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 ho-
"mogenates of rat and human small intestine mu-
cosa and have delineated some of the cofactor re-
quirements 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-C14
and the incorporation of label into lipid from C14-
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-C14 and uniformly labeled glucose-C14
were obtained from the Volk Radiochemical Company,
Chicago, Illinois. The C14-labeled palmitate was puri-
fied 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 re-
sulting solution was diluted with water, acidified, and
the precipitated free bile salt filtered, washed and dried.
In earlier experiments commercial sodium taurocholate
(Planstiel 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 ma-
terial which was used in later experiments.

Female albino rats (Charles River Laboratories, Bos-
ton, 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 oxy-
genated Krebs-Ringer phosphate buffer, pH 7.4, modi-

ted 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 in-
testinal rings. These measured approximately one-
eighth inch (lengthwise) and weighed between 80 to
150 mg (wet weight). Since one of the problems in us-
ing slices or sacs of small intestine in metabolic studies
is the variation in activity along this organ, some pre-
liminary 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 com-
ing from a different site, and 3) by performing all incuba-
tions in duplicate. Incubations were carried out in
25 ml Erlenmeyer flasks and the incubation mixture con-
tained either palmitate-1-C14 or uniformly labeled glu-
cose-C14 plus other agents such as bile salts, Tween “80”

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obtained at hamster all stored at the remaining conditions. In petroleum ether was lipid petroleum ether, presenting petroleum ether within 10 scintillation metabolites. 20 ml in evaporated was 2,500 rpm methanol filtered stand chloroform-methanol (2:1) in Teflon microtorsion balance. in Sloane were placed at the individual transport at 370 in indicated KCl buffer, where further operation of this 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\textsuperscript{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\textdegree C rather than at 37\textdegree C. On the other hand, when taurocholate was added to

TABLE I

| Additions† | Palmitate-1-C\textsuperscript{14} Palmitate-1-C\textsuperscript{14} Palmitate-1-C\textsuperscript{14} 100 mg tissue 100 mg tissue 100 mg tissue | Total Total | Unesterified Unesterified Unesterified | Esterified Esterified Esterified |
|-----------|-------------------------------------------------|-------------|-----------------------------|-----------------------------|-----------------------------|
| None      | 7.2                                             | 7.0         | 0.2                         | 0.2                         | 0.2                         |
| Albumin (5 mg/ml) | 4.8 | 4.2 | 0.6 | 0.6 | 0.6 |
| Tween "80" (0.38 %) | 3.3 | 1.6 | 1.7 | 1.7 | 1.7 |
| Taurocholate (2 x 10\textsuperscript{-4} M) | 5.7 | 1.0 | 4.7 | 4.7 | 4.7 |

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

† Figures in parentheses represent concentration in the incubation medium.
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 $2 \times 10^{-2}$ M taurocholate anaerobically or in the presence of either dinitrophenol ($10^{-4}$ M) or potassium fluoride ($2 \times 2^{-2}$ 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-$\text{C}^{14}$.

The conjugated bile salts studied were taurocholate, glycocholate and taurodesoxycholate and all stimulated the esterification of palmitate-1-$\text{C}^{14}$. 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 $2 \times 10^{-2}$ M while the optimal concentration for taurodesoxycholate was $5 \times 10^{-3}$ 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 $5 \times 10^{-3}$ 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 II**

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

<table>
<thead>
<tr>
<th>Additions</th>
<th>Gas phase</th>
<th>Palmitate-1-$\text{C}^{14}$/100 mg tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cellular debris</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>None</td>
<td>Oxygen</td>
<td>22.9</td>
</tr>
<tr>
<td>None</td>
<td>Nitrogen</td>
<td>22.3</td>
</tr>
<tr>
<td>Taurocholate</td>
<td>Oxygen</td>
<td>6.6</td>
</tr>
<tr>
<td>(2 $\times$ 10^{-2} M)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* All flasks contained palmitate-1-$\text{C}^{14}$, 100 $\mu$moles ($5 \times 10^{4}$ 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 $\times$ G at 4° C of a 0.15 M KCl homogenate of the slices.

**TABLE III**

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

<table>
<thead>
<tr>
<th>Bile salt added</th>
<th>Palmitate-1-$\text{C}^{14}$ esterified/100 mg tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycocholate</td>
<td>$\mu$moles/mlin</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

* Each flask contained palmitate-1-$\text{C}^{14}$, 100 $\mu$moles ($5 \times 10^{4}$ 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-$\text{C}^{14}$ by slices of small intestine of various species *

<table>
<thead>
<tr>
<th>Species</th>
<th>Additions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea pig</td>
<td>None</td>
</tr>
<tr>
<td>Hamster</td>
<td>6.1</td>
</tr>
<tr>
<td>Human</td>
<td>20.0</td>
</tr>
<tr>
<td>Rabbit</td>
<td>2.2</td>
</tr>
<tr>
<td>Rat</td>
<td>1.6</td>
</tr>
</tbody>
</table>

* Results expressed as millimicromoles palmitate-1-$\text{C}^{14}$ esterified per 100 mg intestine, except human tissue which is per 100 mg intestinal mucosa.
† Each flask contained palmitate-1-$\text{C}^{14}$, 100 $\mu$moles ($5 \times 10^{4}$ 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.
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-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 V

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

<table>
<thead>
<tr>
<th>Tween “80”</th>
<th>Taurocholate</th>
<th>Palmitate-1-C14 esterified/100 mg tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>μmoles/ml</td>
<td>mmoles</td>
</tr>
<tr>
<td>0.38</td>
<td>None</td>
<td>1.2</td>
</tr>
<tr>
<td>0.5</td>
<td>None</td>
<td>1.1</td>
</tr>
<tr>
<td>0.38</td>
<td>20</td>
<td>4.2</td>
</tr>
<tr>
<td>None</td>
<td>20</td>
<td>4.6</td>
</tr>
</tbody>
</table>

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

### 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 *

<table>
<thead>
<tr>
<th>Addition</th>
<th>No. of Expt.</th>
<th>Palmitate esterification/100 mg tissue after preincubation with</th>
<th>Mean change in palmitate esterification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Buffer only</td>
<td>Buffer plus wetting agent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>Taurocholate (10−2 M)</td>
<td>6</td>
<td>1.1</td>
<td>0.7 - 1.5</td>
</tr>
<tr>
<td>Glycocholate (10−2 M)</td>
<td>6</td>
<td>1.2</td>
<td>0.9 - 1.7</td>
</tr>
<tr>
<td>Taurodesoxycholate (1.5 × 10−3 M)</td>
<td>2</td>
<td>0.18</td>
<td>0.16 - 0.20</td>
</tr>
<tr>
<td>Tween “80” (0.1 %)</td>
<td>4</td>
<td>0.43</td>
<td>0.28 - 0.60</td>
</tr>
</tbody>
</table>

* 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 μmole s) 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.
cerol. The presence of taurocholate \((2 \times 10^{-2} \text{ M})\) 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 phosphorylated 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 \(\text{C}^{14}\)-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 \(\text{C}^{14}\)-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 \(\mu\) mole of unlabeled palmitate was added to the \(\text{C}^{14}\)-glucose incubation mixture under conditions optimal for the esterification of palmitate (\textit{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 \(5 \times 10^{-4} \text{ M}\) and was almost complete at \(3 \times 10^{-3} \text{ M}\). 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 \(5 \times 10^{-2} \text{ M}\).

### Table VII

<table>
<thead>
<tr>
<th>Addition</th>
<th>Radioactivity/100 mg tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total lipid</td>
</tr>
<tr>
<td>None</td>
<td>4,160</td>
</tr>
<tr>
<td>Taurocholate</td>
<td>16,400</td>
</tr>
<tr>
<td>((2 \times 10^{-2} \text{ M}))</td>
<td></td>
</tr>
</tbody>
</table>

*Each flask contained uniformly labeled glucose, 0.02 \(\mu\) moles \((5 \times 10^9)\) 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 \(\mu\) mole of carrier fructose-1,6-diphosphate was added and the phosphorylated carbohydrate 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.

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**Fig. 1. The effect of conjugated bile salts on the esterification of palmitate-\(\text{C}^{14}\) by slices of rat small intestine.** Standard incubation procedure was used. There were 100 \(\mu\) moles \((5 \times 10^9 \text{ cpm})\) of palmitate-\(\text{C}^{14}\) in each flask and bile salts were added as indicated.
10^{-3} \text{ M} \) (Figure 1). Free cholate, on the other hand, did not inhibit esterification and when its effects were compared to those of its conjugated 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...
with free acids obtained by the hydrolysis of re-
crystallized conjugated bile salts.

The inhibition by desoxycholate was not spe-
cific 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
($5 \times 10^{-3}$ M) had no effect on glucose transport,
free desoxycholate at this concentration decreased
glucose transport by 75 per cent. Cholate ($1.5 \times
10^{-2}$ M) inhibited glucose transport 35 per cent,
while its conjugated derivatives, glycocholate and
taurocholate ($2 \times 10^{-2}$ 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 $^{14}$C-glucose to as-
sess 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 tis-
ue showed generally well preserved villi with
scattered mild mucosal damage. The results were
similar when taurodesoxycholate ($5 \times 10^{-3}$ M)
was in the medium (Figure 5A). On the other
hand, free desoxycholate ($5 \times 10^{-3}$ 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 $1.5 \times 10^{-2}$ M it
caused histological damage to all villi. Intestinal
segments incubated with glycocholate or tauro-
cholate ($2 \times 10^{-2}$ M) were histologically the same.

\[ \text{\textbf{Fig. 5. The effect of free desoxycholate and taurodesoxycholate in the histology of rat small intesti-}} \\
\text{\text{\textit{ne. The sacs of small intestine used to demonstrate glucose transport (Figure 4) were fixed in 10 per cent formal-}} \\
\text{\text{\textit{in and histologic sections prepared and stained with hematoxylin and eosin. A) After incubation with taurodesoxy-}} \\
\text{\text{\textit{cholate, }5 \times 10^{-2} \text{ M (magnification } \times 200); B) after incubation with desoxycholate, }5 \times 10^{-1} \text{ M (magnification } \\
\text{\textit{\times 100).}}} \]
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-C¹⁴ or uniformly labeled C¹⁴-glucose are shown in Table IX. With labeled lipid derived from palmitate-1-C¹⁴ 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 C¹⁴-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-C¹⁴ esterification and the incorporation of label from C¹⁴-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-C¹⁴ complex. Under his conditions, in contrast to our experiments, a significant proportion of esterified palmitate-1-C¹⁴ 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-C¹⁴ 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-C¹⁴ 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 abu-

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**Table IX**

The separation of labeled small intestinal lipids by silicic acid chromatography

<table>
<thead>
<tr>
<th>Eluate</th>
<th>Solvent</th>
<th>Volume</th>
<th>Lipid fraction eluted</th>
<th>Radioactive lipid following incubation with Palmitate-1-C¹⁴</th>
<th>Radioactive lipid following incubation with C¹⁴-glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>ml</td>
<td></td>
<td>cpm</td>
<td>%</td>
</tr>
<tr>
<td>1% Benzene</td>
<td>18</td>
<td></td>
<td>Cholesterol ester</td>
<td>42</td>
<td>0.2</td>
</tr>
<tr>
<td>3% Ether in hexane</td>
<td>60</td>
<td></td>
<td>Triglyceride</td>
<td>19,846</td>
<td>86.8</td>
</tr>
<tr>
<td>30% Ether in hexane</td>
<td>60</td>
<td></td>
<td>Diglyceride</td>
<td>2,360</td>
<td>10.4</td>
</tr>
<tr>
<td>Ether</td>
<td>60</td>
<td></td>
<td>Monoglyceride</td>
<td>224</td>
<td>1.0</td>
</tr>
<tr>
<td>Methanol</td>
<td>100</td>
<td></td>
<td>Phospholipid</td>
<td>336</td>
<td>1.5</td>
</tr>
</tbody>
</table>

*The lipid was prepared from rat small intestine following a standard incubation with either palmitate-1-C¹⁴ or uniformly labeled C¹⁴-glucose in the presence of taurocholate 2 × 10⁻³ 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.
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 palmiteate-1-C\textsuperscript{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\textsuperscript{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 palmiteate-1-C\textsuperscript{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\textsuperscript{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 palmiteate-1-C\textsuperscript{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 \textit{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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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.

**REFERENCES**


