THE ESTERIFICATION OF PALMITATE-1-C¹⁴ BY HOMOGENATES OF INTESTINAL MUCOSA * †

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The esterification of long chain fatty acids has been shown to represent an important phase of fat absorption in the rat and in man (1–4). Although some glycerides are not completely hydrolyzed prior to absorption, a large proportion of the fatty acids is liberated in the intestinal lumen (5, 6) only to appear in the lymph as neutral fat in chylomicrons (7–9). In contrast, short chain fatty acids pass largely unchanged into the portal vein (3, 10, 11). The conversion of long chain fatty acids into neutral fat occurs in the mucosa of the small intestine, but the processes involved have not been clearly demonstrated. Favarger and Gerlach (12) supported the hypothesis that this formation of neutral fat was due to a synthetic action of a mucosal lipase. However, the demonstration in liver that the activation of a fatty acid by Coenzyme A (CoA) and adenosine triphosphate (ATP) is a necessary preliminary step prior to glyceride bond synthesis (13–17) has suggested that a similar mechanism may obtain in the mucosa of the small intestine.

In the present investigation, by using homogenates of rat and human intestinal mucosa, a very active system has been demonstrated which is capable of incorporating long chain fatty acids into neutral fat. This system is dependent upon ATP and CoA and is more active in the jejunum than in the ileum or colon. Short chain fatty acids are not incorporated. It has been observed that homogenates from the jejunum of patients with idiopathic steatorrhea possess greatly diminished activity.

MATERIALS AND METHODS

C¹⁴-labeled fatty acids were obtained commercially (Volk Radio-chemical Company). Both palmitic acid-1-C¹⁴ and stearic acid-1-C¹⁴ had a specific activity of 5×10^6 cpm per μMole. The C¹⁴-labeled palmitic acid contained 6 per cent of a neutral radioactive impurity while the C¹⁴-stearic acid contained 2 per cent of a neutral impurity. These were removed by dissolving the acids in an alkaline solution of 50 per cent ethanol and washing three times with petroleum ether. The alcoholic solution was then acidified and the fatty acid extracted with petroleum ether which was evaporated to dryness. Water was added, the pH adjusted to 9.0 with ammonium hydroxide and the solution heated in a steam bath until clear (13). The final concentration for both acids was 5×10^6 cpm per ml. Sodium octanoate (6.75×10^5 cpm per μMole) contained no radioactive neutral impurity, and was brought into solution in a similar manner.

Tween "80"® (polyoxyethylene sorbitan mono-oleate) was prepared as a 10 per cent (v/v) aqueous solution. Free fatty acids were removed by the method of Minard (18). Coenzyme A was obtained from Pabst Laboratories, Milwaukee, Wisconsin, or Sigma Chemical Company, St. Louis, Missouri, and solutions were freshly prepared before each experiment. L-α-glycerophosphate was a gift of Dr. Erich Baer.

Female albino rats (Charles River Laboratories) weighing 150 to 250 Gm. were fed on Purina® Chow. They were killed by a blow on the head. The small intestine was washed through with ice cold isotonic saline and the mucosa obtained by scraping the serosa with a spatula. A 5 per cent homogenate was prepared with a tissue grinder (Potter-Elvehjem, Teflon Pestle) in 0.15 M KCl and filtered through a double layer of absorbent gauze. Cell debris and nuclei were removed by spinning at $600 \times G$ for 15 minutes. When more concentrated homogenates were used, this step was difficult to perform due to the presence of excessive insoluble mucus. The protein concentration of the supernatant fraction was measured spectrophotometrically (19) and then the fraction was diluted with 0.15 M KCl to a final concentration of less than 1 mg. per ml.

Human intestinal mucosa was obtained from 25 patients of whom 9 were women and 16 men.[1] Their ages ranged from 21 to 68 years. Histologically normal sam-

[1] We are indebted to Dr. John A. Benson, Jr. for his help and assistance in obtaining the human intestinal tissue for these experiments.

ples were obtained from the jejunum of six patients of whom five were undergoing surgery—four for peptic ulcer and one for chronic pancreatitis. In the sixth patient, who had postgastrectomy steatorrhea, a biopsy of the afferent jejunal loop was taken with a Shiner tube (20). Histologically normal terminal ileum was obtained from one patient at operation for carcinoma of the cecum and through a biopsy tube from six patients who had an ileostomy for chronic ulcerative colitis. Histologically normal colon or rectum was obtained from four patients at operation for large bowel tumors and from two patients by using biopsy forceps at routine sigmoidoscopy. Jejunal tissue was removed from six patients with idiopathic steatorrhea—one at operation and five by using a Shiner biopsy tube. This tissue showed the usually accepted histological changes found in this disease (20, 21). Immediately after it was obtained the specimen was placed in 2 to 3 ml. of ice cold 0.15 M KCl and, as soon as possible, homogenized in a tissue grinder (Potter-Elvejhem type). It was then centrifuged at $600 \times G$ in the cold and the supernatant solution assayed for its enzymatic activity.

Incubations of the $600 \times G$ supernatant fractions from the rat and human homogenates were carried out in Pyrex® centrifuge tubes for 30 minutes at 37° C. in air without agitation. The standard incubation mixture contained 0.5 ml. Tris-maleate [2] buffer, 0.5 M, pH 7.0; $MgCl_2$, 10 μMoles; potassium fluoride, 25 μMoles; 0.1 ml. of a 10 per cent solution of Tween "80"®; ATP, 10 μMoles, CoA, 0.5 μMole, C^{14}-labeled fatty acid, 100 mμMoles and 0.5 ml. homogenate containing less than 0.5 mg. protein. The final volume of the mixture was 1.5 ml. A 1 ml. aliquot was removed after incubation and the total lipid extracted by adding it to 20 ml. chloroform-methanol (2:1) (22). After standing 30 minutes, the mixture was filtered through Whatman No. 1 filter paper. The filtrate was evaporated and the lipids taken up in 10 ml. of petroleum ether (B.P. 30 to 60° C.) The free fatty acids and monoglycerides were removed from the petroleum ether by extracting three times with 25 ml. of 50 per cent ethanolic 0.25 N KOH (23). A 2 ml. aliquot of the petroleum ether was then added to 10 ml. of toluene containing 0.01 per cent p-bis[2-(5-phenyloxazolyl)]-benzene and 0.3 per cent 2,5-diphenyloxazole (Pilot Chemicals Inc., Waltham, Mass.) and the radioactivity measured in a Packard Liquid Scintillation Spectrometer. In each experiment the control consisted of a tube to which the radioactive fatty acid was added immediately prior to extraction, and on the average less than 0.3 per cent of the radioactivity remained in the petroleum ether. This blank value was subtracted before expressing the results as micromoles of C^{14}-labeled fatty acid incorporated into neutral fat. Duplicate determinations usually varied less than 10 per cent.

In later experiments, when the distribution of the radioactive fatty acid among the various lipid fractions was measured, an alternative extraction procedure was used. This was used because it was observed that washing with alkaline ethanol not only removed monoglycerides (23) but also changed the behavior of the lipid on silicic acid columns. This alternative extraction procedure consisted of supersaturating the incubation mixture with sodium sulfate crystals followed by the addition of about 10 mg. of sodium lauryl sulfate. The mixture was made alkaline with 0.1 ml. 5 N KOH and extracted four times with 3 ml. of ethyl ether (24). This wet ether extract was passed through a 5 Gm. Amberlite IRA-400 column (23) to remove any remaining free fatty acids. The column was washed with a further 30 ml. of wet ether and the combined ether washings were evaporated to dryness. The lipid was taken up in petroleum ether, washed with 0.1 M KCl and an aliquot counted in the liquid scintillation spectrometer.

The products were analyzed on a small silicic acid column using a modification of the method of Barron and Hanahan (25). Two Gm. of silicic acid (Bio-Rad Laboratories, Berkeley, Calif.) was baked overnight at 120° C. After cooling, 1 Gm. of Hyflo® supercel (Johns Manville Co.) was added and the mixture washed in a frittered glass funnel with 50 ml. each of anhydrous ethyl ether, 15 per cent benzene in hexane, and finally hexane. The hexane was prepared according to Barron and Hanahan (25). The slurry was suspended in 30 ml. hexane and poured into an 8 mm. diameter column. After initial settling, the column was packed by gentle water suction. A small disc of ether-extracted filter paper was placed on top of the silicic acid and the column washed with a further 20 ml. of hexane. The lipid to be analyzed was applied in 1 ml. of hexane. The developing solvents were 1 per cent benzene in hexane, 3 per cent ethyl ether in hexane, 30 per cent ethyl ether in hexane and ethyl ether. The column was finally stripped with 50 ml. methanol. The column was initially standardized with mono-, di-, and triolein and the eluates checked by chromatography on silicone impregnated paper (26).

Triglyceride labeled with palmitate-1-C^{14} was prepared by pooling the labeled products of numerous incubations and isolating the triglyceride on a silicic acid column. This was emulsified by the method of Borgström (27). Sodium taurocholate was obtained from Pfanstiehl Laboratories, Waukeegan, Illinois and cholic acid from Matheson, Coleman, and Bell, Norwood, Ohio. These showed one bile acid spot on paper chromatograms (28).

RESULTS

Properties of the system and cofactor requirements

Under the conditions described, homogenates of the mucosa of rat small intestine were very active in their ability to incorporate palmitate-1-C^{14} into neutral fat. The palmitate incorporation was proportional to homogenate protein up to a protein content of 0.5 mg. Above this amount the curve usually leveled off (Figure 1) and in experiments where the protein exceeded 5 mg. (not shown in
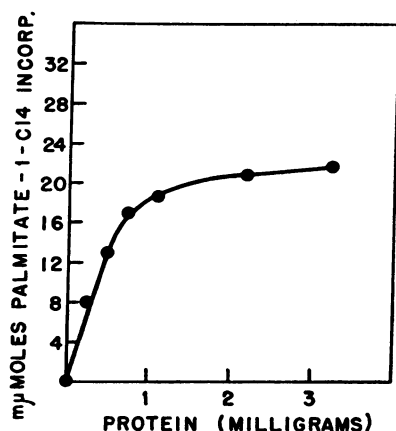
[2] Tris = Tris(hydroxymethyl)aminomethane.

FIG. 1. THE EFFECT OF INCREASING AMOUNTS OF A HOMOGENATE OF RAT SMALL INTESTINAL MUCOSA ON THE INCORPORATION OF PALMITATE-1-C^{14} INTO NEUTRAL FAT

The system contained 0.5 ml. Tris-maleate buffer 0.5 M, pH 7.0, 10 μMoles MgCl$_2$, 25 μMoles KF, 0.1 ml. Tween "80"® (10 per cent solution), 0.5 μMoles Coenzyme A (CoA), 10 μMoles adenosine triphosphate (ATP), palmitate-1-C^{14}, 5 × 10^5 cpm (0.1 μMole) and varying amounts of homogenate protein as indicated in a final volume of 1.5 ml. Incubation was at 37° C. for 30 minutes.

Figure 1) reduction in the incorporation of label actually occurred. Most of the activity in the homogenate was lost by storing at either − 10° C. or + 4° C. for 24 hours.
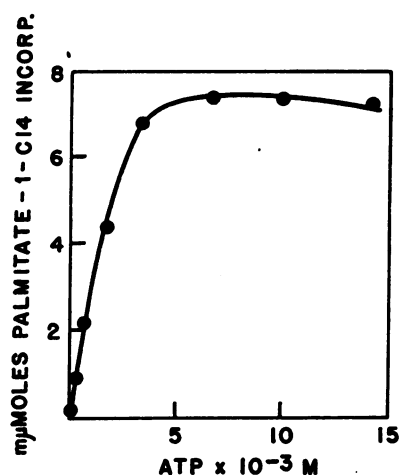

FIG. 3. THE EFFECT OF CoA CONCENTRATION ON THE INCORPORATION OF PALMITATE-1-C^{14} (RAT)

Standard incubation procedure was used except for the varying amounts of CoA. Each tube contained 0.19 mg. homogenate protein.

ATP was essential for the incorporation of palmitate-1-C^{14} into neutral fat and only negligible activity was observed in its absence. The optimal concentration of ATP in this system was 6.6 × 10^{-3} M (Figure 2). Coenzyme A was also necessary and its optimal concentration in the standard incubation mixture was 3.3 × 10^{-4} M (Figure 3). As might be expected, magnesium was required and it is seen in Figure 4 that optimal incorpora-

FIG. 2. THE EFFECT OF ATP CONCENTRATION ON THE INCORPORATION OF PALMITATE-1-C^{14} WITH A HOMOGENATE OF RAT SMALL INTESTINE

Standard incubation procedure was used except for the variation of ATP. Each tube contained 0.18 mg. homogenate protein.
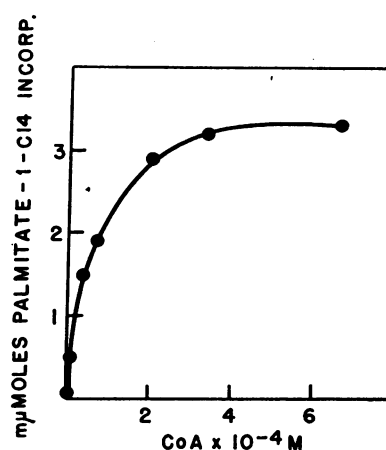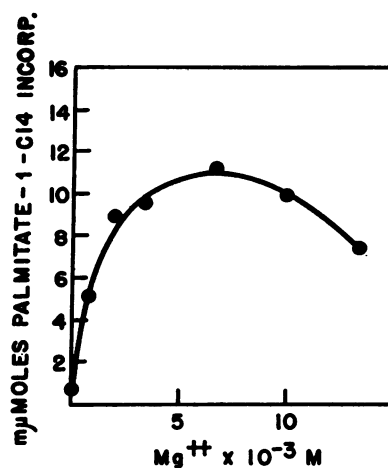

FIG. 4. THE EFFECT OF MAGNESIUM CHLORIDE CONCENTRATION ON THE INCORPORATION OF PALMITATE-1-C^{14} (RAT)

Standard incubation procedure was used except for the variation of Mg^{++} concentration as indicated. Each tube contained 0.36 mg. homogenate protein.

tion of label occurred at the magnesium ion concentration of 6.6×10^{-8} M. Higher concentrations inhibited the reaction.

There was a linear relationship between the time of incubation and palmitate incorporation until the reaction was complete. This took up to 50 minutes for homogenates of high activity, but the reaction was complete within 20 minutes with homogenates of lower activity. The pH optimum for the reaction was found to be a broad one, between 7 and 8 (Figure 5).

Tween "80"® was initially added to the mixture to maintain the lipid components in solution and, indeed, the presence of such an agent has often been necessary to demonstrate some aspects of lipid metabolism *in vitro* (29). In the present

TABLE I

*The effect of potassium fluoride on palmitate-1-C¹⁴ incorporation into neutral fat by homogenates of rat small intestinal mucosa**

	Additions		
KF (1.6×10⁻² M)	ATP	Tween "80"® (final concentration)	Palmitate-1-C¹⁴ incorporated
	μMoles	*% ×10²*	*mμMoles*
0	1	66.0	1.4
+	1	66.0	1.2
0	10	66.0	3.6
+	10	66.0	3.4
0	10	0.66	0.9
+	10	0.66	2.4

* Standard incubation conditions except for variation of potassium fluoride, adenosine triphosphate (ATP) and Tween "80"®. There was 0.40 mg. of homogenate protein in each tube.

system the stimulation of palmitate incorporation into neutral fat by Tween "80"® was impressive (Figure 6), but this stimulation could be partially explained by the inhibitory effect of Tween "80"® on a lipase present in the homogenate. Evidence for this is afforded indirectly by experiments with potassium fluoride and more directly by experiments employing C¹⁴-labeled triglyceride.

Stimulation of palmitate incorporation by fluoride would most likely be due either to the inhibition of an ATPase or of a lipase (30). The results shown in Table I favor the lipase hypothesis. If fluoride stimulation were due to inhibition of ATPase one would expect a stimulation in the presence of suboptimal amounts of ATP and op-
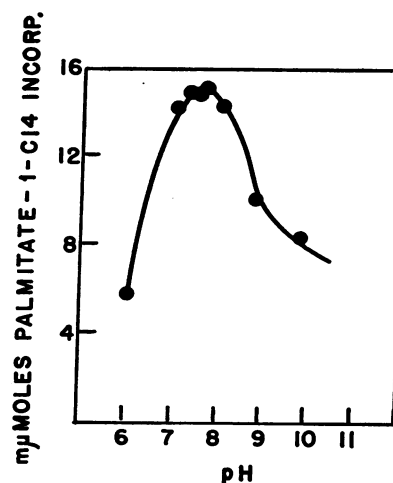

FIG. 5. THE EFFECT OF pH ON THE INCORPORATION OF PALMITATE-1-C¹⁴ (RAT)

Standard incubation procedure was used except that the incubation time was 100 minutes. Between pH 6 and 8, Tris-maleate buffer was used, while glycine buffer was used for pH 9 and 10. Each tube contained 0.15 mg. homogenate protein. The pH of the system was measured after incubation with a glass electrode at room temperature.

timal amounts of Tween "80"®. This was not observed. In contrast, when the system contained suboptimal amounts of Tween "80"® and optimal ATP, the stimulation by fluoride was nearly threefold. This increased incorporation in the presence of fluoride could be explained by lipase inhibition,
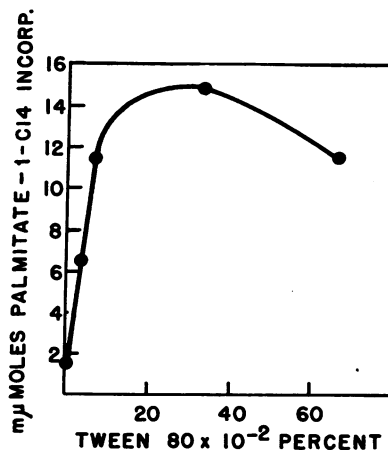

FIG. 6. THE EFFECT OF TWEEN "80"® ON THE INCORPORATION OF PALMITATE-1-C¹⁴ (RAT)

The standard incubation procedure was used except for the variation in the concentration of Tween "80"®. Each tube contained 0.25 mg. homogenate protein.

TABLE II

*Lipase activity in a homogenate of rat small intestinal mucosa**

| | Neutral fat | Glyceride fraction | |
Additions		Tri	Mono +di
	cpm	%	%
1. None (4° incubation)	650	95	5
2. None (37° incubation)	60†		
3. Tween "80"® + KF	530	68	32
4. Tween "80"® + KF + taurocholate	270	33	67

* Each flask contained 0.5 ml. Tris-maleate buffer, 0.5 M, pH 7.0; 0.5 ml. emulsion of palmitate-1-C^{14}-labeled triglyceride; MgCl$_2$, 5 × 10^{-3} M; and 2.2 mg. homogenate protein. Tween "80"®, 0.5 per cent; KF, 1.25 × 10^{-2} M; and taurocholate, 2 × 10^{-3} M were added as indicated. Final volume was 2.0 ml. and the mixture was incubated for 60 minutes at 37° C. After incubation the lipids were extracted and any liberated free fatty acid removed by passing through an IRA-400 column. The remaining lipid was separated into tri- and lower glycerides on a silicic acid column.

† Too few counts for accurate glyceride analysis.

resulting from the additive effect of the fluoride and Tween "80"® (18). The results of a more direct experiment are shown in Table II. Lipase activity was demonstrated when palmitate-1-C^{14}-labeled triglyceride was incubated with a homogenate of small intestinal mucosa at 37° C. This was not observed in the control tube incubated at 4° C. In the presence of Tween "80"® (0.5 per cent) and potassium fluoride (1.25 × 10^{-2} M) the breakdown of the labeled triglyceride was largely but not completely prevented for there was a slight loss of total counts and an appearance of labeled lowered glycerides. This protection was partially

TABLE III

*The effect of cofactors in the esterification of palmitate-1-C^{14} by homogenates of human small intestinal mucosa**

Incubation mixture	Palmitate-1-C^{14} esterified
	mμMoles
Complete	5.1
Minus ATP	0.5
Minus CoA	0.4
Minus Mg^{++}	0.7
Minus Tween "80"®	2.9
Minus KF	4.2

* The incubation mixture contained 0.5 ml. Tris-maleate buffer, 0.5 M, pH 7.0; ATP, 10 μMoles; CoA, 0.5 μmole, MgCl$_2$, 10 μMoles; 0.1 ml. Tween "80"® (10 per cent solution); KF, 25 μMoles; and palmitate-1-C^{14}, 100 mμMoles (5 × 10^5 cpm). Homogenate protein was 0.18 mg. and the final volume 1.5 ml. Incubation was for 30 minutes at 37° C.

overcome by the addition of taurocholate, a known lipase activator (27).

The addition to the standard incubation mixture of either L-α-glycerophosphate (1 μMole), glucose (3 μMoles), glycerol monostearate (1 μMole) or glycerol diolein (1 μMole) produced no consistent increase in the incorporation of palmitate-1-C^{14} into neutral fat.

Results using homogenates of human intestinal tissue were comparable, both qualitatively and quantitatively, to those obtained with the rat intestine. Typical findings showing the dependence upon ATP, CoA and magnesium, as well as the effect of Tween "80"® and potassium fluoride, are shown in Table III. Similar to the observations with the rat homogenates, there was a linear relationship between the esterification of palmitate-1-C^{14} and the amount of homogenate protein in the range studied, which was up to 0.5 mg. (Figure 7). It will be seen that with homogenates of mucosa from patients with idiopathic steatorrhea, the incorporation was significantly lower but a linear relationship between homogenate protein and palmitate esterification still obtained.

Analysis of lipid products formed

The labeled esterified lipid formed during an incubation was stable to 0.1 N KOH at 37° C. for
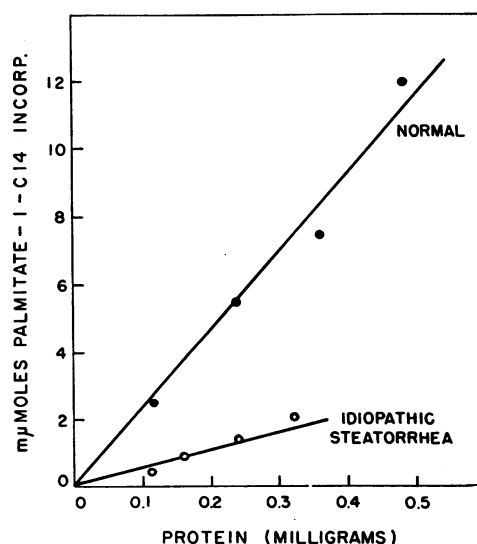

FIG. 7. THE EFFECT OF INCREASING AMOUNTS OF A HOMOGENATE OF HUMAN JEJUNAL MUCOSA (NORMAL AND IDIOPATHIC STEATORRHEA) ON THE INCORPORATION OF PALMITATE-1-C^{14} INTO NEUTRAL FAT
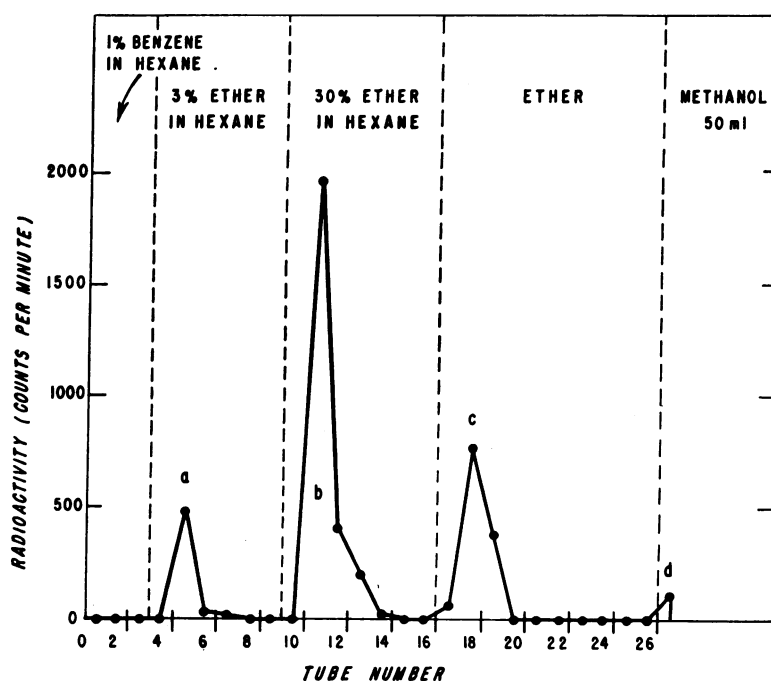
FIG. 8. RESULTS OF SILICIC ACID CHROMATOGRAPHY OF LABELED ESTERI-
FIED PRODUCTS OBTAINED FROM AN INCUBATION WITH A HOMOGENATE OF RAT
SMALL INTESTINE

Each tube contained 6 ml. of eluate. Peak a (triglyceride), 12 per cent
total radioactivity; Peak b (diglyceride), 58 per cent total radioactivity;
Peak c (monoglyceride), 27 per cent total radioactivity; Peak d (phospho-
lipid), 3 per cent total radioactivity.

30 minutes and thus did not merely represent the
formation of palmityl-CoA (13). The result of a
typical analysis of the products obtained from an
incubation with a rat small intestinal homogenate
is shown in Figure 8. Quantitatively similar re-
sults were obtained with homogenates of human
intestinal tissue. It will be observed that most of
the label was incorporated into the glyceride frac-
tions. In analyses of lipid isolated from some other
incubations, about 1 per cent of the radioactivity
was eluted before the glyceride fractions and prob-
ably represented cholesterol esters. After the
glyceride peaks were eluted, a variable but small
proportion of labeled lipid (3 to 8 per cent) re-
mained on the column which could be removed
with 50 ml. of methanol. No attempt was made to
identify this fraction, but on the basis of its polarity
it was assumed to be phospholipid. The glyceride
fraction always consisted of a mixture of tri-, di-
and monoglyceride, of which the diglyceride usu-
ally predominated. Similar results were obtained

by chromatography on silicone impregnated paper,
and it is of interest that the proportion of differ-
ent glycerides in the sample, as shown by the de-
gree of staining of the paper with iodine vapor, was
roughly proportional to the distribution of the
radioactivity. The ratio of glycerides may be al-
tered under certain conditions (Table IV). Thus,
when taurocholate (1.3 × 10⁻³ M) was added to

TABLE IV

The effect of taurocholate on palmitate-1-C^{14} incorporation
into glyceride fractions*

Incubation mixture	Total palmitate-1-C^{14} incorporated	Glyceride fraction		
		Tri	Di	Mono
	mμMoles	%	%	%
Complete	13.2	28	49	23
Plus taurocholate (1.3 × 10⁻³ M)	5.2	13	22	65

* Standard incubation procedure was used. Each tube
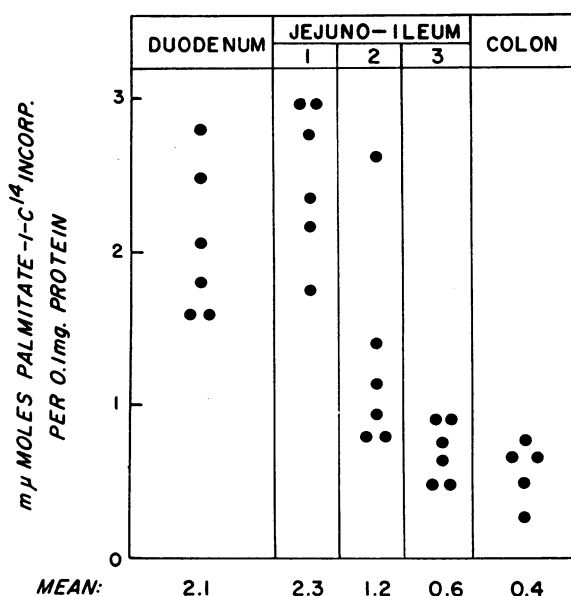contained 0.40 mg. protein.

FIG. 9. A COMPARISON OF PALMITATE-1-C^{14} INCORPORATION BY HOMOGENATES OF RAT SMALL INTESTINAL SEGMENTS AND COLON

The intestine was washed with saline and homogenates were prepared from the mucosa of the duodenum, the jejuno-ileum (divided into three equal segments) and the colon. The numbering of the jejuno-ileal segments is from above downward. The standard incubation procedure was used and less than 0.4 mg. homogenate protein was present in the mixture. Each point represents the mean of duplicate determinations.

the incubation, the total incorporation of palmitate-1-C^{14} was depressed and a significantly greater proportion of monoglycerides was isolated. Similar results were obtained with cholate (1.3×10^{-3} M). These effects may be explained in part by the stimulation of lipase activity by these bile salts.

Effect of fatty acid chain length

In view of the difference in the intestinal absorption of fatty acids depending upon their chain lengths (7–11), the ability of the rat and human homogenates to incorporate C^{14}-labeled octanoate, palmitate and stearate into neutral fat was compared. Taking palmitate incorporation arbitrarily as 100, the incorporation of stearate was 44 ± 9 (mean ± S. E.) and of octanoate 1.2 ± 0.4, using rat intestinal tissue. In homogenates prepared from the jejunum of three human subjects the mean incorporation of stearate was 29 and of octanoate 1.3.

Relative activity of homogenates from different intestinal segments and the effect of abnormal villi

The activity of homogenates prepared from different sites of the rat and human intestine were compared to observe whether the results would correlate with what is known about the sites of fat absorption (31–33). The results shown in Figures 9 and 10 are expressed as the amount of C^{14}-labeled palmitate incorporated into neutral fat by 0.1 mg. of homogenate protein in 30 minutes. It will be seen that, in the rat, the duodenum and upper jejunum were four to five times as active as the terminal ileum or colon. Normal human jejunal tissue was more active than terminal ileum, which in turn was more active than colon. Specimens from the jejunum of six patients with idiopathic steatorrhea, all with histologic criteria of this disease, exhibited a low activity comparable to that usually found in tissue taken from the normal ileum or colon.

DISCUSSION

The system described above for the incorporation of long chain fatty acids into glycerides by homogenates of rat and human intestinal mucosa is similar to that observed in rat liver by Stein, Tietz and Shapiro (15, 16). In both instances incorporation rather than net synthesis was studied. The dependence of this incorporation upon CoA and ATP suggests that the fatty acid is first activated to the acyl-CoA derivative before
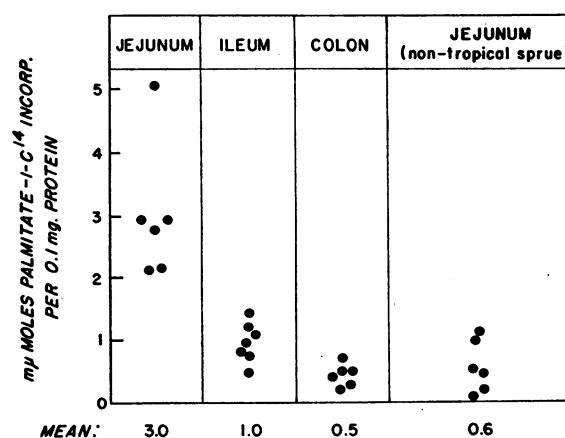

FIG. 10. A COMPARISON OF PALMITATE-1-C^{14} INCORPORATION BY HOMOGENATES OF NORMAL HUMAN JEJUNUM, ILEUM AND COLON AND THE JEJUNUM OF PATIENTS WITH IDIOPATHIC STEATORRHEA

being esterified. These cofactor requirements indicate that the incorporation of fatty acid cannot be explained simply by the synthetic action of a lipase and, indeed, to demonstrate the reaction lipase had largely to be inhibited by Tween "80"® and potassium fluoride. The origin of this lipase is unknown. The studies on rat intestinal tissue (Table II) suggest that it represents an intramucosal rather than a pancreatic enzyme, for the intestine had been repeatedly washed before being homogenized and cell debris, which included cell walls, had been removed from the homogenate by spinning at 600 × G. No experiments were performed on human tissue to demonstrate lipolytic activity. However, the even distribution of label among the glyceride fractions after a standard incubation suggests that a mucosal lipase was probably present in the homogenates of the human biopsy specimens.

The system observed by Favarger and Gerlach in rat small intestinal mucosa, which incorporated palmitate-1-C^{14} into neutral fat, differed from ours (12). It was of lower activity; there was no dependence upon CoA or ATP; and the pH optimum was 5. Thus their results may have represented the synthetic action of a lipase. The differences between the two systems can perhaps be explained by the fact that Favarger and Gerlach did not inhibit lipase activity with Tween "80"® and potassium fluoride, and possibly by their use of far more concentrated homogenates. Such homogenates depressed palmitate incorporation under our experimental conditions.

Kennedy has expanded the early observations of Kornberg and Pricer (13, 14) and demonstrated a series of reactions relating the synthesis of triglycerides and phospholipids (29). These are represented in Figure 11. One will notice that there is no place in this scheme for the formation of monoglycerides and that phosphatidic acid is the precursor of 1,2-diglyceride, which in turn may become either triglyceride or phospholipid. The fact that analyses of the labeled lipid formed in our experiments showed an appreciable quantity of monoglycerides in no way negates the Kennedy scheme, for it will be noted in Table II that the lipolytic activity present in the homogenate was not completely inhibited by the addition of Tween "80"® and potassium fluoride. Thus, the labeled lipid formed during an incubation may be the re-
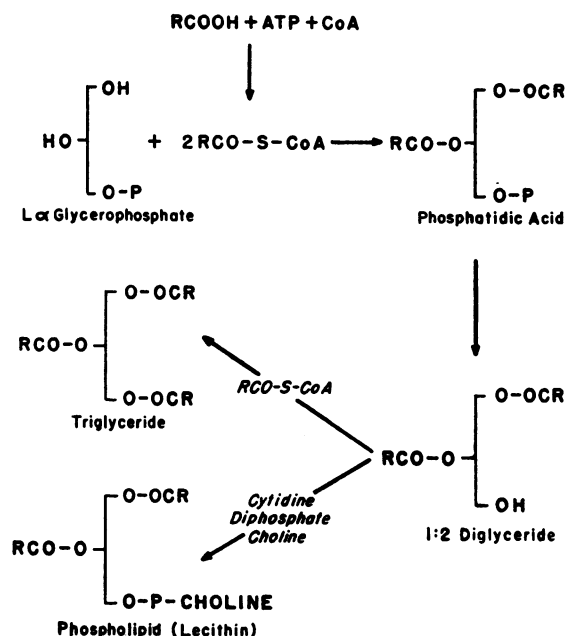

Fig. 11. Pathways for the Enzymatic Synthesis of Phospholipid and Triglyceride

This scheme has been outlined by Kennedy (29). R = long chain fatty acid.

sult of both neutral fat formation on the one hand and unsuppressed lipolysis on the other. This is supported by the experiments in which taurocholate, a known lipase activator, was added to the incubation medium (Table IV). This resulted in a diminished total incorporation of palmitate into neutral fat but in an increased proportion of labeled monoglyceride. A contributing factor to the lowered incorporation of palmitate in the presence of taurocholate may have been the inhibition of acyl-CoA formation by the bile salt (13).

Buell and Reiser have recently studied the incorporation of C^{14}-fructose-1,6-diphosphate into glyceride-glycerol using fortified concentrated homogenates of hog intestinal mucosa (34). They concluded that L-α-glycerophosphate was the immediate glyceride-glycerol precursor which is in accordance with Kennedy's scheme. They also indicated that the addition of L-α-glycerophosphate to the homogenate was necessary before incorporation of palmitate-1-C^{14} into triglyceride could be observed. In our rat intestinal homogenate system L-α-glycerophosphate did not have to be added nor did its addition cause any stimulation. Results similar to ours have been reported by Stein,

Tietz and Shapiro in whole liver homogenates (15). These workers could only demonstrate dependence upon L-α-glycerophosphate with a liver microsomal preparation (16). Buell and Reiser (34), using chromatography on silicic acid impregnated paper, identified triglyceride as the only labeled glyceride formed during an incubation. This may have been due to the fact that they washed their lipid sample with alkaline ethanol to remove fatty acids and monoglycerides. We have found that such treatment also alters the behavior of glycerides on silicic acid columns such that a large proportion of lower glycerides may be eluted prior to or with the triglyceride fraction. On the other hand, treatment with alkaline ethanol does not alter the behavior of glycerides on silicone impregnated paper (35).

The negligible incorporation of octanoate into neutral fat in our experiments is consistent with previous observations (3, 10, 11) that short chain, in contrast to long chain fatty acids, pass unesterified into the portal vein during absorption. This difference in the behavior of short chain fatty acids, both *in vivo* and *in vitro,* may be related either to their greater water solubility or to enzyme specificity. Greater water solubility might reduce their contact with the enzymes involved in activation or esterification. On the other hand, it is possible that one or both of these enzyme systems in the mucosa are specific and act only on long chain fatty acids. Such enzyme specificity has been observed by Kornberg and Pricer (13, 14) in guinea pig liver microsomal preparations. They found that the activation of octanoate was approximately half that of palmitate, but that the esterification of octanoate was only minimal. The relative status of these enzyme systems in the intestine is as yet unknown. In our rat and human experiments, stearate incorporation into neutral lipid was also less than that of palmitate. This correlates with but is probably not causally related to the fact that in the rat stearate is normally absorbed less efficiently than palmitate (36). Again the relative importance of fatty acid solubility and enzyme specificity in explaining the *in vivo* and *in vitro* behavior of stearate remains to be established.

Further evidence that the system we have observed may be involved in fatty acid absorption is provided by the experiments in which the activity of the mucosa from different segments of the rat and human intestine was compared (Figures 9 and 10). The fact that homogenates from the upper small intestine were more active in incorporating palmitate than those from the lower intestine or colon is compatible with the observations that fat absorption in the rat usually occurs in the duodenum and jejunum (31, 32) and the findings of Borgström, Dahlqvist, Lundh and Sjovall (33) that the major area of fat absorption in man is in the upper small intestine. Since the enzymatic activity of the mucosa is probably in the villous epithelial cells, a homogenate containing less villous epithelium per milligram of protein would be expected to be less active. The difference in enzymatic activity along the intestine may thus be due to an *anatomic* factor and may be explained by a decrease in the number of villi as one proceeds from the upper to the lower intestine (37). On the other hand, it is possible that the gradation of activity may in part represent a *functional* differentiation of the cells along the intestine. An example of such specialization of intestinal cell function is the observation that vitamin B_{12} absorption occurs primarily in the ileum (38). Our data do not allow us to conclude at this time whether the differences in palmitate incorporation along the intestine are on an anatomic or a functional basis, or both. It is of interest that the jejunum of patients with idiopathic steatorrhea possessed a very low activity, comparable to that of the normal ileum or colon. Since such jejunum has flattened villi with abnormal epithelial cells, these results may again be a reflection either of a diminution in the proportion of villous epithelial cells or of altered mucosal cell function. It cannot be concluded, nor do we propose that the low activity observed in patients with idiopathic steatorrhea is the primary basis for their malabsorption of fat.

The data presented indicate that the mucosal epithelium of the rat and human intestine is most active in the incorporation of long chain fatty acids into neutral fat. The correlation between the properties of the system described by us and previous physiologic observations (3, 33) lends support to the view that a mechanism similar to that demonstrated *in vitro* is involved in the esterification of long chain fatty acids during their absorption by the intestine.

SUMMARY

1. A very active system has been demonstrated in cell-free homogenates of rat and human intestinal mucosa which incorporates palmitate-1-C^{14} into neutral fat. This incorporation is dependent upon Coenzyme A, adenosine triphosphate and magnesium ions. Short chain fatty acids are not incorporated by this system.

2. Lipase activity had to be suppressed with Tween "80"® and potassium fluoride before significant incorporation could be demonstrated.

3. The labeled neutral fat formed *in vitro* was a mixture of mono-, di- and triglyceride and probably represented a balance between the incorporation of palmitate-1-C^{14} into neutral fat and lipolysis.

4. In both rat and human experiments, homogenates prepared from the duodenum or jejunum, which are the usual sites of fat absorption, were four to five times as active as those from the ileum or colon. Mucosa from the jejunum of patients with idiopathic steatorrhea was found to have a greatly diminished capacity to esterify palmitate-1-C^{14}.

5. It is suggested that a mechanism similar to the one described *in vitro* is involved in the esterification and absorption of long chain fatty acids by the intestine *in vivo*.

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