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THE *IN VITRO* UPTAKE AND KINETICS OF RELEASE OF PALMITIC ACID AND TAURODEOXYCHOLATE FROM HAMSTER SMALL INTESTINAL SEGMENTS

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SUMMARY

1. Everted sacs from hamster proximal and distal small intestine were incubated for 10 min at 37° in a micellar solution containing labeled palmitic acid, sodium taurodeoxycholate and inulin. After incubation, efflux of the labeled compounds from the intestinal mucosa was measured by sequential 1-min rinsings in separate 20-ml volumes of ice-cold Krebs–Ringer phosphate buffer for a total of 25 min. The radioactivity in each rinsing solution, tissue homogenates and serosal fluids was assayed.

2. The uptake of labeled taurodeoxycholate and inulin by the proximal and distal small intestine was not significantly different. However, the amount of [1-^{14}C]palmitic acid taken up by the proximal small intestine was significantly greater.

3. A considerable fraction of the labeled substances taken up during the initial incubation could be released by rinsing. The distal small intestine released a greater fraction of labeled palmitic acid and its release appeared to be inversely related to the esterifying capacity of the intestine. Proportionately greater amounts of [^{3}H]taurodeoxycholate and [^{14}C]inulin than that of [1-^{14}C]palmitic acid were released by both proximal and distal small intestine.

4. Analysis of the kinetics of efflux of each substance indicated that efflux occurred from two compartments, one rapidly and one slowly turning over. The characteristics of efflux of labeled palmitic acid and taurodeoxycholate from the rapidly turning over compartment were similar to those of labeled inulin suggesting that they occupied the extracellular fluid space. The characteristics of efflux from the slowly turning over compartment were different for each substance, and no conclusions could be drawn regarding the location of this compartment. However, the finding that the efflux of [1-^{14}C]palmitic acid and [^{3}H]taurodeoxycholate from this compartment was slower than that of [^{14}C]inulin indicate that superficial binding sites may be involved in the reversible uptake of these substances. In addition the ratio of [^{3}H]taurodeoxycholate to [1-^{14}C]palmitic acid in this compartment was greatly in excess of the 10:1 ratio of the micellar incubation medium indicating that the efflux of [^{3}H]taurodeoxycholate exceeded that of [1-^{14}C]palmitic acid.

INTRODUCTION

It is known from *in vivo* studies in man and experimental animals that the principal site for the intestinal absorption of bile salt is the terminal ileum[1]. In contrast, virtually all ingested lipid is absorbed in the proximal intestine[2]. On the basis of these

findings it is believed that the bile salts in the proximal small intestine are confined predominantly to the intraluminal fluid, where by mediating the micellar solubilization of the end-products of lipid digestion, free fatty acid and β-monoglyceride, ensure their efficient absorption[3]. However, the manner in which the mixed micellar aggregate once formed, comes into contact with the epithelial surface and preferentially releases its free fatty acid–monoglyceride content is as yet unknown.

The results of *in vitro* experiments designed to study this problem have so far been conflicting. While some authors conclude from their results that the intact micellar aggregate penetrates the membrane[4,5] others believe that only the free fatty acid–monoglyceride component penetrates this barrier[6]. Furthermore, it is clear that *in vitro* preparations of the proximal intestine take up significantly more conjugated bile salt than would have been predicted from the findings *in vivo*[7]. In order to account for this apparent discrepancy it has been postulated without direct evidence that bile salt originally taken up by the tissue can subsequently be released back into the intraluminal fluid[4]. Data obtained recently in our laboratory have indicated that a significant proportion of both micellar free fatty acid and bile salt originally taken up by hamster proximal small intestine *in vitro* was reversibly bound to the tissue[8]. The finding that a significantly greater proportion of bile salt than of free fatty acid was available for release, indicated that hamster intestine binds fatty acid more tightly than bile salt. These results were felt to be compatible with the hypothesis that the initial step in the uptake of conjugated bile salt and fatty acid from micellar solution involved the reversible association of these molecules to binding sites, presumably located near or at the surface of the epithelial cell. Once bound, these molecules could proceed in either of two directions: they could be released back into the aqueous medium bathing the villous surface (net efflux) or they could be drawn deeper into the cell along electrochemical gradients (net influx).

In the case of free fatty acid taken up by the proximal intestine it can be expected that the relatively tight surface binding of fatty acid[9] as well as the favourable gradient for absorption created by the continuous removal of intracellular free fatty acid by the process of esterification, which is very active in this region[10], would promote a net influx. With regard to the uptake of bile salt in this region, it can be predicted that the relatively weak surface binding as well as the absence of an active transport mechanism proximally[1], would favour the return of bile salt molecules back into the intraluminal fluid.

It is reasonable therefore, to expect that the handling of free fatty acid and bile salt molecules would be different in the distal intestine. This region is normally not presented with fatty acid for absorption and possesses very little esterifying activity[9] whereas it does contain an active transport mechanism for the absorption of bile salt[1]. At present, however, there is no information about the reversible binding of micellar fatty acid and bile salt by the distal small intestine. This investigation was undertaken to study this aspect by characterizing and comparing the kinetics of *in vitro* efflux of [^{14}C]palmitic acid and sodium [^{3}H]taurodeoxycholate from the proximal and distal intestine.

MATERIALS

Palmitic acid and monoolein were purchased from the Hormel Institute, Austin, Minn. and sodium taurodeoxycholate from Maybridge Research Chemicals, Tintangel,

Cornwall, U.K. These chemicals were certified as at least 99 % pure by the suppliers and confirmatory tests with gas–liquid and thin–layer chromatography were in close agreement with the stated purities. [1-^{14}C]Palmitic acid (New England Nuclear Corporation) was certified to be 98 % pure by the supplier and on thin-layer chromatography 97 ± 1.0 % was present in the free fatty acid fraction. This fraction gave a single peak, corresponding to palmitate on gas–liquid chromatography. Sodium taurodeoxycholate was tritiated by the Wilzbach technique (Tracerlab, Waltham, Mass.), which yielded a chemically pure substance which was stored at 4° in ethanol. In view of the significant exchange of radioactivity which occurred with the dissolving medium, aliquots of sodium [^{3}H]taurodeoxycholate were run twice on thin-layer chromatography, extracted and recrystallized prior to incorporation into each micellar incubation medium. [*carboxy*-^{14}C]Inulin (New England Nuclear Corporation, Boston, Mass.) was found to be chemically pure when run on paper chromatography[11,*]. All chemicals used were reagent grade and all organic solvents were doubly distilled.

ANALYTICAL PROCEDURES

Thin-layer chromatography was carried out on standard glass plates (20 cm × 20 cm) coated with silica gel G (E. Merck Company, Darmstadt, Germany). For the separation of lipid fractions, 2′,7′-dichlorofluorescein was incorporated into the silicic acid and the solvent system used consisted of *n*-hexane–diethyl ether–acetic acid–methanol (90:20:2:3, by vol.)[12]. The lipid fractions were identified by comparison with simultaneously run standards (Hormel Institute). The fractions were visualized by ultraviolet illumination and the corresponding silica gel as well as appropriate blanks were scraped directly into counting vials containing 12 ml of a scintillation solution, made up of 4 g of Omnifluor (New England Nuclear Corporation, Boston, Mass.) dissolved in 1 l of toluene. Bile salts were separated using a solvent system of isooctane–acetic acid–isopropyl alcohol–isopropyl ether (120:60:60:60, by vol.)[13]. After separation, the plates were sprayed with a 10 % solution of phosphomolybdic acid in ethanol and heated briefly to visualize the bile salts. The R_F values for each bile salt were determined with standard bile salts (Maybridge).

Total radioactivity of the different incubation and washing solutions, as well as the tissue homogenates and serosal fluid, was assayed in 1-ml aliquots added to 12 ml of solution according to BRAY[14]. All samples were counted in a Tri-Carb liquid scintillation counter (Model 3375, Packard Instrument Company, Downers Grove, Ill.) with a minimum efficiency of 43 % for ^{14}C and 23 % for ^{3}H in toluene, and 38 % for ^{14}C and 11 % for ^{3}H in solution according to BRAY[14]. The samples were corrected for quenching by the channels ratio method[15], or by internal standardization.

Aliquots of samples containing [^{14}C]inulin were counted in solution according to BRAY[14] as described. In view of the fact that a rapid loss of activity has been found to occur from [^{14}C]inulin in dioxane based scintillation media[16], all such samples were counted within 24 h of preparation, as preliminary experiments indicated that only a negligible loss of radioactivity had occurred during this period.

* Kindly performed by Dr. K. Baerlocher, the deBelle Laboratory for Biochemical Genetics, Montreal Children's Hospital Research Institute, Montreal, Canada.

EXPERIMENTAL PROCEDURE

Female golden hamsters, weighing from 100 to 120 g (Quebec Breeding Laboratories, LaPrairie, Quebec) were fasted overnight and anaesthetised with ether. The small intestine was removed and rinsed with ice-cold saline. The gut was then everted over a chilled glass rod and filled with 0.15 M Krebs–Ringer phosphate buffer (pH 6.3) (Ca^{2+} and Mg^{2+} omitted). Sacs, 3 cm in length, were prepared from the proximal and distal small intestine by cutting between double ligatures. The portions of intestine used were restricted to the proximal 15 cm from the pylorus and terminal 12 cm from the ileocecal valve, as these regions were found in preliminary experiments to manifest a uniform uptake and release of [1-^{14}C]palmitic acid and sodium [^{3}H]taurodeoxycholate and [1-^{14}C]palmitic acid esterification. The everted sacs of each region were randomized and incubated in pairs for 10 min at 37° under O_2–CO_2 (95:5, by vol.) in 10 ml of micellar incubation medium which contained 10 μmoles [1-^{14}C]palmitic acid (0.05μC/μmole), 10 μmoles monoolein, 100 μmoles sodium [^{3}H]taurodeoxycholate (0.05–0.08 μC/μmole) and 100 μmoles glucose made up in 0.15 M Krebs–Ringer phosphate buffer (pH 6.3, Ca^{2+} and Mg^{2+} omitted)[4,17]. In some of the experiments 0.10 μmole of [^{14}C]inulin (0.07 μC/μmole) was added to the incubation medium, which was identical to that described above, except that stable palmitic acid was used in place of [1-^{14}C]-palmitic acid. Following incubation, the sacs were drained along the wall of the incubation vessel and transferred sequentially into a series of unlabeled washing solutions maintained at 4° to ensure that the release of the radioactive compounds was unaffected by the energy-requiring processes of the tissue.

The rinsing procedure was carried out in the following order: (a) a single 20-sec rinse in an identical but unlabeled micellar solution, (b) fifteen separate 1-min rinses followed by five 2-min rinses in Krebs–Ringer phosphate buffer (pH 6.3) (*i.e.* for a total of 25 min). Each successive rinse was carried out in a fresh 20-ml volume of unlabeled rinsing solution, by gently swirling the sacs every 30 sec. Rinsing of the sacs for longer than 25 min or the use of more vigorous rinsing resulted in the histological deterioration of the tissue when examined under the light microscope. The principal changes noted were the shedding of significant numbers of epithelial cells from the villi, as well as the appearance of irregularities in the PAS-positive "brush border region" of the remaining epithelial cells.

The amounts of [1-^{14}C]palmitic acid and [^{3}H]taurodeoxycholate released into any given rinsing solution never exceeded their theoretical solubilities in Krebs–Ringer phosphate buffer [18,19]. In addition, preliminary studies showed that identical results were obtained by rinsing with volumes of 20, 50 and 100 ml of Krebs–Ringer phosphate buffer, indicating that the solubilizing capacity of the rinsing solutions for palmitic acid and sodium taurodeoxycholate was not a limiting factor in the release of [1-^{14}C]palmitic acid and [^{3}H]taurodeoxycholate from the tissue.

After completion of the rinsing sequence, the sacs were drained of serosal fluid, weighed, and homogenized in 10 ml of normal saline in an all-glass Potter–Elvehjem homogenizer. 1-ml aliquots of incubation medium, rinsing solutions, tissue homogenate and serosal fluid were directly counted in solution according to BRAY[14] as described. The remaining samples were extracted with chloroform–methanol (2:1, by vol.)[20], and aliquots of the lipid extract were subjected to thin-layer chromatography to determine the distribution of ^{14}C radioactivity among the free and esterified fatty acid fractions.

The total uptake of [1-^{14}C]palmitic acid, [^{3}H]taurodeoxycholate and [^{14}C]inulin (μmoles $\times$ 10^{-3}/100 mg wet weight) were calculated by addition of the amounts of these respective substances released into all the rinsing solutions to that which remained in the tissue at the end of the rinsing sequence. As no direct measurements of fatty acid or bile salt mass were done in these experiments, all calculations assume that the specific activity of the labeled fatty acid and bile salt which was released into the washing solutions was identical to that present in the initial incubation medium. This assumption was based on the observation that intestinal sacs obtained from fasted animals did not release any determinable amount of fatty or bile acids (gas–liquid and thin-layer chromatography) when subjected to an identical incubation and rinsing sequence in Krebs–Ringer phosphate buffer. Analysis of the tissue immediately after incubation, prior to rinsing, yielded values for total uptake which were within one standard deviation of the mean value obtained by the method described above. The amount of tissue-esterified [1-^{14}C]palmitic acid at the end of incubation and that after rinsing for 25 min at 4°, remained within very narrow limits, indicating that the ^{14}C radioactivity released during the rinsing procedure had not been derived from the lipolysis of this fraction. Furthermore, the substitution of [9,10-$^{3}H_2$]palmitic acid for [1-^{14}C]palmitic acid in the incubation medium did not change the results obtained. This finding is consistent with the conclusion that oxidation of the terminal carboxyl group of palmitic acid had not occurred to any significant extent under our experimental conditions.

STATISTICAL ANALYSIS

The Student's *t*-test for paired values was used for comparing individual differences. Only P values of <0.01 were considered as being significant.

The curves of efflux of tissue radioactivity were obtained by the use of semilogarithmic plots of the amounts of the respective labeled substances released during each successive rinse against time. The exponential components of the curves were resolved according to the method of RIGGS[21] and the resulting straight lines were fitted by the method of least squares adapted for logarithmic values of the amounts released. The regression lines derived in this manner were then compared statistically.

RESULTS

Uptake of [1-^{14}C]palmitic acid, sodium [^{3}H]taurodeoxycholate and [^{14}C]inulin by proximal and distal hamster small intestine

The data shown in Table I indicate that the uptake of each substance by the proximal and distal small intestine was not significantly different except in the case of [1-^{14}C]palmitic acid which was taken up in significantly greater amounts by the proximal small intestine. The uptake of [^{3}H]taurodeoxycholate by the sacs was proportionately less than that of [1-^{14}C]palmitic acid and the ratio of taurodeoxycholate to palmitic acid radioactivity in the tissue was 7.3:1 and 9.3:1 in the proximal and distal small intestine, respectively. These values were found to be significantly smaller than the corresponding ratio of 10:1 present in the micellar incubation medium. Even though the fractional uptake of [^{3}H]taurodeoxycholate by the everted sacs was lower than that of [1-^{14}C]palmitic acid, it was still greatly in excess of that predictable from *in vivo* experiments[7].

TABLE I

UPTAKE OF [1-^{14}C]PALMITIC ACID, SODIUM [^{3}H]TAURODEOXYCHOLATE AND [^{14}C]INULIN BY PROXIMAL AND DISTAL SEGMENTS OF HAMSTER SMALL INTESTINE

Everted gut sacs prepared from proximal and distal hamster small intestine were incubated in duplicate for 10 min at 37° in 10 ml of micellar solution, containing 10 μmoles [1-^{14}C]palmitic acid (0.05 μC/μmole), 10 μmoles monoolein, 100 μmoles sodium [^{3}H]taurodeoxycholate (0.05 μC/μmole) and 100 μmoles glucose made up in 0.15 M Krebs–Ringer phosphate buffer (pH 6.3). Only stable palmitic acid was present in the incubation media containing 0.1 μmole of [^{14}C]inulin (0.07 μC/μmole). The amount of [1-^{14}C]palmitic acid, sodium [^{3}H]taurodeoxycholate, and [^{14}C]inulin present in the tissue immediately after incubation is termed the total uptake. This total uptake is also expressed as the percentage of the amount of labeled compound initially present in the incubation medium (shown in brackets in the first column). The figures represent the mean ± S.E.

Compound	*Intestinal segment*	*Total uptake (μmoles × 10^{-3}/100 mg wet wt.)*	*% of radioactivity taken up per 100 mg wet wt.*
[1-^{14}C]Palmitic acid (10 μmoles)	Proximal	142.4 ± 21.0	1.42 ± 0.21
	Distal	114.0 ± 10.6	1.14 ± 0.11
Sodium [^{3}H]taurodeoxycholate (100 μmoles)	Proximal	1027.4 ± 129.5	1.03 ± 0.13
	Distal	1073.9 ± 130.5	1.07 ± 0.13
[^{14}C]Inulin (0.1 μmole)	Proximal	0.917 ± 0.03	0.92 ± 0.03
	Distal	0.937 ± 0.04	0.94 ± 0.03

TABLE II

COMPARTMENTAL DISTRIBUTION OF [1-^{14}C]PALMITIC ACID, SODIUM [^{3}H]TAURODEOXYCHOLATE AND [^{14}C]INULIN

Everted gut sacs were incubated as described under Table I. The sacs were then subjected to a 20-sec rinse in unlabeled micellar solution and then to twenty five 1-min sequential rinses in separate 20-ml volumes of ice-cold Krebs–Ringer phosphate (0.15 M, pH 6.3). Using the total uptake of [^{14}C]palmitic acid, sodium [^{3}H]taurodeoxycholate and [^{14}C]inulin as 100% for each substance, the table indicates the summated percentages of each labeled substance released into the rinsing solutions, as well as the percentages of the total uptake which remained in the tissue and serosal fluid after rinsing. All calculations were based on the assumptions outlined in EXPERIMENTAL PROCEDURES. The figures represent the mean ± S.E.

Compound	*Intestinal segment*	*% Released into Krebs–Ringer phosphate buffer*	*% Remaining in the*	
			Tissue	*Serosal fluid*
[1-^{14}C]Palmitic acid	Proximal	33.0 ± 3.8	66.7 ± 3.6	0.7 ± 0.1
	Distal	46.2 ± 4.3	52.9 ± 4.2	1.0 ± 0.1
Sodium [^{3}H]taurodeoxycholate	Proximal	79.2 ± 2.6	19.2 ± 2.0	3.3 ± 0.1
	Distal	80.5 ± 1.9	14.8 ± 1.9	4.7 ± 0.5
[^{14}C]Inulin	Proximal	89.0 ± 1.7	5.9 ± 1.5	5.1 ± 0.2
	Distal	90.5 ± 1.6	4.3 ± 1.2	5.2 ± 0.3

The reversible uptake of [1-^{14}C]palmitic acid, [^{3}H]taurodeoxycholate and [^{14}C]inulin by hamster small intestine

A significant amount of each substance was found to be reversibly bound to the tissue and could be removed by sequential rinsing in Krebs–Ringer phosphate buffer (Table II). Approx. 90% of the [^{14}C]inulin and 80% of the [^{3}H]taurodeoxycholate taken up during the 10 min of incubation was released in the rinsing solutions. These values were the same regardless of whether the sacs had been prepared from proximal

or distal small intestine. In contrast, only 33.0 ± 3.8 and 46.2 ± 4.3 % of [1-^{14}C]palmitic acid taken up by the proximal and distal small intestine, respectively, could be removed. The fraction of [1-^{14}C]palmitic acid released from the distal small intestine was significantly greater than that released proximally. This difference was minimized because of the use of randomized segments of proximal and distal small intestine. However, when segments of corresponding levels of jejunum and ileum were compared, a distinct gradient of releasable ^{14}C radioactivity from proximal to distal small intestine was obtained. The fractions released from the first two proximal and the last two distal segments differed by a factor of 2–3. Independent of the intestinal level of the segments used, 93.1 ± 1.1 % of the [1-^{14}C]palmitic acid released into any of the Krebs–Ringer phosphate rinses was in the form of free fatty acid.

Following the 25-min rinse, a significant amount of radioactivity was retained in the tissue. The greatest retention of radioactivity occurred in the case of [1-^{14}C]-palmitic acid in the segments of proximal small intestine, namely 66.7 ± 3.6 %. The corresponding value for the segments of distal small intestine, 52.9 ± 4.2 %, was found to be significantly smaller. Again, when segments of corresponding levels of jejunum and ileum were compared, the difference between the radioactivity retained in the tissue increased progressively from proximal to distal small intestine. In addition, it was found that 59.5 ± 1.9 and 15.9 ± 2.1 % of the tissue radioactivity was in the form of esterified fatty acid in the proximal and distal small intestine, respectively. The findings suggested that an inverse relationship existed between the esterifying capacity of the tissue and the proportion of [1-^{14}C]palmitic acid which was available for release into Krebs–Ringer phosphate buffer. Less than 20 % of the uptake of [^{3}H]taurodeoxycholate and less than 6 % of that of [^{14}C]inulin was retained in the tissue after rinsing. The serosal fluid contained 3.3 ± 0.1 and 4.7 ± 0.5 % of the uptake of [^{3}H]taurodeoxycholate in the proximal and distal small intestine, respectively. These values were significantly different at the 1 % level, and may result from the active transport of bile salt by the distal small intestine, as experiments done at 4° did not show this difference. It was found that approx. 5 % of the [^{14}C]inulin taken up by the proximal and distal small intestine was present in the serosal fluid, while the amounts of [1-^{14}C]palmitic acid in this compartment were very small and never exceeded 1.4 % of the uptake.

These results indicated that [1-^{14}C]palmitic acid and [^{3}H]taurodeoxycholate taken up by the sacs behaved very differently from each other in these experiments. Firstly, the fraction of [^{3}H]taurodeoxycholate released into Krebs–Ringer phosphate buffer exceeded that of [^{14}C]palmitic acid by a factor of 2.4 and 1.8 in the proximal and distal small intestine, respectively. Secondly, the percentage of [^{14}C]palmitic acid retained in the tissue was approximately 3 times greater than that of [^{3}H]taurodeoxycholate, regardless of whether the sacs were prepared from proximal or distal small intestine. Thirdly, the proportion of [^{3}H]taurodeoxycholate in the serosal fluid compartment of both the proximal and distal segments was 4.7 times larger than that of [1-^{14}C]palmitic acid.

Kinetics of release of the labeled substances during sequential washing of the intestinal sacs in Krebs–Ringer phosphate buffer

The semilogarithmic plot of the amounts of each substance released from the tissue as a function of time is shown in Figs. 1 and 2. In all cases each curve was

readily resolved into two exponential functions which were assumed to represent two compartments with significantly different half-times of efflux (Table III).

Compartment A, in all cases, was rapidly turning over and had a half-time of release ($t_{1/2}$) ranging between 0.93 and 1.24 min. In contrast, compartment B, in each case, displayed a much slower and more variable half-time of release (Table III).

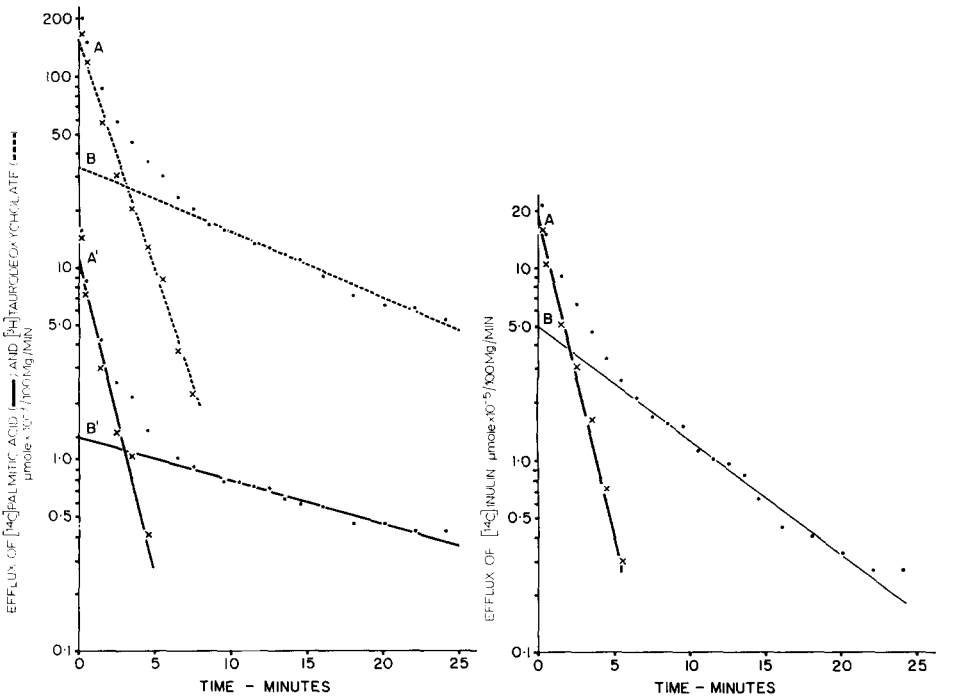

Fig. 1. The efflux curves for [1-^{14}C]palmitic acid and sodium [^{3}H]taurodeoxycholate into Krebs–Ringer phosphate buffer from everted sacs of proximal small intestine. The everted sacs were pre-incubated for 10 min as described under Table I. The sacs were then subjected to a 20-sec rinse in 10 ml of unlabeled micellar solution and to twenty five 1-min sequential rinses in separate 20-ml volumes of ice-cold Krebs–Ringer phosphate buffer. The amounts released during each successive rinse (●) are plotted against time of rinsing (min). Each point represents the mean value of six experiments. The standard error for each point never exceeded 0.50 and 20.9 μmoles $\times$ 10^{-3}/100 mg for [^{14}C]palmitic acid and sodium [^{3}H]taurodeoxycholate, respectively. All calculations were based on the assumptions outlined under EXPERIMENTAL PROCEDURES. The efflux curves of labeled palmitic acid and sodium taurodeoxycholate from the distal small intestine were very similar and are not shown in this figure.

Fig. 2. The efflux curve for [^{14}C]inulin into Krebs–Ringer phosphate buffer from everted sacs of proximal small intestine. The experimental conditions were indentical to those described under Fig. 1. The amount of [^{14}C]inulin released during each successive rinse in Krebs–Ringer phosphate buffer (●) is plotted against time of rinsing (min). Each point represents the mean of four experiments. The standard error of each point never exceeded 0.5 μmole $\times$ 10^{-5}/100 mg. The efflux curve of labeled inulin from the distal small intestine was identical to the curve shown in this figure.

Statistical analysis of the regression lines derived for compartment A indicated that there was no significant difference between the characteristics of efflux of [1-^{14}C]-palmitic acid, [^{3}H]taurodeoxycholate and [^{14}C]inulin from either the proximal or distal small intestine. This suggested that the location of compartment A was the same

TABLE III

EFFLUX OF [1-^{14}C]PALMITIC ACID, SODIUM [^{3}H]TAURODEOXYCHOLATE AND [^{14}C]INULIN INTO KREBS–RINGER PHOSPHATE BUFFER

The everted sacs were incubated as described under Table I. Efflux was into separate 20-ml volumes of Krebs–Ringer phosphate buffer. The figures for $t_{1/2}$ were calculated from the lines A and B for curves drawn as in Figs. 1 and 2. Each curve was drawn from the mean points of six experiments. Compartment size expressed as μmoles $\times 10^{-3}$/100 mg wet tissue was derived by expressing the amounts released into each rinse as in Figs. 1 and 2 as the fraction of the total exchangeable radioactivity in the tissue. The resultant curves were resolved as described in *Statistical analysis* and pool size was derived by extrapolating each line to zero time. All calculations were based on the assumption outlined in EXPERIMENTAL PROCEDURE.

Compound	*Intestinal segment*	($t_{1/2}$ *min*)		*Compartment size*	
		A	*B*	*A*	*B*
[1-^{14}C] Palmatic acid	Proximal	0.93	14.44	15.11	18.20
	Distal	1.05	10.19	15.32	15.14
Sodium [^{3}H]taurodeoxycholate	Proximal	1.24	8.77	201.06	330.76
	Distal	1.22	8.45	198.82	358.74
[^{14}C] Inulin	Proximal	0.96	5.06	0.32	0.23
	Distal	0.95	5.06	0.30	0.24

for all three substances measured, and corresponded to the extracellular fluid space as defined by [^{14}C]inulin.

Statistical comparison of the data derived for compartment B revealed that while no significant difference was found for the efflux of each labeled substance from the proximal and distal small intestine, the efflux characteristics of each compound differed very significantly from each other and especially from [^{14}C]inulin. The half-time of release of [^{14}C]palmitic acid from compartment B was much slower than that of [^{3}H]taurodeoxycholate which in turn was significantly slower than that of [^{14}C]-inulin. These differences may have resulted from the fact that each substance was contained within a different anatomical compartment, or alternately, that each substance was bound with different degrees of tightness to superficial binding sites.

The values derived for compartment size (see Table III) represent the amounts of the isotopic substances present in each compartment before any efflux had occurred. While the ratio for [^{3}H]taurodeoxycholate to [1-^{14}C]palmitic acid in the initial incubation medium was 10:1, the ratio of these substances in compartment A was 13.3:1 and 13.0:1 for the proximal and distal small intestine, respectively. The corresponding ratios for compartment B were 18.2:1 and 23.8:1. The great excess of [^{3}H]taurodeoxycholate to [1-^{14}C]palmitic acid in compartment B is mainly responsible for the finding that approximately twice as much of the bile salt as compared to the palmitic acid taken up by the everted sacs was available for release during the 25 min of rinsing in Krebs–Ringer phosphate buffer (Table II).

DISCUSSION

It has been shown that a significant proportion of fatty acid originally taken up by a variety of *in vitro* preparations is available for release into different aqueous media[22–24]. We have extended these observations to the release of [1-^{14}C]palmitic acid, and sodium [^{3}H]taurodeoxycholate by both proximal and distal small intestine of

hamster. Our results clearly indicate that although both substances were reversibly bound, they behaved very differently with regard to their uptake and availability for release into Krebs–Ringer phosphate buffer. The uptake of micellar [^{3}H]taurodeoxycholate by segments of proximal and distal small intestine was within very narrow limits and the fraction available for release in the washing solution, which was approx. 80 % of the uptake, was independent of the intestinal origin of the segments. In contrast, the uptake and release of [1-^{14}C]palmitic acid was markedly dependent on the level from which the intestinal segments were prepared. Whereas the uptake by the proximal segments was 142.4 ± 21.0 μmoles × 10^{-3}/100 mg, of which 33.0 ± 3.8 % was available for release, the distal segments took up and released significantly different amounts of [1-^{14}C]palmitic acid, *i.e.* 114.0 ± 10.6 μmoles × 10^{-3}/100 mg and 46.2 ± 4.3 %, respectively. These differences became even greater when intestinal segments from corresponding levels of jejunum and ileum, instead of randomized proximal and distal segments, were compared. In view of the finding that a significant difference in the capacity for [1-^{14}C]palmitic acid esterification existed between the proximal and distal small intestine (59.5 ± 1.9 *vs.* 15.9 ± 2.1 %), it appeared that the greater esterification of [1-^{14}C]palmitic acid in the proximal small intestine had contributed to the greater uptake and the diminished fractional release of [1-^{14}C]palmitic acid in this region. This conclusion was supported by the finding that brush border preparations of proximal and distal small intestine, in which no esterifying activity could be detected, took up and released identical amounts of [1-^{14}C]palmitic acid and by the demonstration of a highly significant linear inverse relationship between the capacity for esterification and the fraction of [1-^{14}C]palmitic acid available for release from the small intestinal segments[9]. The factors described above would promote a large net influx of fatty acid, especially in the proximal small intestine, while most of the bile salt taken up in this region would be available for efflux back into the intraluminal fluid bathing the villous surface of the intestine.

The efflux of [1-^{14}C]palmitic acid and [^{3}H]taurodeoxycholate could be described in terms of two functionally distinct compartments regardless of whether the sacs were prepared from proximal or distal small intestine (Fig. 1, Table III). Compartment A, which had a rapid half-time of efflux was identical for both substances, in contrast, compartment B, which had a much slower half-time of efflux, was markedly different for each substance. These results are in agreement with those of HOFFMAN[23], who has also described two compartments for the efflux of [^{14}C]oleic acid from rat jejunum. The half-times of release of [1-^{14}C]palmitic acid derived from our data were much shorter than those obtained for [^{14}C]oleic acid. These results may be attributable to either a difference in the physical properties of the fatty acids tested, to differences in the binding properties of the intestinal mucosa towards different fatty acids, or to variations in the experimental procedures employed. The efflux of [^{14}C]inulin from hamster small intestine also occurred from two analogous compartments (Fig. 2), the first of which, compartment A, had a half-time of efflux which was very similar to that obtained for the efflux of [1-^{14}C]palmitic acid and [^{3}H]taurodeoxycholate from the same compartment. These findings suggest that compartment A for [1-^{14}C]palmitic acid and [^{3}H]taurodeoxycholate was largely, if not entirely, confined to the extracellular space as defined by [^{14}C]inulin. In contrast, the efflux of these substances from compartment B was significantly slower than that of [^{14}C]inulin (Table II). This would imply that either these lipid substances were in a different anatomical com-

partment or that all of them had been bound with different degrees of tightness to superficial binding sites, which as indicated by experiments with brush border preparations[9], may possibly be located in the glycocalyx or the microvillous membrane. It is of interest that the ratio of [^{3}H]taurodeoxycholate to [1-^{14}C]palmitic acid in compartment B was 18.2:1 and 23:8:1 for the proximal and distal small intestine, respectively (Table III). These values were significantly in excess of the corresponding ratios in compartment A, namely 13.3:1 and 13.0:1, as well as the ratio of 10:1 present in the initial micellar incubation medium. It would appear that the excess of bile salt in compartment B as well as the more rapid half-time for its release accounted for the finding that a greater proportion of the uptake of [^{3}H]taurodeoxycholate than [1-^{14}C]palmitic acid was available for release from the tissue (Table II).

The conclusions which may be dawn from the results of these *in vitro* experiments with regard to the process of fat absorption *in vivo* must of course remain speculative. It is conceivable that the distribution of fatty acid and bile salt in the compartments described might be very different in the presence of an intact circulatory system. Furthermore, it is not known to what extent fatty acid and bile salt taken up *in vivo* can be removed by serial washings. Recent preliminary *in vivo* experiments in our laboratory (S. MISHKIN, E. CRYSTAL AND J. I. KESSLER, unpublished observation) in which the same parameters were investigated in ligated intestinal segments with intact circulation seem to support our *in vitro* results and lend further support to the hypothesis that a significant fraction of the micellar fatty acid and bile salt taken up by the intestinal mucosa is contained within the extracellular fluid space of this tissue (compartment A), possibly in the form of micellar aggregates similar in composition to those present in the micellar phase of the intraluminal fluid. In addition, it would appear that one of the initial steps in the absorption of micellar fatty acid and bile salt involves the reversibly binding of these substances to binding sites, located possibly near the surface of the epithelical cell (compartment B). Once present in these two compartments, these molecules can proceed in either of two directions: they can be released back into the aqueous medium bathing the villous membrane or be drawn deeper into the cell along electro-chemical gradients maintained by the continuous esterification of fatty acids. If the Krebs–Ringer phosphate used in our artificial system shares the physical properties of the fluid medium bathing the villous surface *in vivo*, it can be expected that bile salts, because of their weaker association with the intestinal mucosa and the absence in the proximal small intestine of an active mechanism to maintain a proper concentration gradient for inward flow, would be released in excess of fatty acid. This mechanism would ensure the presence of an effective intraluminal concentration of bile salts in the proximal small intestine, resulting in a continuous formation of micelles and the efficient absorption of fatty acid, which once esterified would not be available for intraluminal release. According to this hypothesis, the continuous uptake and release of bile salt would occur repeatedly along the length of the small intestine, until the terminal ileum was reached, where the active mechanism for bile salt absorption would result in its irreversible removal from the lumen.

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REFERENCES

1 J. M. DIETSCHY, *J. Lipid Res.*, 9 (1968) 297.
2 J. R. SENIOR, *J. Lipid Res.*, 5 (1964) 495.
3 A. F. HOFMANN AND D. M. SMALL, *Ann. Rev. Med.*, 18 (1967) 333.
4 S. G. GORDON AND F. KERN, *Biochim. Biophys. Acta*, 152 (1968) 372.
5 J. H. SCHULMAN, M. ROSOFF AND H. ERBRING, *Symp. on Biophysics and Physiology of Biological Transport, Frascati, 1965, Surface Properties of Molecular Aggregates of Micellar Size in Relation to Membrane Permeability and Adhesion*, Springer-Verlag, Wien–New York, 1967.
6 A. G. THORNTON, G. V. VAHOUNY AND C. R. TREADWELL, *Proc. Soc. Exptl. Biol. Med.*, 127 (1968) 629.
7 W. J. SIMMONDS, A. F. HOFMANN AND E. THEODOR, *J. Clin. Invest.*, 46 (1967) 874.
8 S. MISHKIN AND J. I. KESSLER, *Biochim. Biophys. Acta*, 202 (1970) 222.
9 S. MISHKIN AND J. I. KESSLER, in preparation.
10 A. M. DAWSON AND K. J. ISSELBACHER, *J. Clin. Invest.*, 39 (1960) 150.
11 J. C. HAWORTH AND N. H. BARCHUK, *Paediatrics*, 39 (1967) 608.
12 J. L. BROWN AND J. M. JOHNSTON, *J. Lipid Res.*, 3 (1962) 480.
13 J. A. GREGG, *J. Lipid Res.*, 7 (1966) 579.
14 G. A. BRAY, *Anal. Biochem.*, 1 (1960) 279.
15 L. A. BAILLIE, *Intern. J. Appl. Radiation Isotopes*, 8 (1960) 1.
16 F. J. GENNARI, S. CORTELL AND W. B. SCHWARTZ, *J. Appl. Physiol.*, 28 (1970) 105.
17 F. KERN, Jr. AND B. BORGSTROM, *Biochim. Biophys. Acta*, 98 (1965) 520.
18 L. M. JOHN AND J. W. MCBAIN, *J. Am. Oil Chemists' Soc.*, 25 (1948) 40.
19 *Handbook of Chemistry and Physics*, Chemical Rubber Co., Cleveland, Ohio, 46th ed., 1965–66, p. C-559.
20 J. FOLCH AND G. H. SLOANE-STANLEY, *J. Biol. Chem.*, 226 (1957) 497.
21 D. S. RIGGS, *The Mathematical Approach to Physiological Problems, Fitting Experimental Observations by a Multiple Exponential Function*, Williams and Williams, Baltimore, Md., 1963, p. 146.
22 D. PORTE, Jr. AND C. ENTENAMN, *Am. J. Physiol.*, 208 (1965) 607.
23 N. E. HOFFMAN, *Biochim. Biophys. Acta*, 196 (1970) 193.
24 S. B. SHOHET, D. G. NATHAN AND M. L. KARNOVSKY, *J. Clin. Invest.*, 47 (1968) 1096.