rate of uptake of fatty acid 12:0 is a linear function of concentration (8) this clearance term, expressed in ml/min per cm, can be converted to the equivalent apparent permeability coefficient (*P), and therefore the uptake rate of all fatty acids will be expressed in terms of *P.

Diffusion barrier resistance was measured by the diffusion-limited probe method (1, 7, 8). In order for this method to be valid, it must be clearly shown that the probe molecules used are, in fact, diffusion-limited. This can be done by plotting the apparent permeability coefficients for these probe molecules, normalized by their aqueous diffusion coefficients (D), as a function of chain length. A diffusion-limited situation exists when the value of *P/D reaches a maximal and constant value with increasing chain length. In this case it can be shown that

$$\frac{d}{S_w} = \frac{D}{{}^*P} \qquad Eq.\ 6)$$

where *P is the apparent permeability coefficient of the diffusion-limited probe and D is its aqueous diffusion coefficient.

Once the resistance of the diffusion barrier has been measured, it is possible to calculate the concentration of each probe molecule at the MVM (C_2) using equation 3. Simply dividing the measured uptake rate (J_d) by C_2 for each probe (equation 1) will then yield the true membrane permeability coefficient (P) for each molecule.

RESULTS

The first problem encountered is the large variation in aqueous solubilities for these various fatty acids. Ideally, each fatty acid should be totally extracted into an organic solvent prior to injection onto the column. However, since the smaller members of this homologous series have a relatively high aqueous solubility, it was difficult to devise an extraction technique that ensured 100% recovery of each probe molecule. Following extensive preliminary studies, it was found that by lowering the pH of the aqueous solution to less than 1 with HCl and by saturating the aqueous phase with sodium sulfate, acceptable extraction rates could be achieved. Using this solution as a starting point, **Fig. 2** illustrates the efficiency of extraction into various organic solvents for each member of the series following a 2-h extraction. This extraction consisted of 10 min of rapid shaking followed by a 2-h incubation at room temperature. Fatty acid extraction was maximal at the 2-h time point (data not shown) and remained constant for 48 h. Since chloroform provided the best overall extraction efficiency, it was selected as the organic solvent for the remainder of the studies.

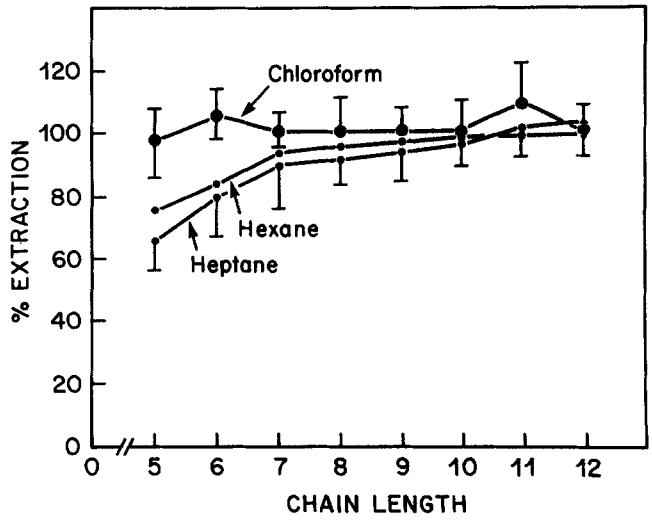

Fig. 2. Fatty acid extraction rates into three organic solvents. The individual points represent the mean value ± SEM for three separate experiments. In each case, the perfusate containing all the probes was acidified to a pH of < 1 with HCl and saturated with sodium sulfate prior to extraction. For each solvent the extractions were performed over 2 h.

From the data in Fig. 2 it is apparent that the extraction efficiency for each member of this homologous series is not uniform. Therefore, there is no single fatty acid that can be conveniently used as an internal standard to account for small variations in extraction for the remaining members of the series. In light of this observation, two separate fatty acids were selected as internal standards, fatty acid 6:0 and 12:0, the former used to judge extraction of the shorter members of the series and the latter for the longer members. For those fatty acids that lie between these two internal standards, a weighted average of the extraction of both standards was employed. Thus, fatty acid 7:0 was assumed to be extracted in a fashion equal to 80% of fatty acid 6:0 and 20% of fatty acid 12:0. Conversely, fatty acid 11:0 was assumed to be extracted in a manner approximating 80% of fatty acid 12:0 and 20% of fatty acid 6:0. Therefore, to each sample of perfusate a known amount of both fatty acid 6:0 and 12:0 was added and, following extraction into chloroform, the concentration of each fatty acid was measured using GLC techniques. A representative chromatogram is shown in **Fig. 3** and illustrates the separation of each fatty acid that can be achieved using this technique.

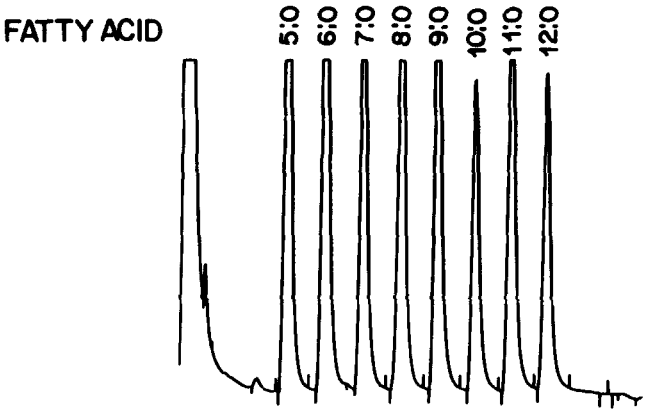

Fig. 3. A representative chromatogram. Using the method described in the text, these fatty acids elute in the order shown and are easily resolved. The total time required is 15 min.

During the course of a perfusion the concentration of each fatty acid decreases with time following a hyperbolic relationship (7). Uptake rates of each fatty acid are determined using the initial decrease in concentration with time and approximating this with a linear relationship. Therefore, in order for this method to be valid, the measured fall in concentration must be as small as possible while maintaining the ability to very accurately measure this decline in concentration. In order to evaluate the ability of this method to measure small changes in concentration of aqueous free fatty acids, similar to those encountered in a perfusion system, a series of precise dilutions was made of a standard perfusate containing all the fatty acids to be used. In **Fig. 4** the measured concentration of each fatty acid is plotted relative to the actual fatty acid concentration as determined from the sequential dilutions. The measured fatty acid concentration was obtained following extraction into chloroform as described, and after correction for variable extraction rates by the use of the dual internal standard method, also as described.

As shown in Fig. 4, the correlation between the measured fatty acid concentration and the actual concentration is remarkably good over a 50% fall in fatty acid concentrations. In fact, the error in each measurement, with the exception of fatty acid 13:0, never exceeded 2% and therefore this method provides a means of both cleanly separating the probe molecules and accurately measuring the concentration of each. Since the concentration of fatty acid 13:0 could not be reliably determined, presumably due to its very low aqueous concentration, it was not used in further studies. Thus, in summary, it is practical to extract free fatty acids from a perfusate and to precisely measure their concentrations over a range that will enable the determination of accurate absorption rates.

Since it has already been shown that the rate of uptake of these fatty acids is a linear function of increasing concentration (7, 8), it is clear that the apparent permeability coefficient (*P) for each probe molecule can be calculated from a well-defined rate of uptake (J_d) at a specific bulk

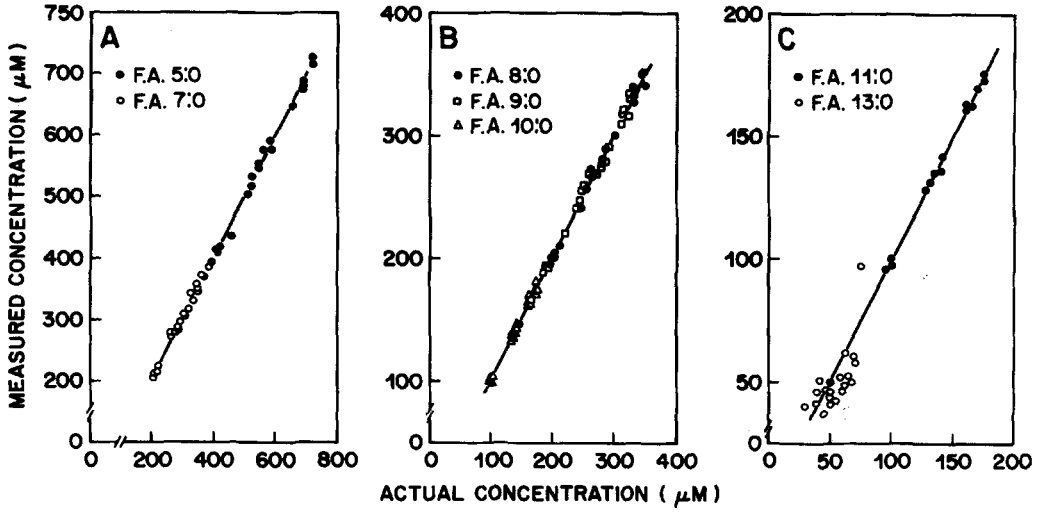

Fig. 4. Fatty acid concentration determinations. Using dual internal standards, as described in the Results section, the concentrations of all seven fatty acids were measured over the range encountered during the course of the perfusion. In each case the actual fatty acid concentration, as determined from sequential dilutions of the perfusate, is plotted on the horizontal axis while the concentration measured by GLC is plotted on the vertical axis. In each panel the line represents a perfect correlation between the measured and actual fatty acid concentration while each individual point represents a single concentration determination. Panel A demonstrates this relationship for fatty acids 5:0 and 7:0, panel B for fatty acids 8:0, 9:0, and 10:0, while panel C illustrates the results for fatty acids 11:0 and 13:0.

phase concentration (C_1), using equation 4. However, before using this technique to measure *P for a variety of probe molecules simultaneously, it must be shown that the rate of uptake for each probe is entirely independent of the remaining fatty acids. Although competition for receptors is unlikely, since these probes are absorbed by a receptor-independent pathway, it is conceivable that complex interactions may occur in a perfusate that contains a mixture of six or more fatty acids. Such systems have not been extensively studied (10) and there is the possibility of fatty acid dimer formation or other reactions that would decrease the concentration of the monomer species. If these occurred, the measured rate of uptake for a fatty acid from the perfusate containing multiple fatty acids would be significantly lower than from a perfusate containing the fatty acid alone. To exclude this possibility, a series of experiments was performed to measure the rate of uptake or each fatty acid from a solution containing all the fatty acids or a perfusate containing only a single probe molecule. These experiments were performed in the rat and representative fatty acid uptake rates (expressed as *P) from either perfusate are shown in **Fig. 5**. It can be seen that in no case did the measured uptake rates differ significantly between either perfusion solution and, therefore, the rate of uptake from a solution containing multiple fatty acids is equivalent to the rates obtained with a single fatty acid.

To demonstrate the usefulness of this technique, **Fig. 6** illustrates the results obtained from such perfusion studies

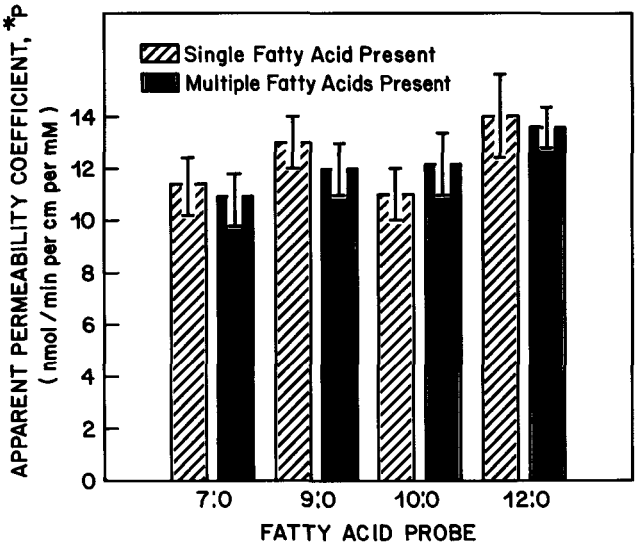

Fig. 5. A comparison of fatty acid uptake rates from a solution containing either a single fatty acid or multiple fatty acids. Four representative fatty acid uptake rates are illustrated with the rate of absorption expressed as nmol/min per cm per mM concentration. In each case the data represent the mean value ± SEM from experiments performed in four to six animals. When a single fatty acid was used, concentrations were measured using either GLC or radioisotopic methods, while for the perfusates containing all seven fatty acids, GLC methods were used as described in the text.

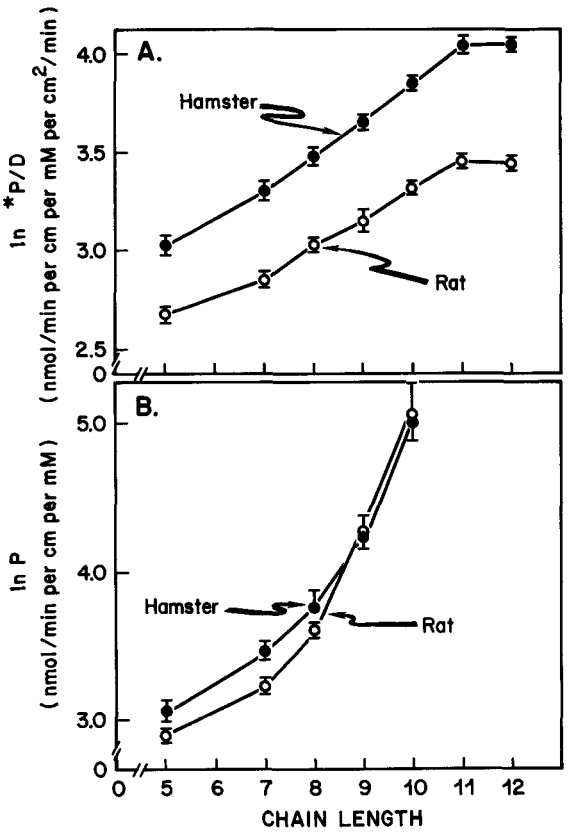

Fig. 6. Perfusion studies carried out in either the rat or the hamster. These studies were performed in eight animals of either species and the data represent the mean values ± SEM. In panel A the apparent permeability of each fatty acid probe has been normalized for its appropriate free aqueous diffusion coefficient (D) and expressed on a logarithmic scale. A diffusion-limited situation has been reached in each species represented by the plateau of fatty acids 11:0 and 12:0. Diffusion barrier resistance can, therefore, be calculated from the rate of uptake of either fatty acid 11:0 or 12:0 as discussed in the text. Panel B illustrates the relationship between fatty acid chain length and the true membrane permeability coefficient (P) for each probe following correction for unstirred layer effects. The significance of these findings is discussed in the text.

carried out in both the rat and the hamster. Using the technique described above, the apparent permeability coefficients for each fatty acid were determined in every animal. In panel A each apparent permeability coefficient is normalized for the aqueous diffusion coefficient (D) of that probe and plotted as a logarithmic function of chain length. From this data several observations may be made. First, the permeability coefficients for any fatty acid in the hamster are much greater than in the rat. Fatty acid 5:0 is taken up 1.5 times faster in the hamster while fatty acid 12:0 is absorbed almost twice as fast. There are three possible explanations for this difference; the hamster must possess either a greater absorptive surface area per linear cm of intestine, and/or a greater intrinsic permeability of the intestinal MVM to these fatty acids or a lower diffusion barrier resistance to the passage of these probe molecules. From the data presented in Fig. 6 it is possible to

discern which of these possibilities is, in fact, responsible for these differences. It can be seen from the data in panel A that a diffusion-limited situation has clearly been reached in the rates of uptake of both fatty acids 11:0 and 12:0 in each species (as *P/D has reached a maximal and constant value). Therefore, the resistance of the diffusion barrier in both species can be directly calculated from these experiments using equation 6. This provides support for the last hypothesis cited since diffusion barrier resistance (d/S_w) measured in the hamster equals 0.017 ± 0.004, a value almost one-half that found in rat of 0.033 ± 0.002. Therefore, at least one explanation for the higher rates of fatty acid uptake seen in the hamster is the presence of an unstirred layer that offers only one-half the resistance of the unstirred layer in the rat under these perfusion conditions. In order to examine the questions of absorptive surface area and the intrinsic permeability properties of the MVM, it is necessary to calculate the true membrane permeability coefficients for each of these fatty acids in both species. This can be achieved once the diffusion barrier resistance is known, as discussed in the Methods section, and these results are illustrated in panel B. If the hamster had a greater available absorptive surface area per linear cm of intestine than the rat, the true membrane permeability coefficients for each fatty acid (expressed per linear cm of intestine) would be greater in the hamster than in the rat. As shown in panel B this is not the case. There appears to be very little difference in this parameter between these two species. The final possible explanation for the observed increase in fatty acid uptake rates seen in the hamster is that this animal has a MVM that is intrinsically more permeable to the fatty acids than that found in the rat. As discussed in the background section, this would imply that the change in incremental free energy associated with the absorption of a methylene group ($\delta\Delta F_{w\to l}$) would be more negative in the hamster than the rat, providing a thermodynamically more favorable setting for fatty acid absorption. This term can be directly measured from these experiments using equation 2 and the difference in permeability coefficients of fatty acids 9:0 and 10:0. The results of this calculation reveal that $\delta\Delta F_{w\to l}$ in the rat equals -442 ± 58 cal/mol while in the hamster a value of -461 ± 34 cal/mol is found, suggesting that no significant difference exists in the underlying permeability properties of MVM in these species. Therefore, the observed difference in fatty acid uptake rates between these two species can be entirely explained by the difference in diffusion barrier resistance. There are no apparent differences in either the intrinsic permeability of the MVM or the available absorptive surface area per cm of intestine between these two experimental animals.

DISCUSSION

In order to understand the regulation of transport mechanisms that occur in any membrane, it is first necessary to have some means available to quantitate these processes. In the case of receptor-dependent pathways, classical techniques are available to quantitate, in kinetic terms, receptor number and receptor affinity (7, 11). However, such techniques are more difficult to apply to those portions of the membrane involved in the receptor-independent uptake of biologically important compounds such as the fatty acids and sterols. As described more completely in the background section, a method has been described in the past to quantitate the effective polarity of this portion of a membrane by measuring the membrane permeability coefficients of a homologous series of either fatty acids or fatty alcohols. Although this method is theoretically appealing, it has suffered from two major drawbacks in the past. First, it was relatively cumbersome. Since the rates of absorption of different fatty acids were measured by isotopic techniques, a large number of studies were required. Therefore, the technique was essentially limited to in vitro studies or, more recently, in vivo work using an animal model (8). It is relatively impractical, using these techniques, to measure multiple fatty acid absorption rates in human perfusion studies since numerous perfusions would be required. Secondly, since multiple studies must be performed in different animals and the individual uptake rates averaged, it has been impossible to derive these curves in either individual animals or man. Therefore, it has been impossible to follow changes in individuals that occur with changes in diet, diseases, or drug therapy.

In this communication a significant extension of this method has been validated. An entire homologous series of fatty acids can be perfused simultaneously and the uptake rates of each individual fatty acid can be quantitated independently of the remainder. These uptake rates are identical to those seen when the fatty acids are perfused individually and, therefore, there is no interaction between individual members of the series either in the perfusate or at the level of the microvillus membrane. The advantages of this technique over previous ones are not trivial. Three major results are obtained from this type of study and, importantly, they are obtained in each individual subject in vivo. First, the resistance of the diffusion barrier that lies external to the microvillus membrane can be accurately assessed. Secondly, the true membrane permeability coefficients, after correction for unstirred layer effects, for an entire homologous series of fatty acids are obtained. Thirdly, from knowledge of these permeability coefficients a term can be calculated that reflects the

relative polarity or hydrophobicity of that portion of the membrane involved in the uptake of these probe molecules. This term, the incremental change in free energy associated with the transfer of a methylene group ($\delta\Delta F_{w\to l}$), is independent of changes in membrane surface area between two animals or species.

Therefore, in conclusion, the method described here provides the basis for rapidly assessing several important physiologic parameters of the intestine in vivo. It is now possible to evaluate the functional effects of manipulations known to induce alterations in the physical structure of the microvillus membrane such as dietary change (4) or disease states. Since this information can now be obtained from a single perfusion study, these parameters can also be measured in human subjects using classical jejunal incubation and perfusion techniques.

This work was supported by National Institutes of Health Grants HL-09610 and AM-19329 and by grants from the Moss Heart Foundation, the Texas affiliate of the American Heart Association and Diabetes Research and Education Foundation. Dr. Jonathan B. Meddings was supported by the Alberta Heritage Foundation.

Manuscript received 17 October 1988 and in revised form 17 February 1989.

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