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Research Article

STUDIES ON LIPID METABOLISM IN THE SMALL INTESTINE WITH OBSERVATIONS ON THE ROLE OF BILE SALTS * †

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It is now well established that the esterification of long chain fatty acids is an important step in their transport across the small intestinal mucosa before they appear in the lymph in chylomicrons. Evidence for this process has been well reviewed recently (1, 2). The main ester formed by the mucosa is triglyceride but a small proportion of the fatty acids is incorporated into phospholipids and cholesterol esters. We have recently reported some *in vitro* studies on the incorporation of long chain fatty acids into glycerides using homogenates of rat and human small intestine mucosa and have delineated some of the cofactor requirements involved (3). This work has now been extended to the intact mucosa of the rat. Observations are presented on factors influencing the uptake and esterification of palmitate-1-C^{14} and the incorporation of label into lipid from C^{14}-glucose. In addition, evidence is presented for the concept that conjugated bile salts directly stimulate glyceride metabolism in the intestinal mucosa.

MATERIALS AND METHODS

Palmitic acid-1-C^{14} and uniformly labeled glucose-C^{14} were obtained from the Volk Radiochemical Company, Chicago, Illinois. The C^{14}-labeled palmitate was purified and made up into a solution as previously described (2). Crystalline bovine albumin was obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio. Cholic acid and desoxycholic acid were obtained from Matheson, Coleman and Bell, Norwood, Ohio and the cholic acid was recrystallized from 70 per cent ethanol. The taurine derivatives of these acids and glycocholic acid were prepared according to Norman (4) and their purity tested by melting point determinations and paper chromatography (5). Hydrolysis of the conjugated bile salts, glycocholate and taurodesoxycholate was carried out by dissolving them in 2 N NaOH and then heating in sealed Pyrex tubes at 140° C for 3 hours. The resulting solution was diluted with water, acidified, and the precipitated free bile salt filtered, washed and dried. In earlier experiments commercial sodium taurocholate (Pfanstiehl Laboratory, Waukegan, Ill.) was used. This contained a faint trace of glycocholate which could only be demonstrated by chromatography when there was heavy overloading of Whatman no. 3 filter paper. No unconjugated bile salts were present but there were some contaminating pigments. The commercial taurocholate gave substantially the same results as the synthetic material which was used in later experiments.

Female albino rats (Charles River Laboratories, Boston, Mass.) weighing 150 to 250 g were fasted overnight. They were killed by a blow on the head and the small intestine washed out at room temperature with oxygenated Krebs-Ringer phosphate buffer, pH 7.4, modified to contain half the usual concentration of calcium. This buffer was used in all the experiments described. The small intestine was everted on a glass rod following the technique of Wilson and Wiseman (6) and then cut across so as to form small cylindrical segments or intestinal rings. These measured approximately one-eighth inch (lengthwise) and weighed between 80 to 150 mg (wet weight). Since one of the problems in using slices or sacs of small intestine in metabolic studies is the variation in activity along this organ, some preliminary experiments were performed to determine the distribution of esterifying activity along the intestine. It was found that under optimal conditions there was moderate (50 per cent) variation of activity along the upper four-fifths of the jejuno-ileum. The terminal ileum showed an abrupt and profound fall off in activity to 5 to 10 per cent that of the upper intestine. Colonic tissue was about as active as terminal ileum. Thus the reproducibility of our method was increased in subsequent studies by *1*) using only the upper two-thirds of the jejuno-ileum, *2*) by using at least two segments of intestine in each incubation flask with each segment coming from a different site, and *3*) by performing all incubations in duplicate. Incubations were carried out in 25 ml Erlenmeyer flasks and the incubation mixture contained either palmitate-1-C^{14} or uniformly labeled glucose-C^{14} plus other agents such as bile salts, Tween "80"

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(polyoxyethylene sorbitan mono-oleate) and albumin as indicated in the individual experiments. The incubation mixture was made up to a volume of 4 ml with buffer. All incubations were performed under oxygen at 25° C in a Dubnoff shaking incubator. It was found that incubating at 25° as compared to 37° C reduced the transport of esterified lipid out of the tissue to less than 0.1 per cent [using the everted sac technique (6)] and thus increased the validity of simply analyzing tissue lipid at the end of the incubation. This temperature also permitted a better demonstration of the effects of bile salts on the system. At the end of the incubation the flasks were placed on ice and the intestinal segments washed in cold Krebs-Ringer buffer, blotted and weighed on a microtorsion balance. The method of extracting lipid from the tissue was based on that of Folch, Lees and Sloane Stanley (7). Segments were homogenized in chloroform-methanol (2:1) in a tissue grinder (Potter-Elvehjem, Teflon pestle) and the mixture allowed to stand at least 30 minutes at room temperature. It was then filtered through Whatman no. 1 filter paper into a 50 ml Pyrex centrifuge tube which was fitted with a ground glass stopper. A volume of water was added which was approximately one-fifth of the chloroform-methanol solution. The mixture was shaken and the separation into two phases facilitated by centrifuging at 2,500 rpm for 5 minutes. The upper aqueous methanol phase was drawn off and discarded. In experiments with radioactive palmitate the resulting chloroform solution was evaporated to dryness and the lipid, after being dissolved in 10 ml of petroleum ether, was washed once with 20 ml of 0.1 M KCl to remove any nonlipid radioactive metabolites. The total lipid radioactivity was assayed by counting 2 ml of this petroleum ether extract in a lipid scintillation spectrometer (3). Duplicate tissue analyses agreed within 10 to 15 per cent. Another aliquot of the petroleum ether solution was evaporated to dryness in a lipped test tube, taken up in 2 ml of wet ether and the unesterified fatty acids removed by passage through an Amberlite IRA-400 column. The ether eluate representing esterified lipid was evaporated, taken up in petroleum ether, and counted. In experiments with C^{14}-labeled glucose the chloroform solution containing the lipid was evaporated and the lipid taken up in 10 ml of petroleum ether and washed three times with approximately 0.1 M KCl before counting an aliquot of the petroleum ether extract. Negligible water-soluble radioactivity remained in the petroleum ether under such conditions. In experiments where further fractionation of the lipid was to be performed by silicic acid chromatography, all evaporations were conducted under nitrogen, the samples were not heated to above 37° C and they were stored at −10° C in tubes previously gassed with nitrogen.

Other methods and the preparations of materials have been described previously (3). Tissue from guinea pig, hamster and rabbit were prepared in a manner similar to that used for rat tissue. Human jejunal tissue was obtained at operation from patients undergoing partial gastrectomy and was immediately placed in oxygenated Krebs-Ringer buffer at room temperature and transported as quickly as possible to the laboratory for incubation. This process took less than 20 minutes.

RESULTS

Studies on binding versus esterification of palmitate-1-C^{14}. When a slice of everted rat small intestine is incubated with only palmitate-1-C^{14} and buffer, there is a large uptake of labeled fatty acid by the tissue, but only a negligible proportion of this fatty acid is esterified. The addition of substances to the incubation medium which are known to maintain fatty acids in solution, such as albumin, Tween "80," and taurocholate, decrease the total uptake of palmitate-1-C^{14} but significantly increase the amount which is esterified (Table I). Of these three agents taurocholate is by far the most effective. It seemed likely that the large uptake of radioactivity in the absence of one of these substances represented binding of the fatty acid to the cell surface. This possibility was investigated by examining the cellular distribution of the radioactivity in intestinal slices after an incubation. The results (Table II) indicate that in the absence of a wetting agent (taurocholate) most of the radioactivity was present in the cell debris, which contained cell walls and nuclei. This radioactivity was in the unesterified lipid fraction. These results were not significantly different when the incubation was performed under nitrogen or at 4° rather than at 37° C. On the other hand, when taurocholate was added to

TABLE I

The uptake and esterification of palmitate-1-C^{14} by slices of rat small intestine *

Additions†	Palmitate-1-C^{14}/100 mg tissue		
	Total	Unesterified	Esterified
	mμmoles	*mμmoles*	*mμmoles*
None	7.2	7.0	0.2
Albumin (5 mg/ml)	4.8	4.2	0.6
Tween "80" (0.38 %)	3.3	1.6	1.7
Taurocholate (2×10^{-2} M)	5.7	1.0	4.7

* All flasks contained in addition to substances listed above palmitate-1-C^{14}, 100 mμmoles (5×10^5 cpm), and Krebs-Ringer phosphate buffer to a final volume of 4 ml. The incubation was for 1 hour at 25° C.

† Figures in parentheses represent concentration in the incubation medium.

TABLE II

The localization of palmitate-1-C^{14} in slices of rat small intestine *

Additions	Gas phase	Palmitate-1-C^{14}/100 mg tissue					
		Cellular debris†			Supernatant†		
		Total	Unesterified	Esterified	Total	Unesterified	Esterified
		mμmoles	*mμmoles*	*mμmoles*	*mμmoles*	*mμmoles*	*mμmoles*
None	Oxygen	22.9	22.0	0.9	0.8	0.6	0.2
None	Nitrogen	22.3	21.8	0.5	0.6	0.4	0.2
Taurocholate (2×10^{-2} M)	Oxygen	6.6	1.1	5.5	2.5	0.2	2.3

* All flasks contained palmitate-1-C^{14}, 100 mμmoles (5×10^5 cpm), and Krebs-Ringer phosphate buffer in a final volume of 4 ml. The incubation was for 1 hour at 25° C.

† Based on the centrifugation at 600 × G at 4° C of a 0.15 M KCl homogenate of the slices.

the incubation medium a considerable proportion of the radioactivity was present in the supernatant fraction and was esterified. Additional experiments with albumin and Tween "80" gave results similar to those with taurocholate. As would be expected from our previous studies on palmitate esterification (3), this process in the intestinal slices was energy-dependent. An energy requirement was shown by performing incubations with 2×10^{-2} M taurocholate anaerobically or in the presence of either dinitrophenol (10^{-4} M) or potassium fluoride (2×2^{-2} M). These conditions resulted in inhibition of palmitate esterification (viz., 85, 96 and 64 per cent inhibition, respectively).

The effect of conjugated bile salts on the esterification of palmitate-1-C^{14}. The conjugated bile salts studied were taurocholate, glycocholate and taurodesoxycholate and all stimulated the esterification of palmitate-1-C^{14}. The respective curves demonstrating the effect of increasing concentrations of these salts are shown in Figure 1. The cholate derivatives had an optimal concentration of 1.5 to 2×10^{-2} M while the optimal concentration for taurodesoxycholate was 5×10^{-3} M. Higher concentrations of taurodesoxycholate inhibited the reaction. These curves also show that there was only negligible stimulation by the cholate derivatives below concentrations of 5×10^{-3} M and by taurodesoxycholate below 10^{-3} M. However, the addition of two bile salts (glycocholate and taurodesoxycholate), in concentrations at which each salt alone produced no effect, resulted in stimulation (Table III). The optimal activity of each of these three bile salts studied was comparable under our experimental conditions.

In view of the fact that rat bile contains predominantly taurocholate (8–10) it was of interest to study the effect of this salt on tissue from species which predominantly have glycine-conjugated

TABLE III

The additive effect of different bile salts on the esterification of palmitate-1-C^{14} by slices of rat small intestine *

Bile salt added		Palmitate-1-C^{14} esterified/100 mg tissue
Glycocholate	Taurodesoxycholate	
μmoles/ml	*μmoles/ml*	*mμmoles*
0	0	0.58
5	0	0.64
0	1	0.48
5	1	3.4

* Each flask contained palmitate-1-C^{14}, 100 mμmoles (5×10^5 cpm), and Krebs-Ringer phosphate buffer to give a final volume of 4 ml. The incubation was for 1 hour at 25° C.

TABLE IV

The effect of taurocholate on the esterification of palmitate-1-C^{14} by slices of small intestine of various species *†

Species	Additions	
	None	Taurocholate (2×10^{-2} M)
Guinea pig	3.1	9.2
Hamster	6.1	27.6
Human	2.0	17.3
Rabbit	2.2	5.5
Rat	1.6	3.8

* Results expressed as millimicromoles palmitate-1-C^{14} esterified per 100 mg intestine, except human tissue which is per 100 mg intestinal mucosa.

† Each flask contained palmitate-1-C^{14}, 100 mμmoles (5×10^5 cpm), 20 mg of albumin and Krebs-Ringer phosphate buffer to a final volume of 4 ml. The incubation was for 1 hour at 25° C.

TABLE V

Comparison of the effect of Tween "80" and taurocholate on the esterification of palmitate-1-C¹⁴ by slices of rat small intestine *

Tween "80"	Taurocholate	Palmitate-1-C¹⁴ esterified/ 100 mg tissue
%	*μmoles/ml*	*mμmoles*
0.38	None	1.2
0.5	None	1.1
0.38	20	4.2
None	20	4.6

* Each flask contained palmitate-1-C¹⁴, 100 mμmoles (5×10^5 cpm), and Krebs-Ringer phosphate buffer to give a final volume of 4 ml. The incubation was for 1 hour at 25° C.

bile salts (hamster, rabbit and guinea pig) and from those which excrete a more equal mixture of glycine and taurine conjugates (man) (8–10). In all these species taurocholate (2×10^{-2} M) stimulated the esterification of palmitate-1-C¹⁴ (Table IV).

Since both taurocholate and Tween "80" are good emulsifying agents, the observation in Table I that taurocholate was 2.5 times as effective as Tween "80" in stimulating the esterification of palmitate was further investigated. The results in Table V demonstrate that at optimal taurocholate concentrations the addition of Tween "80" had no effect. In contrast, at optimal Tween "80" concentrations the addition of taurocholate produced a further stimulation (fourfold). The fact that taurocholate was more effective than Tween "80" in stimulating the esterification of palmitate-1-C¹⁴ suggested that taurocholate might have another role besides that of an intraluminal emulsifier of fat. It seemed probable that if taurocholate were merely acting as an emulsifying agent, preincubation of small intestine for 30 minutes with taurocholate, before the addition of palmitate-1-C¹⁴, would offer no advantage over control tubes preincubated with buffer only. However, if taurocholate also had a cellular effect, preincubation with the bile salt might stimulate palmitate esterification. Such experiments were performed with taurocholate, glycocholate and taurodesoxycholate and compared to the action of a wetting agent such as Tween "80". Table VI demonstrates that preincubation with these bile salts resulted in stimulation of palmitate esterification while Tween "80" had no such effect.

The effect of conjugated bile salts on the incorporation of label into lipid from glucose-1-C¹⁴. Further evidence that conjugated bile salts stimulate lipid metabolism in the mucosal cell is afforded by experiments using C¹⁴-labeled glucose. We have observed that when everted rat small intestine is incubated with a tracer amount of uniformly labeled C¹⁴-glucose, a significant proportion of the radioactivity is incorporated into the tissue lipid (Table VII). Following saponification of such lipids a large proportion (i.e., greater than 95 per cent) of the radioactivity is in the water-soluble fraction, presumably in gly-

TABLE VI

The effect of preincubating slices of rat small intestine with conjugated bile salts and Tween "80" upon the esterification of palmitate-1-C¹⁴ *

Addition	No. of Expt.	Palmitate esterification/100 mg tissue after preincubation with: Buffer only — Mean	Buffer only — Range	Buffer plus wetting agent — Mean	Buffer plus wetting agent — Range	Mean change in palmitate esterification
		mμmoles		*mμmoles*		%
Taurocholate (10^{-2} M)	6	1.1	0.7 – 1.5	1.9	1.0 – 2.4	+71
Glycocholate (10^{-2} M)	6	1.2	0.9 – 1.7	1.8	1.6 – 2.6	+45
Taurodesoxycholate (1.5×10^{-3} M)	2	0.18	0.16 – 0.20	0.30	0.28 – 0.32	+66
Tween "80" (0.1 %)	4	0.43	0.28 – 0.60	0.40	0.24 – 0.61	−7

* Control slices were preincubated in Krebs-Ringer phosphate buffer (column 3) and experimental slices with buffer plus bile salt or Tween "80" (column 4). After 30 minutes, palmitate-1-C¹⁴ (100 mμmoles) was added to both, as well as bile salts or Tween "80" to the controls in order to make the final concentration of the wetting agents equal in each set of flasks. Incubation was then carried out for 30 minutes.

TABLE VII

The effect of taurocholate on C^{14}-glucose metabolism by rat small intestine *

Addition	Radioactivity/100 mg tissue	
	Total lipid	Barium-ethanol ppt.
	cpm	*cpm*
None	4,160	3,430
Taurocholate (2×10^{-2} M)	16,400	3,360

* Each flask contained uniformly labeled glucose, 0.02 μmoles (5×10^5) cpm, and Krebs-Ringer phosphate buffer to give a final volume of 4 ml. The incubation was for 1 hour at 25° C. After incubation the tissue was washed, weighed and then homogenized in 0.15 M KCl. One aliquot was used for lipid analysis. To another aliquot 1 μmole of carrier fructose-1,6-diphosphate was added and the phosphorylated carbohydrates precipitated with barium and ethanol (11), plated on aluminum planchets and counted in a gas-flow counter. Correction was made for the different counting efficiency of the liquid scintillation spectrometer and the gas-flow counter. Each result is a mean of triplicate determinations.

cerol. The presence of taurocholate (2×10^{-2} M) in the medium increases the incorporation of radioactivity into the lipid up to fourfold (Table VII). Such an increase in the radioactivity in tissue lipid could possibly result from an increased labeling of the intracellular glucose pool, secondary to a greater transport of the tracer glucose into the cell. If this were the explanation, one would also expect an increase in the labeling of other products of glucose metabolism, such as phosphorlylated carbohydrate intermediates. The phosphorylated carbohydrate intermediates were therefore isolated by barium-ethanol precipitation (11) and did not show any increase in radioactivity when taurocholate was present in the incubation medium (Table VII). Glycocholate and taurodesoxycholate also stimulated the incorporation of radioactivity into lipid when rat small intestine was incubated with C^{14}-labeled glucose. The effect of varying concentrations of these salts on this process is shown in Figure 2. A comparison of the two sets of curves in Figures 1 and 2 shows that the optimal concentration for each bile salt was similar for both the labeled palmitate and labeled glucose experiments.

The formation of glycerol-labeled lipid from C^{14}-labeled glucose in our experiments lends support to the hypothesis that glucose is a glyceride-glycerol precursor in the mucosal cell during fatty acid absorption (12, 13). This was further substantiated by observing a twofold increase of label in tissue lipid when 1 μmole of unlabeled palmitate was added to the C^{14}-glucose incubation mixture under conditions optimal for the esterification of palmitate (*vide supra*).

The effect of unconjugated bile salts. In order to assess the role of the conjugation of bile salts, we investigated the effect of free bile salts on the small intestine. Specifically, we investigated the influence of cholate and desoxycholate on palmitate esterification, glucose transport and tissue histology. Free desoxycholate inhibited palmitate esterification (Figure 3), and this inhibition was demonstrable at a concentration as low as 5×10^{-4} M and was almost complete at 3×10^{-3} M. This inhibition was more readily demonstrated when palmitate esterification had been increased by the presence of a wetting agent (e.g., albumin, Tween "80" or taurocholate). The *inhibition* by free desoxycholate is in striking contrast to the *stimulation* by taurodesoxycholate which occurs at an optimal concentration of $5 \times$

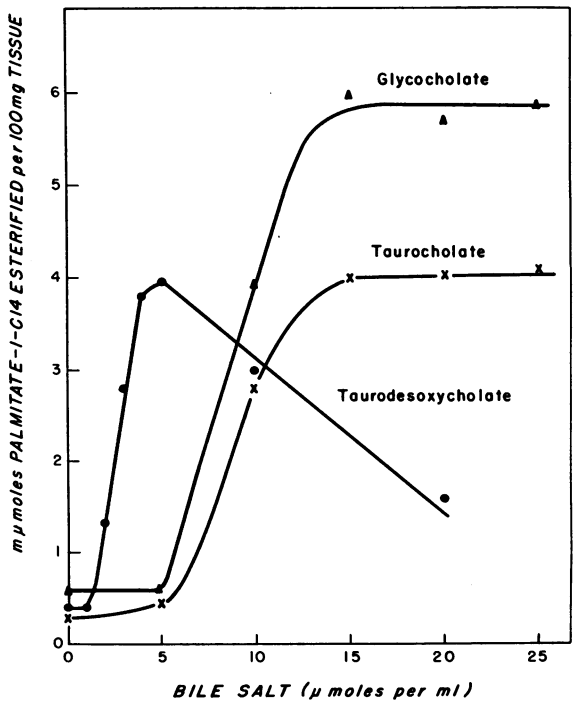


FIG. 1. THE EFFECT OF CONJUGATED BILE SALTS ON THE ESTERIFICATION OF PALMITATE-1-C^{14} BY SLICES OF RAT SMALL INTESTINE. Standard incubation procedure was used. There were 100 mμmoles (5×10^5 cpm) of palmitate-1-C^{14} in each flask and bile salts were added as indicated.

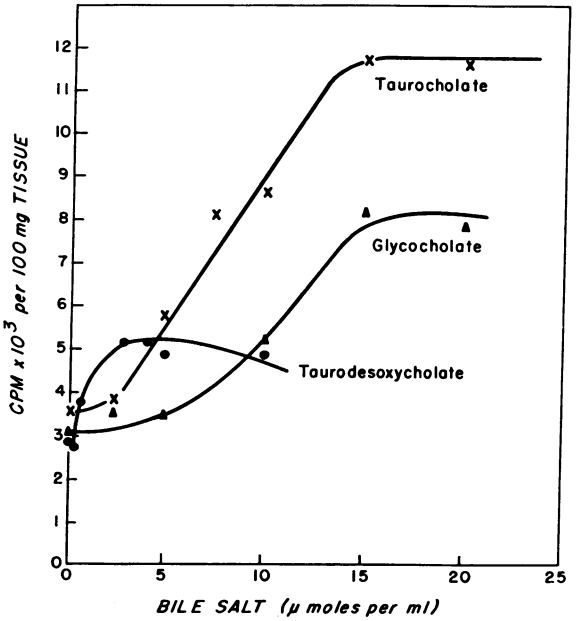


FIG. 2. THE EFFECT OF CONJUGATED BILE SALTS ON THE INCORPORATION OF RADIOACTIVITY FROM C^{14}-GLUCOSE BY SLICES OF RAT SMALL INTESTINE. Each flask contained uniformly labeled C^{14}-glucose, 0.1 μmole (2.7×10^5 cpm), and conjugated bile salts as indicated.

10^{-3} M (Figure 1). Free cholate, on the other hand, did not inhibit esterification and when its effects were compared to those of its conjugated

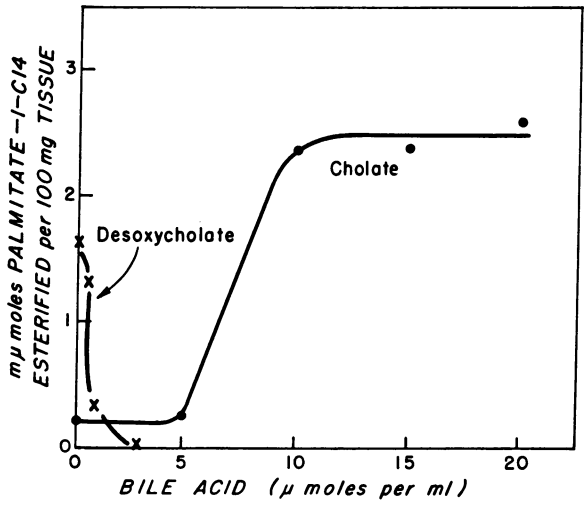


FIG. 3. THE EFFECT OF FREE BILE SALTS ON THE ESTERIFICATION OF PALMITATE-1-C^{14} BY SLICES OF RAT SMALL INTESTINE. Standard incubation conditions were used. Each flask contained palmitate-1-C^{14}, 100 mμmoles (5×10^5 cpm), and free bile salts as indicated. Each flask in the desoxycholate experiments contained Tween "80" (0.38 per cent) to facilitate esterification.

TABLE VIII

Comparison of the effect of cholate and its conjugated derivatives on the esterification of palmitate-1-C^{14} by slices of rat small intestine *

Bile salt: Type	Bile salt: Optimal concentration	Maximal palmitate-1-C^{14} esterified/ 100 mg tissue
	$\times 10^{-3}$ M	*mμmoles*
Cholate	10	2.8
Glycocholate	15	3.9
Taurocholate	15	3.7

* Standard incubation procedure. Data in column 2 are derived from Figures 1 and 3. Data in column 3 are derived from the action of the bile salts on tissue from the same rat.

derivatives (Table VIII, Figures 1 and 3) there were two obvious differences: *1*) the maximal palmitate esterification produced by cholate was less than that produced by its conjugate when tested on tissue from the same animal (Table VIII, column 3); and *2*) the concentration at which this maximum was reached was lower for cholate (Table VIII, column 2). To be certain that these observations were not due to unrecognized contaminants, these results were confirmed

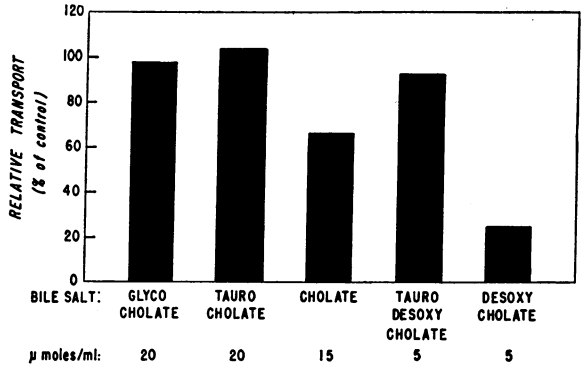
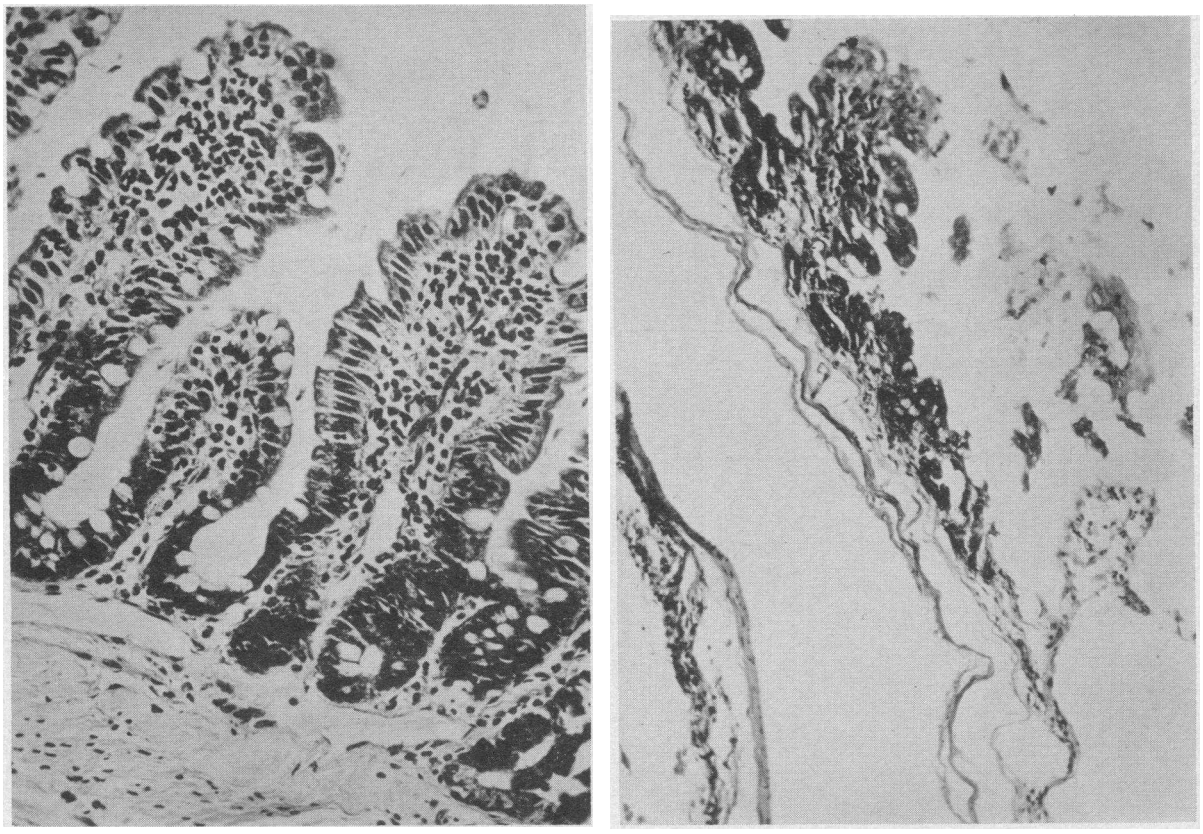


FIG. 4. THE EFFECT OF BILE SALTS ON THE TRANSPORT OF THE C^{14}-GLUCOSE BY EVERTED SACS OF RAT SMALL INTESTINE. Krebs-Ringer phosphate buffer containing unlabeled glucose (10^{-3} M) was used for the incubation medium and for the sac contents. A tracer dose of C^{14}-glucose (0.02 μmole, 2.25×10^5 cpm) was added to the incubation medium. Bile salts were added as indicated. The final volume of the incubation medium was 4 ml and the incubation was for one hour at 25° C under oxygen. At the end of the incubations, aliquots of the sac contents were dried on planchets and counted in a gas-flow counter. The control sacs without bile salts contained on an average 6.5×10^3 cpm per ml at the end of the incubation. The results of the incubation with bile salts are expressed as per cent of the controls. Each result represents the mean value of at least eight sacs.

with free acids obtained by the hydrolysis of recrystallized conjugated bile salts.

The inhibition by desoxycholate was not specific for the esterifying activity of the mucosa as shown by experiments on glucose transport using everted sacs of small intestine. It was found (Figure 4) that whereas taurodesoxycholate (5×10^{-3} M) had no effect on glucose transport, free desoxycholate at this concentration decreased glucose transport by 75 per cent. Cholate (1.5×10^{-2} M) inhibited glucose transport 35 per cent, while its conjugated derivatives, glycocholate and taurocholate (2×10^{-2} M), had no effect on this process. These results on the interference of intestinal glucose transport by free bile salts emphasize the limitation of using C^{14}-glucose to assess intracellular metabolism when the results depend upon the initial entry of the label into the cell.

Normally, at the end of a one hour incubation of rat small intestine at 25° C in buffer containing glucose (10^{-3} M), histological sections of this tissue showed generally well preserved villi with scattered mild mucosal damage. The results were similar when taurodesoxycholate (5×10^{-3} M) was in the medium (Figure 5A). On the other hand, free desoxycholate (5×10^{-3} M) caused dissolution of the tissue with loss of whole villi (Figure 5B). Even at concentrations of 10^{-3} M all villi had necrotic tips. The effect of cholate was far less pronounced, but at 1.5×10^{-2} M it caused histological damage to all villi. Intestinal segments incubated with glycocholate or taurocholate (2×10^{-2} M) were histologically the same



A B

FIG. 5. THE EFFECT OF FREE DESOXYCHOLATE AND TAURODESOXYCHOLATE IN THE HISTOLOGY OF RAT SMALL INTESTINE. The sacs of small intestine used to demonstrate glucose transport (Figure 4) were fixed in 10 per cent formalin and histologic sections prepared and stained with hematoxylin and eosin. A) After incubation with taurodesoxycholate, 5×10^{-3} M (magnification $\times 200$); B) after incubation with desoxycholate, 5×10^{-3} M (magnification $\times 100$).

TABLE IX

The separation of labeled small intestinal lipids by silicic acid chromatography *

Eluate		Lipid fraction eluted	Radioactive lipid following incubation with			
Solvent	Volume		Palmitate-1-C^{14}		C^{14}-glucose	
	ml†		cpm	%	cpm	%
1% Benzene	18	Cholesterol ester	42	0.2		
3% Ether in hexane	60	Triglyceride	19,846	86.8	23,913	92.0
30% Ether in hexane	60	Diglyceride	2,360	10.4	1,691	6.5
Ether	60	Monoglyceride	224	1.0	200	0.7
Methanol	100	Phospholipid	336	1.5	242	0.9

* The lipid was prepared from rat small intestine following a standard incubation with either palmitate-1-C^{14} or uniformly labled C^{14}-glucose in the presence of taurocholate 2×10^{-2} M. All extractions were performed under nitrogen and the lipid was not heated to more than 37° C. The lipid from the glucose experiment was not passed through an Amberlite IRA-400 column prior to silicic acid chromatography.

† The eluate was collected in 6 ml fractions and good separation of the respective lipid peaks was demonstrated. For the sake of brevity the total volume of each solvent fraction and the total radioactivity in each fraction are given.

as the controls. Similar experiments were also performed with everted segments of rat colon. The free bile salts again caused some mucosal damage but to a considerably lesser extent than that seen in the rat small intestine.

Identification of labeled incubation products. The results of silicic acid chromatography of the labeled lipid formed during an incubation with either palmitate-1-C^{14} or uniformly labeled C^{14}-glucose are shown in Table IX. With labeled lipid derived from palmitate-1-C^{14} experiments, 0.2 per cent of the counts were eluted with 1 per cent benzene in hexane and probably represented cholesterol ester. Over 85 per cent of the label was in the triglyceride fraction, about 10 per cent in the diglyceride fraction, and 1 to 2 per cent in both monoglyceride and phospholipid. In view of the very small proportion of counts in the lower glyceride fractions, their identity was also confirmed by chromatography on silicone impregnated paper (14). Some of the monoglyceride was found to come from hydrolysis of higher glycerides on the IRA-400 column. The monoglyceride due to such hydrolysis amounted to 0.5 per cent of the total counts in the tri- and diglyceride fractions. Nevertheless, it is evident in Table IX that in the C^{14}-glucose experiments (where IRA columns were not used) the labeled lipid still showed a definite, though small (0.7 per cent), monoglyceride fraction.

DISCUSSION

These observations on factors affecting lipid metabolism in rat small intestine have been facilitated by using a reproducible technique for measuring both palmitate-1-C^{14} esterification and the incorporation of label from C^{14}-glucose into intestinal lipid. A somewhat similar system for measuring palmitate esterification has recently been described by Johnston (15). He used everted sacs of hamster small intestine which were incubated at 37° C for 2.5 hours with a carefully prepared albumin-palmitate-1-C^{14} complex. Under his conditions, in contrast to our experiments, a significant proportion of esterified palmitate-1-C^{14} was transported into the serosal medium. This difference may be related to the fact that he used a higher temperature, incubated for a longer time, and used a different species. From our observations the species difference is probably the most important, for Table IV shows that hamster jejunum is approximately seven times as active as that of the rat.

The observation that in the absence of a wetting agent palmitate-1-C^{14} adheres to the mucosal cell surface and that this process is not energy-dependent has been observed in other cells (16, 17). Goodman (16) found that red blood cells have a strong binding affinity for palmitate-1-C^{14} and that this property was also demonstrable with red cell ghosts or cyanide-poisoned cells. The reason for the negligible transport of palmitate into the mucosal cell, despite its adherence to the cell surface, is not known but was also described by Fillerup, Migliore and Mead (17) using ascites tumor cells. They facilitated the transport of palmitate into these cells by using an albumin-palmitate complex. In our system taurocholate and Tween "80" had this effect, as well as albu-

min, which suggests that albumin acts by increasing the solubility of the fatty acid rather than by any other specific property. Johnston's observations that only 10 to 20 per cent of the palmitate-1-C^{14} present in the tissue was unesterified may be explained by the fact that he used an albumin-palmitate complex in which all the palmitate was bound to the albumin. In our experiments not all the palmitate present was bound to albumin since we added the palmitate and albumin to the incubation medium for only 15 minutes before the tissue was added.

Bile salts are known to be of importance in fat absorption (10). Their postulated role is usually confined to that of a natural emulsifier of fat in the intestinal lumen. Their detergent action may or may not also explain their ability to activate pancreatic lipase (1). However, Verzar and McDougall (18) did suggest that bile salts might be of importance on the mucosal cell surface and Borgström (19), on the basis of balance studies in rats with bile and lymph fistulae, inferred that these salts had an intracellular function. Further evidence for such an action is that 24 hours after an injection of C^{14}-labeled cholate the major portion of the label remaining in the tissues was present in the small intestinal mucosa (20).

In view of the fact that Tween "80" and albumin increase the esterification of palmitate-1-C^{14} in our system, it was not surprising that bile salts which are also active surface agents would behave in a similar manner. But this cannot be the sole explanation of their effect. The preincubation experiments demonstrate that the conjugated bile salts tested, namely, taurocholate, glycocholate and taurodesoxycholate, have the property of stimulating the cellular phase of palmitate esterification. The site of such action might be on the cell surface, inside the cell or both. Recently pinocytosis has been described as an important mode of membrane transport for widely differing substances (21–24). The electron microscopic studies of Palay and Karlin (24) have suggested that pinocytosis is involved, at least in part, in the transport of fat across the rat small intestine following the feeding of corn oil. Pinocytosis may be stimulated by various factors in different tissues; e.g., insulin has been shown to induce pinocytosis in fat cells (23). It is possible that conjugated bile salts have an analogous effect on the intestinal mucosa and so stimulate the transport of palmitate into the cell making it available for esterification. Regardless of this highly speculative cellular surface role of conjugated bile salts, the experiments with C^{14}-glucose show that these salts affect intramucosal lipid metabolism. A recent report by Whitehouse and Staple (25) provides further evidence that conjugated bile salts have an intracellular metabolic role.

It is of interest that at low concentrations these bile salts had no obvious effects. The observation that two conjugated bile salts can act in a complementary manner when present at low concentrations is of interest, for the bile of most species contains a mixture of these salts.

The difference between the biological properties of free bile salts and their conjugates has not been emphasized in the past, although pharmacological differences have been described (26). This difference was best demonstrated in our experiments by comparing the effect of taurodesoxycholate and desoxycholate on palmitate-1-C^{14} esterification, glucose transport, and tissue histology. The derangement in cell structure should perhaps not be surprising since this agent is frequently used to disrupt cell fractions *in vitro* (27). The difference between cholate and its conjugates was less impressive but nevertheless definite, especially with regard to the effect on glucose transport and cell structure. Whitehouse and Staple (25) also found that cholate and its conjugated derivatives differed in their metabolic effects in a cell-free system in that cholate inhibited pyruvate oxidation by rat liver mitochondria while its conjugated derivatives had no such effect.

Investigations on the physiology of absorption when crude bile salts are used must be interpreted with caution. Unless stringent tests of purity are employed the results may be misleading, for traces of unconjugated bile salts (especially desoxycholate) can alter mucosal function. We encountered this situation when testing both a crude ox bile preparation and a so-called "chemically pure" glycocholate preparation. Both substances contained free bile salts and both inhibited palmitate esterification. These findings might also partially explain some of the confusion concerning the therapeutic usefulness of bile salts

in correcting impaired fat absorption in patients and rats with biliary fistulae.

The conjugation of bile salts which occurs in the liver immediately after they are synthesized from cholesterol serves to convert potentially harmful substances into physiologically useful ones. Normally the small intestine contains only conjugated bile salts. Some of these are absorbed by the small intestine while others pass into the large bowel where they are rapidly hydrolyzed and degraded by bacteria (8, 9, 28).

In the clinical condition of intestinal "blind loops," it has been assumed that an altered small bowel flora contributes to the associated malabsorption (29). It is tempting to speculate that in circumstances where bacteria infest the small bowel conjugated bile salts may be converted in the lumen of the small intestine to toxic unconjugated derivatives. These products might then interfere with absorption, especially since it is known that one of the initial bacterial degradation products of cholate is desoxycholate.

In our analyses of the labeled lipid formed during an incubation, we consistently found a small proportion of the label (1 to 2 per cent) in the monoglyceride fraction. This monoglyceride fraction is of interest for it would not be expected as an intermediate in the formation of triglyceride from fatty acids if Kennedy's scheme for the conversion of fatty acids to neutral glycerides obtains in the mucosa (30). In fact the monoglyceride probably represents a product of lipolysis rather than of lipogenesis. Active mucosal lipolysis has been demonstrated in the rat *in vivo* (12) and in rat mucosal homogenates (3).

The action of the conjugated bile salts in the homogenate system (3) was different from that of the tissue slice. In the homogenate, taurocholate depressed the incorporation of palmitate-1-C^{14} into neutral fat. This was interpreted as being due to the activation of lipase. The difference between the results using these two techniques probably is due to the abnormal structural relationships of cellular particles in the homogenate system. Thus, although homogenates are of use in delineating reactions and their cofactor requirements, the use of intact cells is mandatory to evaluate the possible physiological role of such reactions.

SUMMARY

1. A reproducible method has been described for studying aspects of lipid metabolism in slices of rat small intestine *in vitro*.

2. When slices of rat small intestine are incubated with only palmitate-1-C^{14} and buffer, fatty acid predominantly adheres to the cell surface and negligible esterification occurs. This binding is independent of the metabolic activity of the cell.

3. Esterification of palmitate by slices of intestine is facilitated by adding a wetting agent such as albumin, Tween "80" or taurocholate to the medium. This esterification is dependent upon the metabolic activity of the cell.

4. Conjugated bile salts stimulate the esterification of palmitate-1-C^{14} by directly affecting mucosal cell metabolism in addition to their effect on fatty acid solubility in the incubation medium. This was demonstrated by preincubation studies and by experiments in which conjugated bile salts stimulated the incorporation of radioactivity from C^{14}-glucose into mucosal lipid.

5. The free bile salts, cholate and desoxycholate, behave differently from their conjugated derivatives. The free salts inhibit glucose transport by the small intestine and cause histological damage. Desoxycholate also inhibits palmitate esterification, but cholate stimulates this process. However, the stimulation of palmitate esterification by cholate is less than that produced by glycocholate or taurocholate.

6. Some possible physiological and pathological implications of these findings have been discussed.

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