Influence of site and unstirred layers on the rate of uptake of cholesterol and fatty acids into rabbit intestine

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Abstract Passive permeation into the intestine is influenced by resistance of the unstirred water layer (UWL) and the microvillus membrane. Failure to account for UWL leads to gross underestimation of apparent passive permeability coefficients, and to qualitative errors in the interpretation of the nature of absorptive processes. A previously validated in vitro technique was used *1*) to determine the permeability characteristics of the rabbit jejunum (J) and ileum (I) towards a homologous series of saturated fatty acids; *2*) to measure UWL in jejunum and ileum under conditions of variable effective resistance of the UWL; and *3*) to estimate the preferential site of absorption of cholesterol in the intestine. The rate of uptake, J_d, of fatty acids was similar in J and I when UWL was high; although J_d was higher in J than I when UWL was low, the UWL was smaller in I than J at each rate of stirring of the bulk phase, and the incremental free energy change for the uptake of medium chain-length fatty acids was similar in J and I, indicating similar passive permeability characteristics along the length of the intestine. However, the intercept of the relationship between chain length and uptake was significantly different for the two sites, suggesting a greater surface area, Sm, of the membrane in J than I. These findings persisted over a wide range of different periods of incubation and of concentrations of cholesterol and bile acids. The results suggest that i) cholesterol uptake in the J and I occurs from an aqueous monomolecular phase into the microvillus membrane; and ii) reported variations in the J_d of cholesterol along the intestine are likely due to differences in UWL, Sm, or availability of substrate and not to differences in the permeability properties of the intestine.—**Thomson, A. B. R.** Influence of site and unstirred layers on the rate of uptake of cholesterol and fatty acids into rabbit intestine. *J. Lipid Res.* 1980. **21:** 1097–1107.

Supplementary key words unstirred water layers · surface area.

The proximal intestine is said to be the preferential site of absorption of many nutrients, including fatty acids and cholesterol (1–5). However, it is now recognized that the uptake of many passively transported substances is influenced by the resistance of the unstirred water layer (6–12). This resistance is determined by the thickness and surface area of the lamellae of unstirred water which are interposed between the bulk phase in the intestinal lumen, and the microvillus membrane (11). This study was undertaken to determine the permeability characteristics of the rabbit jejunum and ileum under conditions of variable resistance of the unstirred layer, in an attempt to establish the basis for the apparent preferential absorption of fatty acids and cholesterol by the proximal intestine. The results suggest that *1*) the passive permeability characteristics of the jejunum and ileum are similar for a homologous series of saturated fatty acids; *2*) the mechanism of cholesterol uptake is qualitatively similar in both sites; *3*) the effective resistance of the unstirred water layer and the functional surface area of the membrane are greater in the jejunum than in the ileum; and *4*) reported variations in the rate of uptake of cholesterol along the intestine are likely due to differences in the effective resistance of the unstirred water layer or the functional surface area of the membrane, and not due to differences in the permeability properties of the intestine.

Abbreviations: C_1, concentration of the probe molecule in the bulk phase; C_2, concentration of the probe molecule at the aqueous-membrane interface; C_3, concentration of the probe molecule in the cytosolic compartment; d, effective thickness of the unstirred water layer; D, free diffusion coefficient of the probe molecule; FA, saturated fatty acid; $\delta\Delta Fw \rightarrow 1$, incremental free energy change; J_d, rate of uptake of probe molecule into intestine; K, partitioning coefficient of a solute between aqueous phase and cell membrane; KRB, Krebs Ringer bicarbonate buffer; N, chain length of fatty acid in the homologous series; Pd, membrane passive permeability coefficient (nmol/100 mg/min); P, passive permeability coefficient (cm/sec); P^+/P^0, passive permeability coefficients with (P^+) and without (P^0) the substituent group; R, gas constant; rpm, revolutions per minute at which stirring bar was driven; SEM, standard error of the mean; Sm, surface area of membrane; Sw, effective surface area of unstirred water layer; T, absolute temperature; TDC, taurodeoxycholic acid.

[1] With the technical assistance of B. Philips, M. Yuen, and C. Hotke.

METHODS OF PROCEDURE

Chemicals

Unlabeled and (1-^{14}C)-labeled long-chain fatty acids, fatty alcohols, and cholesterol were used as supplied by Applied Science Laboratories, Inc., State College, PA; unlabeled taurodeoxycholic acid was from Sigma Chemical Co., St. Louis, MO. [G-^{3}H]-Dextran with an approximate molecular weight of 15,000 was obtained from New England Nuclear, Boston, MA and was used as a nonpermeant marker of adherent mucosal volume. All other compounds were of reagent grade and were obtained from Fisher Scientific Co.

Preparation of incubation solutions

The technique used to prepare the micellar solutions has been published (13). Briefly, an appropriate amount of both the ^{14}C-labeled and unlabeled cholesterol probe molecule was dissolved in an exact volume of chloroform–methanol 2:1 (v/v) in an incubation beaker. The chloroform–methanol phase was evaporated and 63 ml of a taurodeoxycholate solution in Krebs-bicarbonate buffer (with Ca^{2+} omitted) at pH 7.4 was added to the beaker and the solution was stirred with a magnetic bar for 2 hr at 37°C. The solution was then further diluted by the addition of 63 ml of Krebs-bicarbonate buffer to give a final volume of 126 ml and a final taurodeoxycholate concentration ranging from 1.25 to 20 mM and a final cholesterol concentration of 0.125 to 0.2 mM. The solution was gassed with 95% O_2–5% CO_2 for 2 hr at 37°C and, if necessary, the pH was readjusted to 7.4. A trace amount of the radiolabeled volume marker, [G-^{3}H]dextran was then added and the solution was ready to be used for determination of tissue uptake rates.

The saturated fatty acid and fatty alcohol solutions were prepared in KRB at pH 7.4, as previously described (11). The concentration of each fatty acid or fatty alcohol was below its limit of solubility and ranged from 0.2 to 5.0 mM.

Tissue preparation

The technique used to measure the rates of uptake has been published. Briefly, as described in detail elsewhere (10, 11), the 6–8-week-old rabbit was killed and a 10-cm length of proximal jejunum and a 10-cm length of terminal ileum were rapidly removed, rinsed with 80 ml of cold saline, opened along the mesenteric border, and the mucosal surface was carefully washed with a stream of cold saline from a syringe to remove visible mucus and detritus. Circular pieces of intestine 2 cm in diameter were cut from the segment using a sharpened steel punch. They were mounted as a flat sheet in incubation chambers, and clamped between two plastic plates so that the serosal surface was 1 cm in diameter. To the serosal compartment was added 1.2 ml of Krebs-bicarbonate buffer, and each chamber was then placed in a beaker containing Krebs-bicarbonate buffer at 4°C and constantly oxygenated by a stream of 5% CO_2 in oxygen until used in the various experiments. The chambers were first transferred to identical beakers containing oxygenated Krebs-bicarbonate buffer at 37°C for a preincubation of 30 min to allow the tissue to equilibrate to this temperature and to allow for closure of the intervillus spaces (11); then they were transferred to other beakers for specific experiments. The preincubation and incubation solutions were mixed at identical stirring rates with circular magnetic bars, and the stirring rates were precisely adjusted by means of a strobe light; stirring rates are reported as the revolutions per minute, rpm, at which the stirring bar was driven.

Determination of rates of uptake of fatty acids and cholesterol

After preincubation in KRB for 30 min, the chambers were transferred to other beakers containing [^{3}H]dextran and various concentrations of ^{14}C-labeled fatty acid or cholesterol in oxygenated Krebs-bicarbonate buffer at 37°C. After incubation for 6 min, the experiment was terminated by removing the chamber and quickly rinsing the jejunal or ileal tissue in cold saline for approximately 5 sec. The exposed mucosal tissue was then cut out of the chamber with a circular steel punch, blotted on filter paper, and placed in a tared counting vial. The tissue was dried overnight in an oven at 75°C and the dry weight was determined. The sample was then saponified with 0.75 N NaOH, scintillation fluid was added, and radioactivity was determined by means of an external standardization technique to correct for variable quenching of the two isotopes (10, 14). The rate of uptake, J_d, was calculated after correcting the total tissue ^{14}C radioactivity for the mass of the probe molecule present in the adherent mucosal fluid: these uptake rates were expressed as the nanomoles of the probe molecule taken up into the mucosa per minute per 100 mg dry weight of tissue.

Determination of rates of uptake of fatty alcohols

The method for the measurement of the rate of uptake of the fatty alcohols was similar to that used for the fatty acids and cholesterol, except that the intestinal tissue was weighed wet, placed in 1 ml

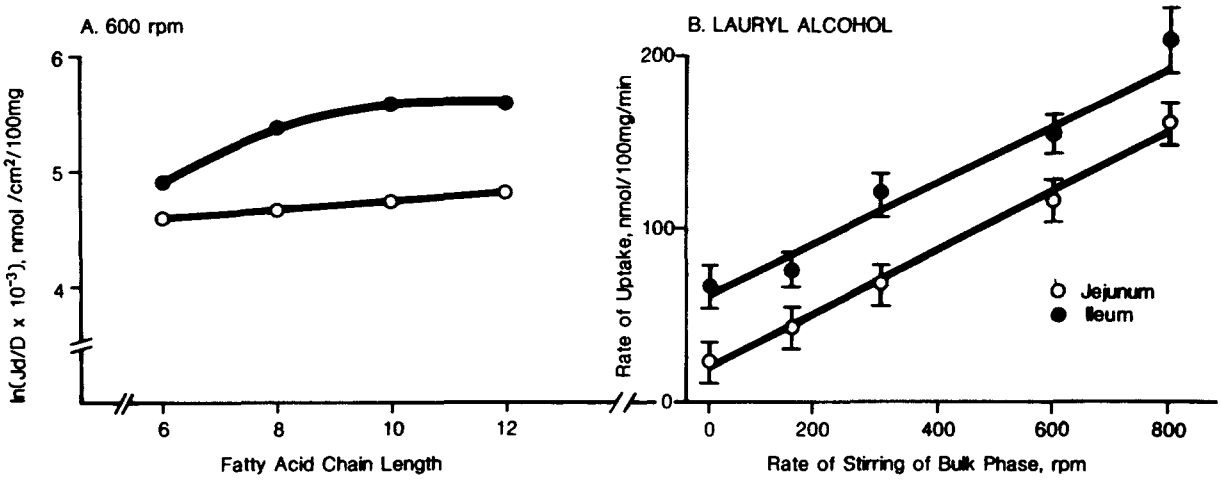

Fig. 1. Effect of rate of stirring of the bulk phase and chain length on the rate of uptake of fatty alcohols into the jejunum and ileum of rabbits. In panel A, the bulk phase was stirred at 600 rpm and the rate of uptake of fatty alcohols 6:0 (hexanol), 8:0 (octanol), 10:0 (decanol), and 12:0 (dodecanol, lauryl alcohol) was measured into the jejunum and ileum after a 6-min incubation period. In panel B, the rate of uptake of lauryl alcohol (12:0) was measured at different rates of stirring of the bulk phase (0, 150, 300, 450, 600, and 800 rpm). Each point represents the mean ± SEM for determinations in 9–12 animals.

Protosol® (New England Nuclear Corp.) at 56°C overnight, and then cooled to room temperature. Scintillation fluid was added and radioactivity was determined as described above. Separate pieces of jejunum and ileum were weighed wet and reweighed after drying overnight at 75°C. From the ratio of intestinal dry to wet weight, the rate of uptake of the fatty alcohols was changed from nmol/min per 100 mg wet weight, to nmol/min per 100 mg dry weight. These latter units are similar to those used for the fatty acids and cholesterol.

In one group of experiments the bulk phase was stirred at 600 rpm and the rate of uptake of fatty alcohols 6:0 (hexanol), 8:0 (octanol), 10:0 (decanol), and 12:0 (dodecanol, lauryl alcohol) was determined after a 6-min incubation period. Since the rate of uptake (in J_d/D) of the fatty alcohols reached a plateau between 10:0 and 12:0, the rate of uptake of lauryl alcohol was determined at different rates of stirring of the bulk phase (0, 150, 300, 450, 600, 800 rpm).

Determination of effective resistance of the intestinal unstirred water layer

It has previously been shown that the rate of uptake of fatty alcohols 10:0 and 12:0 is limited by the effective resistance of the unstirred water layer (11). This study has also demonstrated that the uptake of these fatty alcohols was limited in both the jejunum and ileum by diffusion across the unstirred water layer (Fig. 1A), and thus the rate of uptake of lauryl alcohol at different stirring rates may be used to estimate the effective resistance of the unstirred layer (11), d/SwD, as described in the discussion.

RESULTS

The rate of uptake of fatty alcohols 6:0 to 12:0 was higher in the ileum than in the jejunum when the bulk phase was stirred at 600 rpm (**Fig. 1A**). When the number of $-CH_2-$ units in the fatty alcohol chain length was plotted against ln J_d/D, a plateau was achieved between fatty alcohols 10:0 and 12:0. At each rate of stirring of the bulk phase (0–800 rpm), the rate of uptake of lauryl alcohol (12:0) was higher in the ileum than in the jejunum.

The rate of uptake of lauryl alcohol into the jejunum and ileum was limited by its rate of diffusion across the unstirred water layer (Fig. 1A), and thus the rate of uptake at each rate of stirring of the bulk phase reflected the reciprocal of the effective resistance of the unstirred water layer (11). These values for the effective resistance of the unstirred layer, measured with lauryl alcohol, are given in **Table 1.** At each rate of stirring, the effective resistance of the unstirred layer was lower in the ileum than in the jejunum. Increasing the rate of stirring of the bulk phase from 0 to 600 rpm was associated with a reduction of the effective resistance of the unstirred layer from 375 ± 20 to 79 ± 4 min. 100 mg/cm³ in the jejunum, and from 167 ± 9 to 62 ± 3 min. 100 mg/cm³ in the ileum (Table 1).

A number of other parameters of intestinal structure are also shown in Table 1. The mean dry weight of the ileal tissue per unit serosal surface area was approximately 30% lower than the jejunal dry weight (8.4 ± 0.3 and 11.9 ± 0.6, $P < 0.05$). The proportion of the total tissue dry weight comprised of scraped

TABLE 1. Dimensions of diffusion barriers and morphology of rabbit jejunum and ileum

Parameter of Intestinal Structure	Jejunum	Ileum
mg Dry weight of intestine/ unit serosal surface area	11.9 ± 0.6	8.4 ± 0.3
Proportion of total tissue dry weight comprised of scraped mucosa, %	32.4 ± 4.6	27.6 ± 2.4
mg Dry weight of intestinal mucosa/unit serosal surface area	3.9 ± 0.2	2.3 ± 0.2
Ratio of intestinal dry/wet tissue weight, %	16.9 ± 0.7	14.6 ± 0.3
Adherent mucosal fluid volume, μl/100 mg	40 ± 4	48 ± 5
Effective resistance of the intestinal unstirred water layer, min. 100 mg/cm^3		
0 rpm	375 ± 20	167 ± 9
600 rpm	79 ± 4	62 ± 3

mucosa was similar at both sites, but the mean of the mg dry weight of intestinal mucosa per unit serosal surface area was significantly higher in the jejunum than in the ileum (3.9 ± 0.2 and 2.3 ± 0.2, respectively, $P < 0.05$). The adherent mucosal fluid volume was measured using [^{3}H]dextran as a nonabsorbable extracellular fluid marker, and similar volumes were measured for the jejunum and ileum (Table 1).

The rate of uptake, J_d, of saturated fatty acids 2:0 to 12:0 was enhanced by reducing the effective resistance of the unstirred water layer (**Table 2**): increasing the rate of stirring of the bulk phase from 0 rpm to 200 rpm was associated with a 3-fold increase in J_d of fatty acids 8:0 to 12:0, and increasing the rate of stirring to 600 rpm was associated with a further 3-fold change in J_d. The J_d of fatty acids 8:0 to 12:0 was greater in the jejunum than in the ileum, but only when the bulk phase was stirred at 600 rpm (Table 2). The relative rates of uptake of the homologous series of fatty acid compounds is best compared by establishing the relationship between chain length and absorption (11, 15); this is done by plotting ln J_d/D versus N, where J_d is the rate of uptake normalized to 1 mM, D is the free diffusion coefficient of the probe molecule, and N is the number of carbons in the appropriate fatty acid chain. Note that when the bulk phase was stirred at 600 rpm, the slope of the linear relationship for fatty acids 8:0 to 12:0 was similar in the jejunum and ileum, but the intercepts were different (**Fig. 2**).

To calculate the incremental free energy change, $\delta\Delta Fw \rightarrow 1$, the rates of uptake J_d must be corrected for unstirred layer effects (11). From the values of d/SwD for lauryl alcohol shown in Table 1, the values of the effective resistance of the unstirred water layer in the jejunum and ileum were determined for each fatty acid, at 600 rpm. Those values were then used to calculate the concentration of each fatty acid at the aqueous–membrane interface, from C_1, the concentration of each probe molecule in the bulk phase (11). The equation for the relationship between ln J_d/D versus N, corrected for unstirred layer effects, was ln $J_d/D = 0.69N - 1.8$ for the jejunum, and ln $J_d/D = 0.68N - 2.4$ for the ileum. The slopes of the lines were similar for both sites, and similar values were therefore obtained for $\delta\Delta Fw \rightarrow 1$ in jejunum and ileum, −425 cal/mol and −419 cal/mol, respectively ($P > 0.05$). The difference between the intercepts, −1.8 and −2.4 for the jejunum and ileum, respectively, was statistically significant, $P < 0.05$.

When the bulk phase was stirred at 600 rpm, and the test solution contained 0.2 mM cholesterol and 20 mM taurodeoxycholic acid (TDC), the rate of uptake of cholesterol into jejunum and ileum was similar over incubation periods of 2–8 min (**Fig. 3A**). This similarity was present both when the bulk phase

TABLE 2. Rates of uptake[a] of a homologous series of saturated fatty acids into rabbit jejunum and ileum under conditions of variable effective resistance of the unstirred water layer

Saturated Fatty Acid	Stirring Rate—600 rpm		Stirring Rate—200 rpm		Stirring Rate—0 rpm	
	Jejunum	Ileum	Jejunum	Ileum	Jejunum	Ileum
2:0	5.4 ± 0.4 (16)	5.2 ± 0.7 (14)	4.5 ± 1.0(8)	3.6 ± 0.6(8)	1.9 ± 0.3(9)	4.0 ± 0.6(13)
5:0	9.7 ± 1.2 (10)	5.8 ± 1.1 (21)	3.4 ± 0.5(8)	3.6 ± 0.5(8)	3.4 ± 0.4(14)	2.5 ± 0.3(14)
6:0	24.1 ± 2.8 (14)	18.8 ± 1.4 (16)	4.7 ± 0.5(7)	4.4 ± 0.6(7)	2.4 ± 1.3(15)	5.5 ± 0.9(14)
8:0	22.7 ± 1.8 (15)	16.3 ± 1.6 (16)	9.4 ± 1.4(8)	7.9 ± 1.0(8)	3.1 ± 0.3(14)	5.2 ± 0.8(15)
10:0	64.6 ± 5.0 (15)	46.2 ± 5.8 (16)	14.1 ± 1.9(6)	30.8 ± 4.2(6)	8.2 ± 0.9(14)	7.6 ± 1.4(13)
12:0	150.1 ± 12.0(16)	121.3 ± 13.0(16)	35.8 ± 5.0(8)	36.8 ± 5.8(8)	12.6 ± 1.2(11)	7.0 ± 1.1(12)

[a] Mean values ± SEM are shown. The rates of uptake, J_d, were determined from solutions containing 0.25–1.0 mM concentrations of the test molecules, but all were normalized to a concentration of 1.0 mM; thus J_d has the units nmol·100 mg^{-1}·min^{-1}·mM^{-1}. The figure in parentheses gives the number of animals studied. The term "jejunum" is used to refer to the proximal 10-cm segment of the small intestine beginning at the Ligament of Treitz, and the term "ileum" is used to refer to the terminal 10-cm segment of small intestine proximal to the ileocecal valve. Note that the values of J_d were not corrected for unstirred layer effects in this Table.

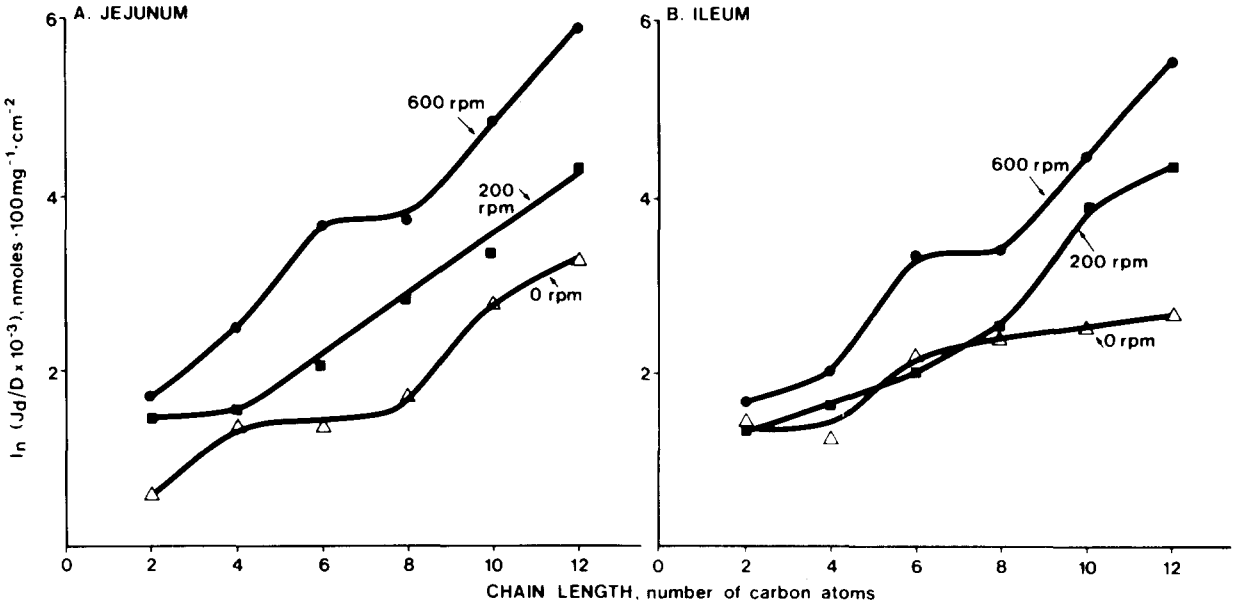

Fig. 2. The relationship between the rates of uptake in jejunum and ileum of a homologous series of saturated fatty acids and the number of carbon atoms in each compound under conditions of variable effective resistance of the unstirred water layer. In these panels, the ln of the quantity $J_d/D \times 10^{-3}$ determined for a series of fatty acids (Table 1) is plotted as a function of the chain length of each compound. Panels A and B show such data in the jejunum and ileum, respectively. Each point represents the mean ± 1 SEM for determination of 9–16 animals, shown in Table 1.

was stirred at 600 rpm, and when the bulk phase was unstirred (0 rpm). The rate of uptake of cholesterol into the jejunum and ileum was also similar at cholesterol concentrations ranging from 0.25–0.4 mM in the presence of 20 mM TDC (Fig. 3B), and over a range of concentrations of added taurodeoxycholic acid and a 0.2 mM concentration of cholesterol (Fig. 3C). In a different series of experiments, when the bulk phase was stirred at 600 rpm, cholesterol uptake into the jejunum was greater than into the ileum when both the cholesterol and bile acid concentrations were varied from 0.025–0.2 mM and 2.5–20 mM, respectively (Fig. 3D); although the difference between the means was statistically significant ($P < 0.05$), the numerical difference between the values was small. When the bulk phase was unstirred (0 rpm), uptake was lower than when the bulk phase was stirred (600 rpm), but once again J_d was similar in the jejunum and ileum (Figs. 3B, 3C, and 3D).

DISCUSSION

The magnitude of the concentration gradient across the unstirred layer is given by the equation (11);

$$C_2 = C_1 - \frac{J_d \cdot d}{Sw \cdot D} \qquad 1)$$

where C_1 is the concentration of the probe molecule in the bulk phase, C_2 is the concentration at the aqueous-membrane interface, J_d is the rate of uptake, D is the free diffusion coefficient of the probe molecule, and d and Sw are the effective thickness and surface area of the unstirred layer, respectively. For a passively absorbed solute, J_d is given by the equation:

$$J_d = Pd \cdot Sm\ (C_2 - C_3) \qquad 2)$$

where Pd is the true passive permeability coefficient, Sm is the surface area of the membrane, and C_3 is the concentration of the probe molecule in the cytosolic compartment. Assuming that $C_2 \gg C_3$ and that $C_2 - C_3 \rightarrow C_2$, then equation *2* may be written:

$$J_d = Pd \cdot SmC_2 \qquad 3)$$

Substituting equation 1 for the value of C_2,

$$J_d = Pd \cdot Sm\ (C_1 - J_d \cdot d/Sw \cdot D) \qquad 4)$$

Thus, for a given value of C_1, the rate of uptake J_d is increased when P, Sm, Sw and D are higher, and when d is lower. The differences in passive absorption between jejunum and ileum must be explained in terms of these five variables. The value of the free diffusion coefficient D is a property of the probe molecule itself and is assumed to be unaffected by the unstirred layer. Therefore, attention must be focused on the potential influence of those constants that reflect the properties of the microvillus membrane (P, Sm), and

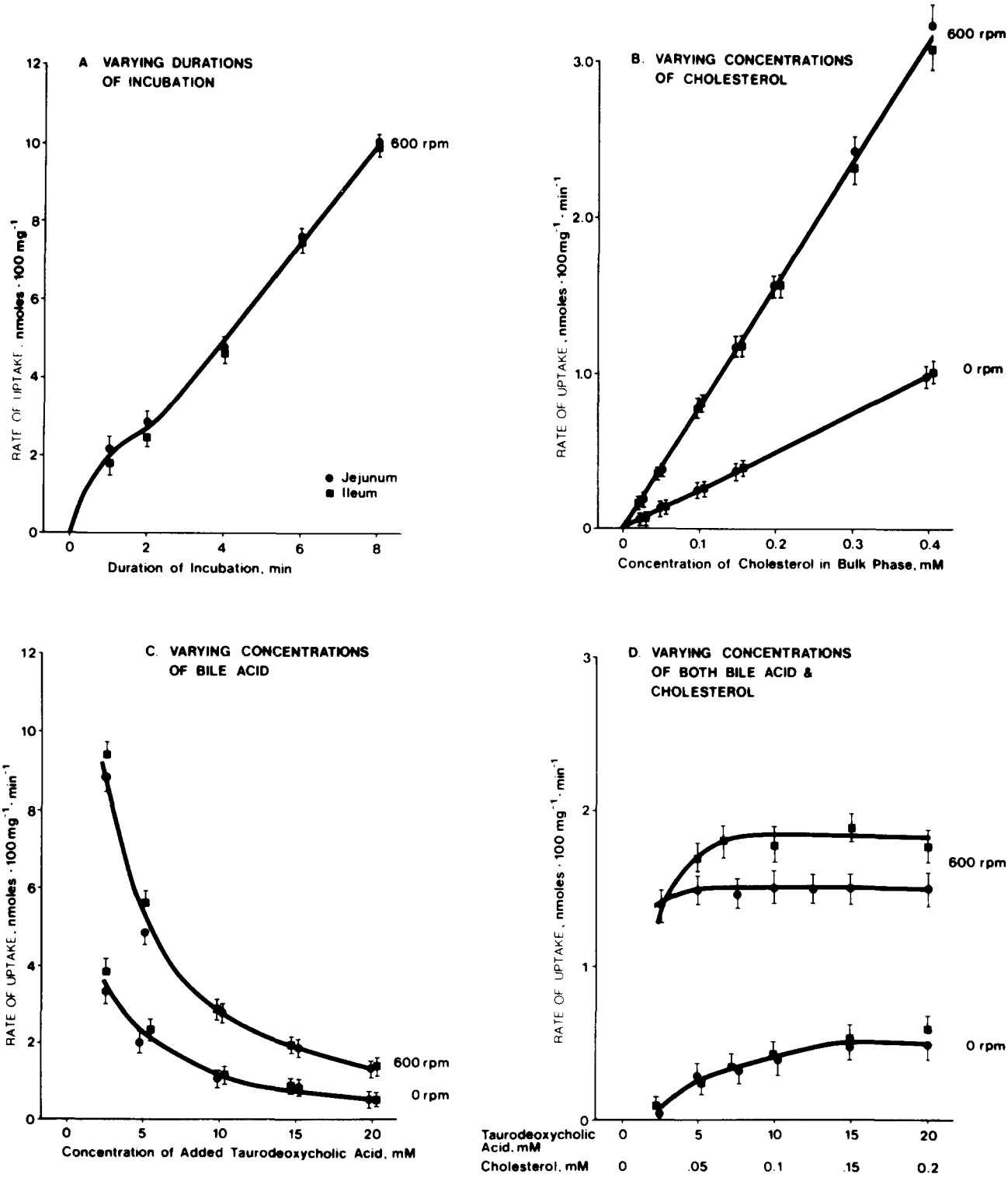

Fig. 3. The rates of uptake of cholesterol into rabbit jejunum and ileum under conditions of variable effective resistance of the unstirred water layer. In Panel A is shown the effect of increasing the duration of incubation from 1 to 8 min on the uptake of 0.2 mM cholesterol in the presence of 20 mM taurodeoxycholic acid. In Panel B is shown the effect of increasing the concentration of the cholesterol relative to that of the bile acid on the mucosal uptake of cholesterol. Varying concentrations of cholesterol (0.025–0.4 mM) were dissolved in the incubation solution in the presence of 20 mM taurodeoxycholic acid; the tissues were preincubated in this labeled test solution for 6 min. In one group of experiments the bulk phase was stirred at 600 rpm (low unstirred layer resistance) and in another group of experiments the bulk phase was unstirred, 0 rpm, (high unstirred layer resistance). In Panel C is shown the effect of increasing the concentration of bile acid relative to that of cholesterol. In these experiments the uptake of cholesterol was measured under circumstances where the concentration of cholesterol was

those constants that reflect the properties of the overlying unstirred water layer (d, Sw).

The rate of penetration of fatty alcohols 10:0 and 12:0 through the membrane is potentially faster than their rate of diffusion across the unstirred water layer (11). This conclusion is also reached in this study for the jejunum and ileum, and is presented in Fig. 1A. In this diagram the natural logarithm of J_d of each fatty alcohol divided by its appropriate D has been plotted against the fatty alcohol chain length. In this type of plot those membranes of the homologous series whose uptake is limited by diffusion through the unstirred water layer will manifest no increase in ln J_d/D with increasing chain length. The slope of this relationship was zero for the uptake of fatty alcohols 10:0 and 12:0 in the ileum, and for fatty alcohols 6:0 to 12:0 in the jejunum (Fig. 1A). When the unstirred water layer becomes totally rate-limiting, C_2 must equal essentially zero, and the limiting value of J_d/D can be calculated by rearranging equation *1*:

$$\frac{J_d}{C_1} = \frac{Sw \cdot D}{d} . \qquad 5)$$

Thus for lauryl alcohol, the value of $Sw \cdot D/d$ may be calculated from the experimental values of J_d and the known values of C_1. In Fig. 1B are shown the rates of uptake of lauryl alcohol at different stirring rates. From these values of J_d, $Sw \cdot D/d$ was calculated, and from the reciprocal of this number the effective resistance of the unstirred water layer was determined at the different rates of stirring of the bulk phase. As seen in Table 1, the effective resistance of the unstirred layer was higher in the jejunum than in the ileum, at each rate of stirring of the bulk phase.

The value of the passive permeability coefficient Pd is unique for a particular solute passing through a given membrane, and is determined by the polar characteristics of both the solute and the membrane. Recently a method has been employed to describe the effective polarity of biological membranes; it involves determination of the manner in which the addition of a particular substituent group to any solute molecule alters the rate of movement of that solute across a biological membrane or alters the partitioning of the solute into a bulk organic solvent (11, 15, 16). The relationship between the partitioning of a solute between the aqueous phase and the cell membrane is given by the equation:

$$K = e^{-\Delta Fw \rightarrow 1/RT} \qquad 6)$$

where K is the partitioning coefficient between the cell membrane and the aqueous phase, $\Delta Fw \rightarrow 1$ is the free-energy change associated with the movement of 1 mol of solute from the bulk aqueous phase to the membrane, R is the gas constant, and T is the absolute temperature. A detailed consideration of the intermolecular forces that provide the thermodynamic explanation for the effect of various substituent groups on permeability coefficients is discussed in detail elsewhere (15, 16). Although it is difficult to obtain absolute values of $\Delta Fw \rightarrow 1$ for a solute, the manner in which the thermodynamic parameter is changed by the addition of a substituent group to the solute can be experimentally measured: thus, the change in $\Delta Fw \rightarrow 1$, i.e., the incremental free-energy change ($\delta\Delta Fw \rightarrow 1$), brought about by the addition of the $-CH_2-$ substituent group to the solute is given by the expression (15):

$$\delta\Delta Fw \rightarrow 1 = -RT \ln \frac{P^+}{P^0} \qquad 7)$$

when the passive permeability coefficients for the solute with and without the $-CH_2-$ substituent group are P^+ and P^0, respectively. In practice, the ratio P^+/P^0 is given by the slope of the linear component of the relationship between ln J_d/D and N = 8 to 12 $-CH_2-$ groups, as shown in Figs. 2A and 2B. The nonpolar methylene group that is forced out of the aqueous phase by entrophy effects and undergoes hydrophobic interactions within the membrane; the addition of each $-CH_2-$ group gives a negative value for the incremental free-energy change. Therefore the measurement of $\delta\Delta Fw \rightarrow 1$ values provides a sensitive method for characterizing the effective polarity of cell membranes. An estimate of the approximate value of the incremental free energy was determined experimentally (Figs. 2A and 2B) and best approximates the true value when it is estimated under conditions of low unstirred layer resistance, such as when the bulk phase is stirred (11). Since the values of the effective resistance of the unstirred

constant at 0.2 mM while the concentration of the taurodeoxycholate in the KRB solution was increased from 2.5 to 20 mM; once again the duration of incubation of the intestinal tissue was 6 min, and the bulk phase was either unstirred, or stirred at 600 rpm so as to vary the effective resistance of the unstirred layer. In Panel D is shown the effect of increasing the concentration of both the TDC and cholesterol on the mucosal uptake of cholesterol. In these experiments the uptake of cholesterol was measured under circumstances where the concentration of cholesterol was varied from 0.0125 to 0.2 mM, and the concentration of taurodeoxycholate was varied from 1.25 to 20 mM, so as to maintain a constant molar ratio of TDC/cholesterol of 100:1 in the bulk solution. Each point represents the mean ± 1 SEM for determinations in 6 animals in the unstirred (0 rpm) group, and 9–12 animals in the stirred (600 rpm) group.

layer at each stirring rate were known (Fig. 1B and Table 1), it was possible to correct these rates of uptake determined at 600 rpm (Table 2), and to obtain accurate values for the true permeability of the jejunum and ileum. When the appropriate corrections were made for unstirred layer effects, the slope of the relationship between ln J_d/D versus chain length was similar in jejunum and ileum (0.69 and 0.68 respectively, $P > 0.05$). This represents an incremental free energy change of −425 and −419 cal/mol for jejunum and ileum, respectively (11); there was no statistical difference between these values ($P > 0.05$). Thus the addition of each $-CH_2-$ to the fatty acid required similar amounts of energy for the uptake of that probe molecule into the brush border membrane of the proximal and distal intestinal sites. Note that the values of incremental free energy have the units cal/mol but are corrected for tissue dry weight per unit serosal surface area exposed in the incubation chamber. This weight, per unit serosal area, was higher in the jejunum than ileum (Table 1). It must be appreciated then that incremental free energies represent a ratio of relative rates of uptake of the homologous series of saturated fatty acids. Thus, these results strongly suggest that the relative passive permeability characteristics of the rabbit intestine are similar in proximal and distal locations.

What is the meaning of the significantly different y-axis intercept of the relationship between ln J_d/D versus N? The units of the unidirectional flux rate J_d are nmoles·100 mg^{-1}·min^{-1} (10, 11); J_d has been normalized per unit tissue dry weight, but it is assumed that there is a constant relationship between this tissue weight and the surface area of the membrane, Sm. It is likely that the relationship between Sm and tissue dry weight is different for the jejunum and ileum. Any change in Sm will influence the value of J_d (equation *4*). Dividing equation *3* by D and taking the natural logarithm:

$$\ln J_d/D = \ln PdSmC_2/D \qquad 8)$$

and

$$\ln \frac{J_d/C_2}{D} = \ln PdSm/D \qquad 9)$$

The product ln PdSm/D may be substituted for the term ln $J_d/C_2/D$ and the equation for the linear relationship between ln PdSm/D versus chain length may be calculated to be $y = 0.69N - 1.8$ for the jejunum and $y = 0.68N - 2.4$ for the ileum, where y is ln PdSm/D and N is the number of carbons in the fatty acid chain. The value of the passive permeability coefficients Pd of the jejunum and ileum are similar (similar slopes of the relationship between ln J_d/D and N, and similar values of $\delta\Delta Fw \rightarrow 1$), as also are the values of D for a given probe molecule. The values of C_2 will vary for a given probe in jejunum and ileum, but since the values of d/Sw·D are known, then the values of C_2 may be determined. Thus the ratio of the functional surface area of the membrane of the jejunum is proportional to the surface area of the ileum by the ratio of this y-axis intercept: the intercepts are −1.8 for the jejunum and −2.4 for the ileum, and thus Sm jejunum (0.75) = Sm ileum. This value agrees closely with the correction factor of 0.8 obtained from the comparison of the structural surface area of the villus and microvillus surfaces of the proximal and distal intestine of the rat (17, 18). Thus, the greater uptake of fatty acids in the jejunum and the ileum under conditions of low effective unstirred layer resistance (Table 2 and Fig. 2), is likely due to the greater surface area of the proximal intestine relative to that of the distal intestine, rather than due to any difference in the inherent permeability characteristics of the jejunal and ileal brush border membrane.

However, two points of caution must be made with regard to these postulated relationships between the morphological and the functional surface area of a microvillus membrane. In the jejunum, the tissue morphology changes with time because of dilation of lacteals and closing off of much of the intervillus space (11); we have confirmed this finding for the jejunum and ileum. Under these circumstances, the functional surface area of the epithelial membrane may be the surface area of the tips of the villi, rather than being comprised of the total villus, the tips plus the sides of the villus. Thus the relative surface area of the jejunum to the ileum, because of the close approximation of the villi, is a complex subject; the functional surface area considered in this discussion may not necessarily be closely related to the total surface area of the villus as measured morphologically. However, because of occlusion of the intervillus spaces in the tissue preincubated 30 min, the surface area of the jejunum and ileum available for uptake of probe molecule in these studies approached a planar surface, thereby allowing for the use of equation *1*.

Second, the surface area of the villus available for uptake may vary for each probe molecule. As discussed by Winne (19), it is probable that the functional surface area of the membrane varies for different solutes such that the more rapidly penetrating solutes would be taken up from the tip of the villus, and the less permeant solutes would be taken up by a greater surface area of the villus. Several points follow from this consideration. In the assessment of incremental changes in free energy, it is assumed that

under a given experimental condition the relationship between the effective surface area of the unstirred water layer (Sw), and the functional surface area of the membrane (Sm) is constant (Sm/Sw is constant). With this suggestion that the magnitude of Sm may vary for different solutes, then the relationship between permeation coefficients and the number of carbons in the homologous series of fatty acids may give misleading incremental free energies. Thus, a morphological assessment of membrane surface area does not necessarily apply to the area of membrane functionally available for transport, and the ratio Sm/Sw may vary from one probe to another. Because only the tips of the villi are available for uptake using this method, it is likely that the values of Sm/Sw were approximately equal for each probe in a given intestinal site and under specified conditions of stirring of the bulk phase. However, under experimental conditions where the sides of the villi are available for uptake, then the values of both Sm and Sw may vary for different lipid probes. This variation in Sm/Sw would obviously distort the relationship between permeation coefficients and the number of carbons in the homologous series of fatty acids, and give misleading values for $\delta\Delta Fw \rightarrow 1$.

The bile acid micelle serves to overcome the resistance of the unstirred layer and to maintain a maximum monomer concentration at the microvillus membrane (13). Once the micelle reaches the vicinity of the aqueous-membrane interface, the constituent lipid molecules carried in the micelle can theoretically be absorbed into the mucosal cell by at least three different mechanisms: *1*, the micelle might be taken up into the cell intact; *2*, the constituent lipids might partition into the cell membrane; or *3*, absorption might occur through the monomer phase of lipid molecules present in the aqueous environment in equilibrium with the lipids in the micelle. There are adequate data in the literature to exclude the first possibility since the various constituent molecules in the mixed micelle are absorbed at essentially independent rates and therefore there is no evidence that the uptake step occurs through a process akin to pinocytosis (20–22).

On the basis of theoretical considerations, it is possible to present lines of evidence that differentiate between the second model, in which it is assumed that the micelle and the membrane interact in such a way that water is excluded from the interface between the two structures, following which there is direct transfer of cholesterol from the micelle to the microvillus membrane, and the third model, in which it is assumed that there is no direct interaction between the micelle and the microvillus membrane and that uptake occurs only by way of the lipid molecules present in aqueous solution at the interface (13). These models can be differentiated experimentally by examining whether the observed rates of uptake J_d of cholesterol vary in a predictable manner with either the mass of the lipid in the micellar phase, or the mass of the lipid in the aqueous phase.

The linear increase in J_d observed when the concentration of cholesterol is increased while the concentration of the bile acid is kept constant (Fig. 3B) is compatible with both models. However, the values of J_d for cholesterol obtained when the concentration of the bile acid is increased but that of the probe molecule is kept constant (Fig. 3C) suggest that cholesterol uptake occurs through an obligatory aqueous phase, first from micelle to aqueous phase, and then from aqueous phase into the membrane. Furthermore, when the concentration of both cholesterol and bile acid is increased in parallel, J_d remains essentially constant (Fig. 3D); these experimental results are compatible with the third model, and confirm the experimental data obtained previously by Westergaard and Dietschy (13) for the rabbit jejunum.

In addition, J_d of cholesterol is qualitatively similar in the ileum, and supports the conclusion that cholesterol uptake occurs through a monomer phase in both proximal and distal intestine. In the presence of high unstirred layer resistance, (no stirring of the bulk phase), the quantity of cholesterol taken up was less than when the resistance was low (stirring of the bulk phase at 600 rpm), (Figs. 3B, 3C, and 3D). However, the same qualitative relationships were observed at low as well as high unstirred layer resistances: increasing J_d with increasing cholesterol concentration but fixed concentration of TDC (Fig. 3B), reciprocal curvilinear decrease in J_d with increasing bile acid concentration but fixed concentration of cholesterol (Fig. 3C), and essentially constant J_d when the concentrations of both the bile acid and cholesterol are increased in parallel but maintaining the ratio of TDC to cholesterol constant (Fig. 3D).

The rate of uptake J_d of cholesterol into the intestine is influenced by its relative concentration in the aqueous and lipid phases, and thus the rate of uptake J_d of cholesterol is affected by the concentration of cholesterol in the bulk phase, the concentration of bile acid available for micelle formation, and the ratio of cholesterol to bile acid (13). When the resistance of the unstirred layer was lowered by stirring the bulk phase at 600 rpm, similar amounts of cholesterol were absorbed in the jejunum and ileum over a wide range of concentrations of cholesterol and bile acid (Figs. 3A, 3B, 3C and 3D).

The effective resistance of the unstirred water layer was about 20% higher in the jejunum than in the ileum when the bulk phase was stirred at 600 rpm (Table 1), and the surface area of the jejunum is about 20% higher than in the ileum (17, 18). Thus the ratio Sm/Sw will be similar in both sites and it is likely that the similar rates of uptake of cholesterol in each site (Figs. 3A to 3D) are a reflection of the similar permeability properties of the jejunum and ileum towards cholesterol. Thus, the proximal and distal intestine appear to be equally permeable to cholesterol over a range of different conditions, with similarities in partitioning of cholesterol between the aqueous and lipid phases.

The net movement of solute from the bulk phase into the cytosolic compartment is given by equation *2*. In our considerations of the rate of penetration of cholesterol through the membrane, we have assumed that $C_2 \gg C_3$, that $C_2 - C_3 \rightarrow C_2$, and accordingly have ignored the contribution of C_3. The concentration of the various lipids just inside the mucosal cell is presumably maintained at very low levels because of rapid esterification (1). The intracellular esterification of cholesterol is by a mechanism dependent upon CoA, is of a microsomal origin, and the reaction is highest in the duodenum (23). This would serve to maximize the value of $C_2 - C_3$, thereby providing a constant high gradient for partitioning of cholesterol out of the mixed micelle into the cytosol.

Differences in cholesterol uptake in the proximal and distal intestine may also be influenced by the availability of bile acid monomers in solution in equilibrium with the bile acid molecules present in the micelle, so that there is also uptake of these substances; the rate of bile acid absorption is low in the proximal intestine, but very high in the distal small bowel (24). Since the critical micelle concentration should increase at the aqueous–membrane interface as lipids are absorbed from the mixed micelle (25, 26), the concentration of bile acid monomers is presumably higher at the aqueous–membrane interface than in the bulk phase of the intestinal contents. The bile acid monomers could then back-diffuse into the intestinal contents to participate in the creation of new mixed micelles as additional amounts of cholesterol are generated from dietary lipids. If the availability of the bile acid monomers was constantly minimized in the distal intestine as a result of their rapid absorption, then this shuttling of micelles between the bulk intestinal content and the aqueous–microvillus interface might also be limited, thereby impairing further uptake of cholesterol. Thus, previous reports of a site-related difference in cholesterol absorption may be due in part to variations in the uptake of bile acids at different locations along the intestine, rather than due to any difference in the permeability properties of the jejunum and ileum towards this probe molecule. Finally, the preferential absorption of lipids in the proximal intestine demonstrated under oral dosing conditions is related to the large capacity of the proximal intestine to absorb dietary lipid (1–3, 5), leaving relatively little of the oral load available for absorption more distally. This is in distinction, of course, to the experimental design of the studies reported here, in which we were attempting to distinguish between the factors determining the rate of lipid absorption when a particular area of the intestine is exposed to a constant concentration of lipid.

In summary, these studies suggest that the passive permeability characteristics of the rabbit jejunum and ileum for fatty acids are similar, and that differences in the rates of uptake of a homologous series of saturated fatty acids at these two sites are due to differences in the surface area of the microvillus intestinal membrane, and to differences in the effective resistance of the overlying unstirred water layer. The permeability of the intestine to cholesterol in vitro is unaffected by variations in the location along the intestine, or by differences in aqueous-membrane partitioning of cholesterol from the bile acid micelle into the microvillus membrane.

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REFERENCES

1. Johnston, J. M. 1968. Mechanisms of fat absorption. *In* Handbook of Physiology. Section 6. Alimentary Canal. American Physiological Society, Washington, DC, **III:** 1353–1375.
2. Treadwell, C. R., and G. V. Vahouny. 1968. Cholesterol absorption. *In* Handbook of Physiology. Section 6. Alimentary Canal. American Physiological Society, Washington, DC. **III:** 1407–1438.
3. Thomson, A. B. R. 1978. Intestinal absorption of lipids: influence of the unstirred water layer and bile acid micelle. *In* Disturbances in Lipid and Lipoprotein Metabolism. J. M. Dietschy, A. M. Gotto, Jr., and J. A. Ontko, editors. Clinical Physiology Series.

American Physiological Society, Washington, DC. 29–56.
4. Salle, V. L., and J. M. Dietschy. 1973. Determinants of intestinal mucosal uptake of short- and medium-chain fatty acids and alcohols. *J. Lipid Res.* **14:** 475–484.
5. Borgstrom, B. 1960. Studies on intestinal cholesterol absorption in the human. *J. Clin. Invest.* **39:** 809–815.
6. Wilson, F. A., V. L. Sallee, and J. M. Dietschy. 1971. Unstirred water layers in intestine: rate determinant of fatty acid absorption from micellar solutions. *Science.* **174:** 1031–1033.
7. Dietschy, J. M., V. L. Sallee, and F. A. Wilson. 1971. Unstirred water layers and absorption across the intestinal mucosa. *Gastroenterology.* **61:** 932–934.
8. Dietschy, J. M. 1973. Mechanisms of bile acid and fatty acid absorption across the unstirred water layer and brush border of the intestine. *Helv. Med. Acta.* **37:** 89–102.
9. Wilson, F. A., and J. M. Dietschy. 1974. The intestinal unstirred layer: its surface area and effect on active transport kinetics. *Biochim. Biophys. Acta.* **363:** 112–126.
10. Lukie, B. H., H. Westergaard, and J. M. Dietschy. 1974. Validation of a chamber that allows measurement of both tissue uptake rates and unstirred layer thickness in the intestine under conditions of controlled stirring. *Gastroenterology.* **67:** 652–661.
11. Westergaard, H., and J. M. Dietschy. 1974. Delineation of dimensions and permeability characteristics of the two major diffusion barriers to passive mucosal uptake in the rabbit intestine. *J. Clin. Invest.* **54:** 718–732.
12. Dietschy, J. M., and H. Westergaard. 1975. The effect of unstirred water layers on various transport processes in the intestine. *In* Intestinal Absorption and Malabsorption. T. Z. Csaky, editor. Raven Press, New York. 197–207.
13. Westergaard, H., and J. M. Dietschy. 1976. The mechanism whereby bile acid micelles increase the rate of fatty acid and cholesterol uptake into the intestinal mucosal cell. *J. Clin. Invest.* **38:** 97–108.
14. Sallee, V. L., F. A. Wilson, and J. M. Dietschy. 1972. Determination of unidirectional uptake rates for lipids across the intestinal brush border. *J. Lipid Res.* **13:** 184–192.
15. Diamond, J. M., and E. M. Wright. 1969. Biological membranes: the physical basis of ion and non-electrolyte selectivity. *Annu. Rev. Physiol.* **31:** 581–646.
16. Diamond, J. M., and E. M. Wright. 1969. Molecular forces governing non-electrolyte permeation through cell membranes. *Proc. R. Soc. London Ser. B* **172:** 273–316.
17. Fisher, R. B., and D. S. Parsons. 1950. The gradient of mucosal surface area in the small intestine of the rat. *J. Anat.* **84:** 272–282.
18. Boyne, R., B. F. Fell, and I. Robb. 1966. The surface area of the intestinal mucosa in the lactating rat. *J. Physiol.* **183:** 570.
19. Winne, D. 1978. The permeability coefficient of the wall of a villous membrane. *J. Math. Biol.* **6:** 95–108.
20. Hoffman, N. E. 1970. The relationship between uptake in vitro of oleic acid and micellar solubilization. *Biochim. Biophys. Acta.* **196:** 193–203.
21. Simmonds, W. J. 1972. The role of micellar solubilization in lipid absorption. *Aust. J. Exp. Biol. Med. Sci.* **50:** 403–421.
22. Wilson, F. A., and J. M. Dietschy. 1972. Characterization of bile acid absorption across the unstirred water layer and brush border of the rat jejunum. *J. Clin. Invest.* **51:** 3015–3025.
23. Haugen, R., and K. R. Norum. 1976. Coenzyme-A-dependent esterification of cholesterol in rat intestinal mucosa. *Scand. J. Gastroenterol.* **11:** 615–621.
24. Schiff, E. R., N. C. Small, and J. M. Dietschy. 1972. Characterization of the kinetics of the passive and active transport mechanisms for bile acid absorption in the small intestine and colon of the rat. *J. Clin. Invest.* **51:** 1351–1362.
25. Carey, M. C., and D. M. Small. 1969. Micellar properties of dihydroxy and trihydroxy bile salts: effects of counterion and temperature. *J. Colloid Interface Sci.* **31:** 382–396.
26. Small, D. M. 1968. Size and structure of bile salt micelles. Influence of structure, concentration, counterion concentration, pH, and temperature. *Adv. Chem. Ser.* **84:** 31–52.