BBA 75 169

STUDIES ON TRANSMURAL POTENTIALS *IN VITRO* IN RELATION TO INTESTINAL ABSORPTION

V. KINETIC CHARACTERISTICS OF LIPID INTERACTIONS WITH RAT GUT*

IRVING LYON**

Department of Biochemistry, The Chicago Medical School, Chicago, Ill. and Science Faculty, Bennington College, Bennington, Vt. (U.S.A.)

(Received March 1st, 1968)

SUMMARY

The *in vitro* studies reported here are concerned with the interactions of bile salt alone and of bile salt, fatty acid, monoglyceride and triglyceride in various combinations, with the mucosal surface of paired everted sacs prepared from the rat small intestine. Lipid-induced changes were noted in transmural potential (ΔPD) and in short-circuit current (ΔI_{sc}) during incubation in buffered media. Increments in PD and I_{sc} appeared to be saturable functions of graded concentrations of taurocholate and of glyceryl monooleate in the mucosal medium. This was confirmed by Lineweaver–Burk plots of the data and apparent K_m values for the interaction of taurocholate and of monooleate with the ileal mucosal epithelium were estimated by extrapolation to be 3.6 and 6.0 mM, respectively.

The lipid–membrane interactions are Na^+-dependent. For example, apparent K_m values increased as the Na^+ concentration of the medium was decreased by isosmolar replacement with $Tris^+$ or K^+. Moreover, K^+, compared with $Tris^+$, seemed to inhibit markedly the bile salt interaction, but had little effect on the interaction of monoglyceride other than deprivation of Na^+. Furthermore, this Na^+ dependency is reflected in a mutual competitive inhibition between glucose and these lipids as well as between the lipids themselves.

A comparison of ΔPD and ΔI_{sc} data, obtained with mixed lipids containing oleic acid or triolein, suggests that 3-component preparations (with taurocholate and monoglyceride) are more effective in interacting with the mucosal surface of the sac than are the corresponding 2-component preparations (without monoglyceride).

The studies with [^{14}C]triolein indicate that (1) a small but significant fraction of the lipid may actually enter the mucosal tissues and (2) uptake, determined under open-circuit and short-circuit conditions, appears to be an active, Na^+-dependent process.

Abbreviation: PD, potential difference.

* A preliminary report[1] of this work was presented at the 51st Annual Meeting of the Federation of American Societies for Experimental Biology held in Chicago, Ill., April 16–21, 1967.

** Present address: Bennington College, Bennington, Vt. 05201, U.S.A.

The results are discussed in an attempt to define the mode of intestinal lipid transport. It is suggested that lipids may be transported across the brush border (microvillar) membrane of the intestinal epithelial cell by a Na^+-dependent, energy-independent process exhibiting kinetic characteristics reminiscent of a saturation phenomenon.

INTRODUCTION

Recent micromorphological studies of the intestinal absorption of lipids suggest that, in addition to micropinocytosis[2–8], this class of substances may cross the microvillar membrane as particulates in the form of droplets or micelles[9–15]. Although the major portion of ingested lipid is absorbed in the upper jejunum in humans and in animals[16–18], the lower intestine also has the capacity for the uptake of micellar lipids[12]. As the higher glycerides, present in an oil phase, move down the small intestine, digestion transforms them into di- and monoglycerides and fatty acids, facilitating formation of an intraluminal micellar phase, from which they are readily absorbed[19]. Micellar monoglycerides and fatty acids appear to be taken up as intact molecules[12,20] by a process which seems to be unaffected by metabolic inhibitors or temperature changes[12]. These findings imply that the mechanism by which micellar lipids cross the brush border membrane of the intestine may be energy-independent. *In vitro* studies of triglyceride absorption suggest that the rate of entry is essentially the same for mono, di- and triglycerides, and that entry, rather than subsequent intracellular hydrolysis of triglycerides into glycerol and fatty acids, is the rate-limiting step[21]. Though the latter concept is disputed[12], the mechanism of glyceride and fatty acid entry is, as yet, unknown.

The transformation of lipids into a micellar phase is brought about in the presence of bile salts[19] which appear to be actively transported, at least in the ileum of the rat and of other species[22–28]. Jejunal transport of bile salts, however, does not seem to be active[22–26,28] and may, in fact, be diffusion-limited. Active transport of bile salt requires Na^+ (refs. 24 and 25) and is inhibited by metabolic poisons[24,25], causing serosal–mucosal accumulation ratios to approach a limiting value of one[28]. Intestinal uptake of bile salts seems to be a saturable function of their concentration in the medium, and Lineweaver–Burk plots of accumulation data yield linear relations[24,25,27]. Furthermore, bile salts exhibit mutual competitive inhibition[24,25,27,28], and they appear to interfere with the uptake of sugars and of amino acids[27,28]; the latter effects, however, have been ascribed to cholate and deoxycholate, present as contaminants in conjugated bile salt preparations[29].

The *in vitro* studies reported here are concerned with some of the kinetic characteristics of interaction of several lipids with the mucosal surface of paired everted sacs, prepared from the small intestine of the rat and incubated in KREBS–HENSELEIT bicarbonate buffer[31], by noting changes in transmural potential (ΔPD) and in short-circuit current (ΔI_{sc}). Since stable readings are attained within 15 sec, usually in less than 5 sec, it seems reasonable to assume that the measurements are primarily a reflection of initial interactions of lipids relatively uncomplicated by the consequences of intracellular accumulation or efflux of previously entered lipids. That lipid may in fact be taken up by the mucosal epithelial cell is indicated by the data obtained in studies with [^{14}C]triolein.

METHODS

Incubation media

Everted sacs[30] were incubated at 37° in KREBS–HENSELEIT bicarbonate buffer[31] (pH 7.4 ± 0.2) or in Tris–bicarbonate buffers (25 mM) containing different amounts of Tris–chloride or KCl, in isosmolar replacement for NaCl, *plus* graded concentrations of sodium taurocholate* (Calbiochem, Los Angeles), alone or with various lipids. Lipid mixtures were freshly prepared before use as stable emulsions using taurocholate as the emulsifying agent. After the bile salt was thoroughly mixed with the lipid(s) to be tested, the mixture was gently warmed and solubilized in warm buffer. The required volume of these preparations was then pipetted into the buffer bathing the mucosal (outer) surface of the sac. Corrections for osmotic disequilibria were made against comparable concentrations of D-mannitol.

Tissue preparations

Everted sacs[30] were prepared, as previously described[32], from Sprague–Dawley MRC rats of either sex, weighing 150 ± 20 g, after a fast of 48 h during which water was available *ad libitum*.

Incubation apparatus and measurement of transmural potentials

The apparatus and the method of measurement of PD and I_{sc} values have been described in detail in earlier reports of this series[32,33].

[^{14}C]*Triolein studies*

Experiments were carried out with emulsions containing carboxyl-labeled [^{14}C]triolein** (Nuclear–Chicago), taurocholate and glyceryl monooleate (Calbiochem, Los Angeles) (12, 8 and 12 mM, respectively) to (a) determine whether the lipid is taken up by the intestinal mucosal cells and to (b) distinguish between total uptake and exchange uptake. Total uptake is defined as the amount of radioactivity extracted from mucosal scrapings of everted sacs incubated, for 1 or 5 min, in the presence of 12 mM radioactive triolein. For exchange uptake this procedure was modified to include a prior incubation for 5 min in non-radioactive triolein (12 mM). Incubations in radioactive lipid were followed by three 1-min rinses in non-radioactive lipid at the same concentration to remove adventitiously adsorbed lipid. Incubations and rinses were conducted under open-circuit or short-circuit conditions and the monitored ΔPD and ΔI_{sc} values were the same as those obtained earlier with non-radioactive triolein and reported herein. Lipid was extracted according to the procedure of BLIGH AND DYER[34] and samples were prepared for liquid scintillation counting as described by PATTERSON AND GREENE[35].

Statistical variation of the data

The data reported here represent mean values; the standard deviations in percent for all values ranged from ± 5 % to ± 13 %.

* Stated by supplier to be homogeneous by thin-layer chromatography, essentially free of deoxycholic acid and to contain less than 0.5 % cholic acid as a contaminant.

** Radiochemical purity of this product was assayed by Nuclear–Chicago at > 98 % by thin-layer chromatography and by reversed phase thin-layer chromatography on silica gel plates using the solvent system petroleum ether–ether–acetic acid (90:10:1, by vol.). Chemical purity was assayed at > 99 % by paper chromatography with the solvent system chloroform–methanol–formic acid–water (40:60:1:5, by vol.).

RESULTS AND DISCUSSION

Tissue conductance

The relationship between PD and external current, both in the absence of lipids and in their presence (Fig. 1), indicates that the tissue acts as an ohmic resistor. As noted earlier with sugars and with phlorizin[33], tissue conductance, $\Delta I_{sc}/\Delta PD$, appears to depend primarily upon the concentration of Na^+ in the medium, approaching 25 $m\Omega^{-1}\cdot cm^{-2}$ at 145 mequiv Na^+ and falling to 15 $m\Omega^{-1}\cdot cm^{-2}$ at 24 mequiv with $Tris^+$ in isosmolar replacement for Na^+.

Effect of taurocholate and of glyceryl monooleate on PD and I_{sc}

Increments in PD and I_{sc} were observed with ileal sacs in the presence of graded concentrations of glyceryl monooleate and of taurocholate (Fig. 2). Above the saturation concentration of taurocholate, about 8–10 mM, ΔPD and ΔI_{sc} values fell suggesting the influence of non-specific inhibitory effects. These data are suggestive of saturation phenomena and an apparent K_m value for the interaction of taurocholate with the ileal mucosal cell membrane has been estimated by extrapolation to be 3.6 mM (Fig. 3). In a similar manner the apparent K_m for glyceryl monooleate has been estimated at 6.0 mM (Fig. 4). With jejunal sacs ΔPD and ΔI_{sc} values appear to be linear functions of the taurocholate concentration in the medium (Fig. 5) but saturable functions of the monoglyceride concentration.

Influence of Na^+ on apparent K_m values for lipids

Apparent K_m values for taurocholate and for monooleate (Fig. 6) increased as the Na^+ concentration in the medium was decreased by isosmolar replacement with $Tris^+$ or K^+. These findings suggest that Na^+ is required for the interaction of these

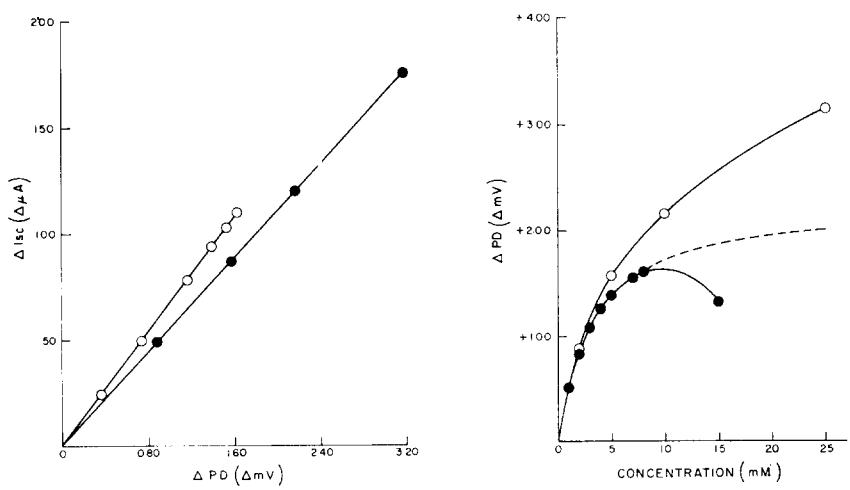

Fig. 1. The relationship between ΔPD and external current, ΔI_{sc}, in the presence of graded concentrations of taurocholate (○—○; 76 paired sacs) and of glyceryl monooletate (●—●; 92 paired sacs).

Fig. 2. The effect of graded concentrations of glyceryl monooleate (○—○; 92 paired sacs) and of taurocholate (●—●; 76 paired sacs) on ΔPD values in paired everted ileal sacs. – – – –, obtained from a Lineweaver–Burk plot of the taurocholate data (see Fig. 3).

substances with the mucosal epithelial surface. Furthermore, K^+, compared with $Tris^+$, appears to exert a marked inhibitory effect on the taurocholate interaction, but very little effect on the interaction of monoglyceride beyond the inhibition due to Na^+ removal.

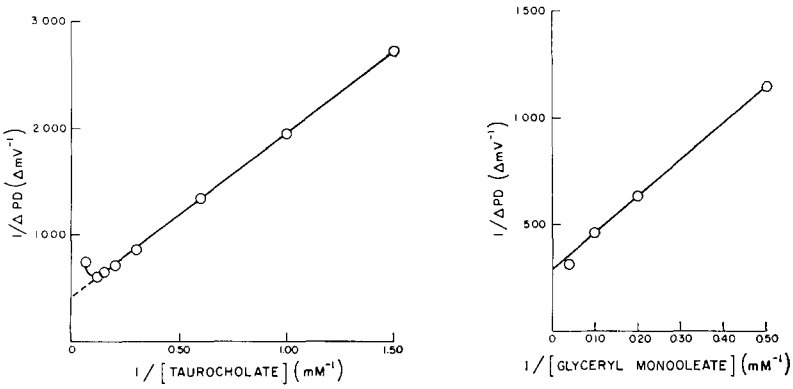

Fig. 3. Double reciprocal plot of $1/\Delta PD$ *vs.* 1/taurocholate concentration from the data of Fig. 2 indicating apparent conformity to Michaelis–Menten kinetics except at higher concentrations of the bile salt. Estimated apparent K_m value is 3.6 mM.

Fig. 4. Double reciprocal plot of $1/\Delta PD$ *vs.* 1/monooleate concentration from the data of Fig. 2. Apparent K_m value estimated to be 6.0 mM.

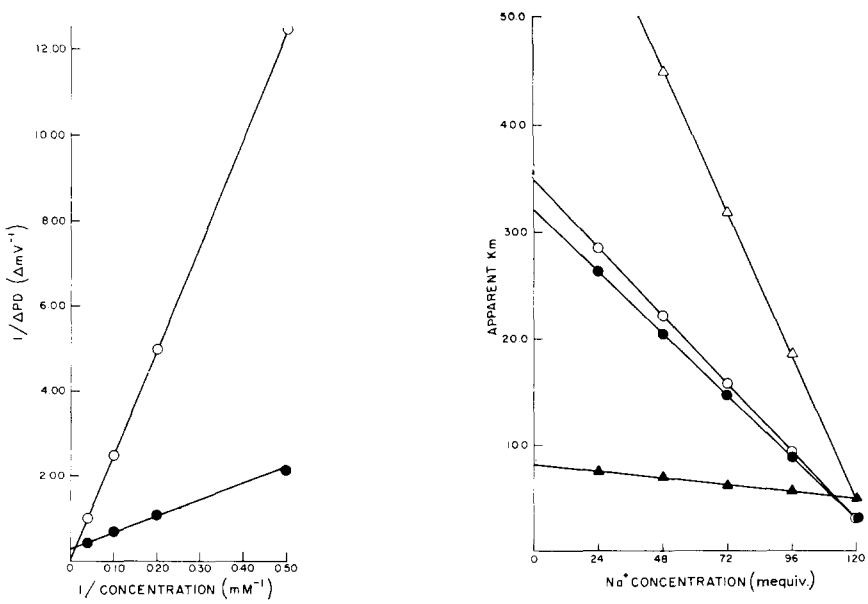

Fig. 5. Double reciprocal plot of jejunal ΔPD values obtained in the presence of taurocholate (○—○; 16 paired sacs) and of monooleate (●—●; 16 paired sacs).

Fig. 6. Influence of Na^+ concentration in the medium upon apparent K_m values for taurocholate (▲—▲, $Tris^+$ replacement; △—△, K^+ replacement) and for glyceryl monooleate (●—●, K^+ replacement; ○—○, $Tris^+$ replacement). Each replacement experiment was carried out with 16 paired sacs and double reciprocal plots of the data yielded the values shown here.

Mutual Na^+ dependency and competitive inhibition

At this point it seemed possible that competition, based upon Na^+ dependency, may be observed among various lipids and between lipids and sugars or amino acids. The effect of taurocholate upon the monooleate-dependent potential was determined in a medium containing Na^+ at 145 mequiv (Fig. 7). The data indicate that the bile salt is approx. 80 (79 ± 6) % effective as a competitor against monoglyceride. In other experiments monooleate was equally effective (87 ± 5 %) as a competitor against taurocholate. Similar studies with glucose and glyceryl monooleate (Fig. 8) revealed

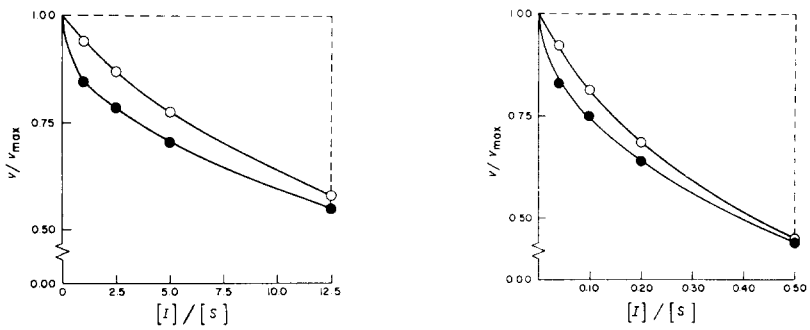

Fig. 7. The effect of taurocholate (I) upon the increment of transmural PD induced by monooleate (S). ●—●, full competition. Relative areas were determined by planimetry. Experimental data obtained with 32 paired sacs.

Fig. 8. The influence of glucose (I) upon ΔPD values obtained with monooleate (S). Experimental data obtained with 32 paired sacs.

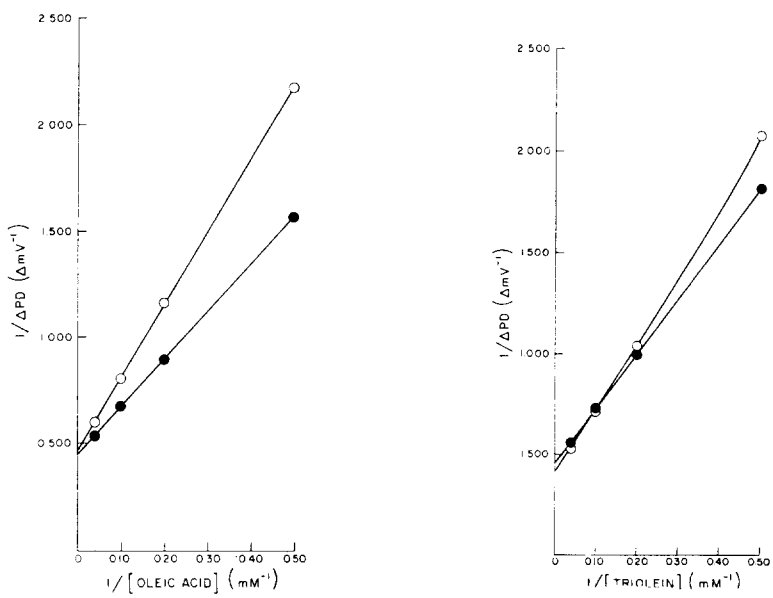

Fig. 9. Double reciprocal plots of $1/\Delta$PD *vs.* 1/oleic acid concentrations. ●—●, with taurocholate and monooleate (16 paired sacs); ○—○, with taurocholate only (16 paired sacs).

Fig. 10. Double reciprocal plots of $1/\Delta$PD *vs.* 1/triolein concentrations. ●—●, with bile salt and monoglyceride (16 paired sacs); ○—○, with bile salt only (16 paired sacs).

a mutual competitive inhibition of about 85 % (glucose *vs.* monooleate, 89 ± 5 %; monooleate *vs.* glucose, 83 ± 6 %). With jejunal preparations the reciprocal competitive inhibition between glucose and the monoglyceride was approx. 90–95 %.

Influence of lipid preparation composition on interaction of specific lipid

In studies with oleic acid (Fig. 9) and with triolein (Fig. 10) transmural PD and I_{sc} data yield linear relationships in Lineweaver–Burk plots. With either of these substances the simultaneous presence of bile salt and monoglyceride showed an apparent activation, *i.e.* a K_m-lowering effect, as compared with bile salt alone. Moreover, triolein does not show a time lag relative to oleic acid for interaction with the mucosal surface of the sac.

[^{14}C]*Triolein studies*

The data obtained in studies with [^{14}C]triolein are summarized in Table I. Under the specified conditions of incubation (see METHODS), it seems clear that triolein and any liberated [^{14}C]oleic acid were rapidly taken up by an easily reversible process, designated exchange uptake, and more slowly taken up by a process other than exchange, designated net uptake. It is interesting to note that exchange accounted for 95 % of the total lipid uptake during incubation for 1 min and for 44 % of the total uptake in a 5-min incubation period. Furthermore, the increase in net uptake values from 1 to 5 min is suggestive of a saturation process, *i.e.* possibly the result of an

TABLE I

[^{14}C]TRIOLEIN DATA

Each figure represents the mean value of data obtained with 8 paired ileal sacs.

Na^+ concn. (mequiv·l^{-1})	*Incubation time (min)*	*Circuit condition*	*Uptake (nanomoles·h^{-1}·g^{-1})*		
			Total	*Exchange*	*Net*
145	1	Open	3195	3044	151
		Short	2269	2167	102
	5	Open	612	272	340
		Short	408	179	229
48	1	Open	456	—	
		Short	764	—	
	5	Open	376	—	
		Short	612	—	

adsorption or carrier-mediated process. Given the nature of the rinse and extraction procedures, it seems likely that a small but significant fraction of the lipid actually entered into the mucosal tissues.

Total lipid uptake during a 1-min incubation under short-circuit conditions, in which the transmural PD is suppressed to and held at zero during the incubation and rinse periods, appears to be directly dependent upon the concentration of Na^+ in the medium, *i.e.* the ratio of ^{14}C-labeled lipid taken up to the Na^+ concentration remains the same both at 145 and 48 mequiv (2269/145 = 15.6; 764/48 = 15.9). Under open-circuit conditions total uptake was higher at 145 than at 48 mequiv of Na^+ in the medium. However, the increased open-circuit uptake at the higher concentration of

Na^+ (from 2269 to 3195 and from 408 to 612 nmoles·h^{-1}·g^{-1}) and the decreased open-circuit uptake at the lower Na^+ concentration (from 764 to 456 and from 612 to 376 nmoles·h^{-1}·g^{-1}) suggest a relationship between lipid uptake and the direction of the Na^+ gradient. Reversal of this gradient, as indicated by a change from positive to negative of the transmural PD measured in everted rat ileal sacs, apparently occurs at a Na^+ concentration of about 55 mequiv (ref. 36). These findings may be related to the influence of the Na^+ concentration of the medium upon the level of activity of a basally located, ouabain-sensitive Na^+ extrusion mechanism[37,38]. Under open-circuit conditions at 145 mequiv of Na^+ the mucosal epithelial membrane is negatively charged[32,39]. In spite of this effective electrical barrier for negatively charged mixed lipid micelles (see below), more lipid was taken up under open-circuit than under short-circuit conditions in which the electrical barrier would be decreased toward zero.

Does the mixed lipid micelle move as a counterion accompanying Na^+?

The answer to this question requires information regarding the distribution of lipid between two or more phases and the size and net surface charge of lipid micelles. During fat digestion and absorption the intraluminal contents of the intestine would be expected to contain an oil phase consisting of higher glycerides and some fatty acids, largely unionized, in equilibrium with an aqueous, micellar phase consisting of bile salt, monoglyceride and both ionized and unionized fatty acids[40]. Insoluble amphipaths, such as triglycerides, may be found in low concentration in micelles of bile salt and monoglyceride from which absorption of triglyceride *in vitro* may take place without prior hydrolysis[41]. For example, in the rat, tripalmitin is absorbed even when pancreatic lipase and bile salt are diminished below normal concentrations. Absorption takes place both of intact tripalmitin molecules and of the di- and monoglycerides and palmitic acid liberated by tripalmitin hydrolysis. While in transport across the mucosal membrane barrier or immediately upon entering into the mucosal cell these products of hydrolysis are rapidly resynthesized, primarily into triglyceride[42].

From their behavior on gel filtration columns mixed micelles, containing taurodeoxycholate and varying proportions of triolein, monoolein, oleic acid and cholesterol, were estimated to have a micellar radius of about 27 to 35 Å (ref. 43). Here, it is pertinent to point out that the physical characteristics of the epithelial membrane of rat ileum indicate that it is equivalent to a membrane having uniform circular pores with a radius of 36 Å and occupying 0.001 % of the surface area[39]. Micelles containing bile salt, monoglyceride and fatty acid have been described as aggregates of various shapes stabilized by adsorbed bile salt molecules oriented with their ionic heads projecting into the aqueous phase[44]. Although the pK_a of taurocholate is approx. 2 due to the strongly acidic sulfonate group[40], the pK_a of fatty acid present in a micelle containing bile salt approaches 6.4 (ref. 45). Therefore, at pH 7.4 such mixed lipid micelles would be expected to have a net negative surface charge.

With the above considerations in view it seems reasonable to suppose first, that negatively charged mixed lipid micelles would be present in preparations similar to those used in the studies reported here and second, that their size would permit entry through the mucosal epithelial surface. If such a micelle did accompany Na^+ as a counterion, then lipid uptake under short-circuit conditions would be expected to exceed open-circuit uptake. This follows from the fact that transmural transfer of Na^+ is increased in the absence of the opposing electrical potential barrier, a state which is

approached during short-circuit conditions[46]. The data in Table I indicate that this did not occur. However, this may mean only that the mixed micellar lipid is not crossing the mucosal membrane primarily in the form of counterions to Na^+ although some fraction of the lipid may be actually involved in this way.

The mode of intestinal lipid transport

There are a number of findings which, when considered together, point toward certain conclusions, however tentative, regarding the mode of lipid transport across the brush border membrane of the mucosal epithelial cell. There is rather clear electron-micrographic evidence which indicates that fine droplets of lipid (linseed oil) may cross the microvillar membrane in rat jejunum and be transported inside rather than between the microvilli in passing to the interior of the mucosal cell[15]. Other evidence implies that the mechanism by which micellar lipids cross the brush border membrane may be energy-independent[12]. Moreover, it was suggested earlier[21], and indicated here by no difference in the time taken to reach plateau ΔPD values induced by oleic acid and by triolein, that the rate of lipid entry may be independent of intracellular hydrolysis.

Several findings suggest the involvement of Na^+ in lipid transport. Adrenalectomy depressed the absorption of [^{14}C]triolein and [^{14}C]oleic acid in rats[47]. The studies reported here, showing mutual competitive inhibition between glyceryl monooleate and taurocholate and between each of these substances and glucose, suggest a common requirement for Na^+ probably mediated at the mucosal brush border surface. Furthermore, the experiments with [^{14}C]triolein under open- and short-circuit conditions also point toward a dependency upon Na^+ for the uptake of lipid.

At this point, therefore, it would appear that lipids may be transported across the mucosal epithelium by a process involving Na^+-dependent, energy-independent entry accompanied and/or followed by hydrolysis and intracellular resynthesis into glycerides. The kinetic characteristics of the entry step may be described as typical of a saturation phenomenon and they are consistent with carrier-mediated transport. The latter possibility, however, remains an open one.

ACKNOWLEDGEMENTS

This work was supported by Grants AM-08359 and AM-12424 from the National Institutes of Health.

I thank Mr. J.-D. WALDMAN for his skillful technical assistance with many of the experiments reported herein.

REFERENCES

1 I. LYON, *Federation Proc.*, 26 (1967) 767.
2 S. L. PALAY AND L. J. KARLIN, *J. Biophys. Biochem. Cytol.*, 5 (1959) 363.
3 C. T. ASHWORTH, V. A. STEMBRIDGE AND E. SANDERS, *Am. J. Physiol.*, 198 (1960) 1326.
4 R. JONES, W. A. THOMAS AND R. F. SCOTT, *Exptl. Mol. Pathol.*, 1 (1962) 65.
5 E. W. STRAUSS, *J. Cell Biol.*, 17 (1963) 597.
6 C. T. ASHWORTH AND J. M. JOHNSTON, *J. Lipid Res.*, 4 (1963) 454.
7 A. J. LADMAN, H. A. PADYKULA AND E. W. STRAUSS, *Am. J. Anat.*, 112 (1963) 389.
8 S. L. PALAY AND J.-P. REVEL, in H. C. MENG, *Lipid Transport*, Thomas, Springfield, 1964, p. 33.
9 C. A. M. HOGBEN, *Ann. Rev. Physiol.*, 22 (1960) 381.

10 A. F. HOFMANN AND B. BORGSTRÖM, *Federation Proc.*, 21 (1962) 43.
11 T. H. WILSON, *Intestinal Absorption*, Saunders, Philadephia, 1962, p. 176.
12 J. M. JOHNSTON AND B. BORGSTRÖM, *Biochim. Biophys. Acta*, 84 (1964) 412.
13 P. C. PHELPS, C. D. RUBIN AND J. H. LUFT, *Gastroenterology*, 46 (1964) 134.
14 R. J. BARRNETT AND J. ROSTGAARD, *N.Y. Acad. Sci.*, 131 (1965) 13.
15 M. SHINER, *Lancet*, 1 (1966) 349.
16 B. BORGSTRÖM, A. DAHLQVIST, G. LUNDH AND J. SJÖVALL, *J. Clin. Invest.*, 36 (1957) 1521
17 J. M. JOHNSTON, *Proc. Soc. Exptl. Biol. Med.*, 100 (1959) 669.
18 A. M. DAWSON AND K. J. ISSELBACHER, *J. Clin. Invest.*, 39 (1960) 150.
19 E. B. FELDMAN AND B. BORGSTRÖM, *Biochim. Biophys. Acta*, 125 (1966) 148.
20 G. CLÉMENT AND R. PARIS, *Bull. Soc. Chim. Biol.*, 47 (1965) 249.
21 R. J. C. BARRY, M. J. JACKSON AND D. H. SMYTH, *J. Physiol. London*, 185 (1966) 667.
22 L. LACK AND I. M. WEINER, *Am. J. Physiol.*, 200 (1961) 313.
23 L. LACK AND I. M. WEINER, *Federation Proc.*, 22 (1963) 1334.
24 M. R. PLAYOUST AND K. J. ISSELBACHER, *J. Clin. Invest.*, 43 (1964) 467.
25 P. R. HOLT, *Am. J. Physiol.*, 207 (1964) 1.
26 J. E. GLASSER, I. M. WEINER AND L. LACK, *Am. J. Physiol.*, 208 (1965) 359.
27 P. R. HOLT, *Am. J. Physiol.*, 210 (1966) 635.
28 L. LACK AND I. M. WEINER, *Am. J. Physiol.*, 210 (1966) 1142.
29 J. L. POPE, T. M. PARKINSON AND J. A. OLSON, *Biochim. Biophys. Acta*, 130 (1966) 218.
30 T. H. WILSON AND G. WISEMAN, *J. Physiol. London*, 123 (1954) 116.
31 H. A. KREBS AND K. HENSELEIT, *Z. Physiol. Chem.*, 210 (1932) 33.
32 I. LYON AND R. K. CRANE, *Biochim. Biophys. Acta*, 112 (1966) 278.
33 I. LYON, *Biochim. Biophys. Acta*, 135 (1967) 496.
34 E. G. BLIGH AND W. J. DYER, *Can. J. Biochem. Physiol.*, 37 (1959) 911.
35 M. S. PATTERSON AND R. C. GREENE, *Anal. Chem.*, 37 (1965) 854.
36 I. LYON AND R. K. CRANE, *Biochim. Biophys. Acta*, 135 (1967) 61.
37 S. G. SCHULTZ AND R. ZALUSKY, *J. Gen. Physiol.*, 47 (1964) 1043.
38 I. LYON AND R. K. CRANE, *Biochim. Biophys. Acta*, 126 (1966) 146.
39 P. F. CURRAN AND A. K. SOLOMON, *J. Gen. Physiol.*, 41 (1957) 143.
40 A. F. HOFMANN AND D. M. SMALL, *Ann. Rev. Med.*, 18 (1967) 333.
41 E. B. FELDMAN AND B. BORGSTRÖM, *Lipids*, 1 (1966) 128.
42 N. J. GREENBERGER, J. B. RODGERS AND K. J. ISSELBACHER, *J. Clin. Invest.*, 45 (1966) 217.
43 E. B. FELDMAN AND B. BORGSTRÖM, *Biochim. Biophys. Acta*, 125 (1966) 136.
44 K. D. DREHER, J. H. SCHULMAN AND A. F. HOFMANN, *J. Colloid Interface Sci.*, 25 (1967) 71.
45 T. MAKI AND N. SUZUKI, *Tohoku J. Exptl. Med.*, 84 (1964) 259.
46 R. J. C. BARRY, D. H. SMYTH AND E. M. WRIGHT, *J. Physiol. London*, 181 (1965) 410.
47 W. C. WATSON AND E. MURRAY, *J. Lipid Res.*, 7 (1966) 236.