Intestinal Metabolism of Fatty Acids

By M. ENSER

Department of Biochemistry, University of Oxford

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1. The effect of concentration on the oxidation and incorporation into lipids of lauric acid and linoleic acid by rings of rat small intestine has been studied *in vitro*. 2. In the absence of glucose, the oxidation of lauric acid in the range 0.01–5.0 mM showed a maximum at 0.1 mM. In the presence of glucose the maximum was at 0.5 mM. The oxidation of linoleic acid in the presence of glucose increased throughout the concentration range 0.01–5.0 mM. 3. The incorporation of lauric acid into lipids was maximal at 0.5–0.6 mM in the presence of glucose, but at 10 mM in the absence of glucose. At 0.8 mM-lauric acid, in the presence of glucose, over 75% of the incorporated lauric acid was in triglycerides, but at 10 mM they only contained 30%. The incorporation of glucose carbon into glycerides paralleled the incorporation of lauric acid. 4. In the range 0.01–2.5 mM-linoleic acid the quantity incorporated into lipids increased. In the range 0.01–0.4 mM linoleic acid was incorporated predominantly into triglycerides, but between 0.4 and 1.0 mM most was in diglycerides, and between 2.5 and 5.0 mM most was in monoglycerides. 5. The relationship of fatty acid concentration to the mechanism of absorption is discussed, together with the correlation between the distribution of the absorbed fatty acids within the tissue lipids and the lipase activity of intestinal mucosa.

The absorption and metabolism of fatty acids by preparations of intestine *in vitro* has been studied by several workers (Johnston, 1958; Johnston & Bearden, 1960; Tidwell & Johnston, 1960, 1961; Kronl, Skála, Michalec, Andryšek & Hromádková, 1963). In these studies the albumin complex of the fatty acids was used as the substrate. However, the albumin complex is not a substrate for intestinal absorption *in vivo*. Moreover, calculations based on the results of Borgström, Tryding & Westöö (1957) showed that in man the fatty acid concentration in the small intestine reached very high levels after a balanced meal. The metabolism of lauric acid and linoleic acid by rings of rat intestine has therefore been investigated in the absence of albumin, and over a wide concentration range, including those concentrations that have been shown to inhibit respiration (Enser, 1964).

EXPERIMENTAL

Materials

Animals. Wistar strain rats (approx. 200 g. each), fed *ad lib.* on a diet of rat cubes (Oxo Ltd., London), were starved for 24 hr. before use.

Chemicals. All solvents were of analytical grade. Fatty acids were obtained from L. Light and Co. Ltd., Colnbrook, Bucks. [$1\text{-}^{14}\text{C}$]Lauric acid, [$1\text{-}^{14}\text{C}$]linoleic acid and [$\text{U-}^{14}\text{C}$]-

glucose were obtained from The Radiochemical Centre, Amersham, Bucks. Glyceryl tripalmitate, glyceryl tri-stearate and cholesteryl stearate were obtained from British Drug Houses Ltd., Poole, Dorset. Glyceryl monostearate was obtained from the California Corp. for Biochemical Research, Los Angeles, Calif., U.S.A. 2,5-Diphenyloxazole was obtained from Thorn Electronics Ltd., Tolworth, Surrey. Florisil, a synthetic magnesium silicate, for chromatography (60–100 mesh), was obtained from L. Light and Co. Ltd.

Fatty acid solutions were prepared by taking up the requisite quantity of fatty acid, to which the calculated equivalent of NaOH had been added, in the buffered saline medium, pH 6.8, with heating on a water bath if necessary to disperse the material.

Fatty acid emulsions were prepared with lecithin and monostearin. The fatty acid solution was warmed to 60° in the vessel of an overhead-drive homogenizer (Measuring and Scientific Equipment Co. Ltd., London). A solution of lecithin and monostearin in ether (0.2 ml.) was added rapidly, and the mixture was homogenized at 14000 rev./min. for two 1 min. periods. The mixture was cooled to 15° and rehomogenized. The emulsion was stable for 24 hr. at 4°, and phase-contrast microscopy showed that all the particles were below 0.5 μ in diameter. For the preparation 0.3 mg. each of lecithin and monostearin were used/ μ mole of fatty acid.

Methods

Analytical procedures. Fatty acid esters were determined by a modification of the method of Rapport & Alonzo (Enser

& Bartley, 1962). Glycerol was determined by the method of Hanahan & Olley (1958). Respiration was measured by following the O₂ uptake by standard manometric techniques at 37° in an atmosphere of O₂.

Tissue preparations. Rings of small intestine were prepared by the method of Enser (1964). A tissue wet wt. of 300–500mg. (10–30 rings) was used/manometer vessel. Tissue was incubated in a phosphate-buffered saline medium (Krebs, 1933), gassed with O₂, at pH 6.8. Calcium and magnesium salts were omitted.

Determination of radioactivity. The methods of Enser (1964) were used. ¹⁴CO₂ was plated as BaCO₃ by using carrier Na₂CO₃ (Sakami, 1955). The plates were counted to 10000 counts, corrected for background and calculated for infinite thinness by using a calibration curve. Lipid samples were counted in a solution of 5.0ml. of sulphur-free toluene containing 12mg. of 2,5-diphenyloxazole in an Ekco scintillation counter. They were counted to 5000 counts and corrected for background and quenching.

Extraction of lipids. The incubation medium and intestinal rings were freeze-dried after the addition of 0.8ml. of 10% (w/v) sucrose. The dry material was extracted with two 5.0ml. portions of chloroform-methanol (2:1, v/v) and the lipid purified by the method of Folch, Lees & Sloane-Stanley (1957), except that during the washing procedures the phases were mixed by shaking vigorously to ensure the removal of radioactive contaminants. The solution was finally evaporated to dryness at 60° under N₂ and the lipid re-extracted into chloroform.

Removal of substrate fatty acids from lipid extracts. Lauric acid was removed by extracting the lower phase, remaining after the washing of the lipid extract, with three 4ml. portions of 0.5% (w/v) K₂CO₃ in 50% (v/v) methanol and one portion of 50% (v/v) methanol to remove any remaining K₂CO₃. Linoleic acid was removed by the same extraction procedure, but with the lipid dissolved in hexane.

Separation of neutral-lipid classes. Neutral lipids were separated on a column (10mm. × 160mm.) of Florisil (about 7g.). The Florisil was prepared by placing 7g. in a measuring cylinder and making the volume up to 30ml. with dry hexane; 0.2ml. of water was then added, and, after the mixture had been shaken to break up the clumps, it was left to equilibrate overnight. The slurry was poured into the column, and settled by tapping the sides of the column. It was then washed with 20ml. of dry hexane, and the lipid was applied in hexane, warmed if necessary to keep it in solution. Carrier was added to the lipid extract, usually 5mg. each of cholesteryl stearate, glyceryl tristearate or tripalmitate, glyceryl dioleate, glyceryl monostearate and palmitic acid. The lipids were eluted with the system of Carroll (1961). The identity of the lipid eluted in each fraction was confirmed by the use of pure lipids and by determination of the ester/glycerol ratio in fractions containing glycerides. All fractions were over 90% pure except for the cholesterol fraction, which contained varying quantities of triglycerides. Since no radioactivity could be detected in cholesterol, the radioactivity of this fraction was added to that of the triglycerides. All column runs were checked by determination of the natural esters present in the eluates. The recovery of lipids from the column was 95–105% by natural ester determination, and the recovery of ¹⁴C was 90–110%.

RESULTS

Oxidation of lauric acid by intestinal rings. Intestinal rings were incubated in phosphate-buffered saline medium, pH 6.8, containing lauric acid labelled with [¹⁴C]lauric acid in manometer vessels, and the radioactivity of the carbon dioxide produced was measured. The effect of concentration on the oxidation of lauric acid is shown in Table 1.

Table 1. *Metabolism of lauric acid by intestinal rings in the presence and absence of glucose*

Intestinal rings were incubated in phosphate-buffered saline, pH 6.8, containing [1- ¹⁴ C]lauric acid (specific activity 5.72 × 10 ⁶ counts/min./μmole) in the presence or absence of 5 mm-glucose at 37° for 90 min.											
Expt. 1											
Concn. of lauric acid (mm) ...		0.01	0.05	0.1	0.5	1.0	5.0				
		¹⁴ CO ₂ produced (counts/min./mg. wet wt. of tissue/hr.)									
With glucose		11	—	55	—	103	68				
Without glucose		—	57	89	—	68	54				
		¹⁴ C incorporated into lipids (counts/min./mg. wet wt. of tissue/hr.)									
With glucose		100	984	1705	10800	4950	5650				
Without glucose		—	448	951	1310	1060	5220				
Expt. 2											
Lauric acid incorporated into neutral lipids in the presence of glucose (mμmoles/500 mg. wet wt. of tissue/90 min.)											
Concn. of		0.01	0.05	0.135	0.2	0.4	0.6	0.8	2.5	5.0	10.0
Lipid fraction		0.01	0.05	0.135	0.2	0.4	0.6	0.8	2.5	5.0	10.0
Cholesteryl esters		0.2	1.5	3.8	4.0	7.7	21	27	33	67	92
Triglycerides		14.4	80	228	503	983	1435	996	113	125	123
Diglycerides		2.3	12.8	31	58	82	106	100	63	90	91
Monoglycerides		0.7	4.7	5.9	11	11.1	12.7	4.6	8.3	11.4	13.8

The production of $^{14}\text{CO}_2$ increased by 57% as the lauric acid concentration was raised from 0.05 to 0.1 mM, but then decreased when the concentration was raised to 5.0 mM. In the presence of 5.0 mM-glucose the oxidation of lauric acid at concentrations below 0.1 mM was less than in the absence of glucose. At 1.0 mM, however, the oxidation of lauric acid was stimulated and was 51% greater than in the absence of glucose. Between 0.5 and 5.0 mM the oxidation gradually decreased and the stimulation by glucose was almost abolished at 5.0 mM.

Incorporation of lauric acid into lipids. The effect of 0.1–5.0 mM-lauric acid on the incorporation of ^{14}C from $[1\text{-}^{14}\text{C}]$ lauric acid into lipids of intestinal rings in the presence and absence of 5.0 mM-glucose is shown in Table 1. In the presence of glucose the incorporation increased 108-fold from 0.01 mM- to 0.5 mM-lauric acid, but fell by half at 1.0 mM and increased again slightly at 5.0 mM. In the absence of glucose there was a threefold increase in the incorporation between 0.05 and 0.5 mM with a slight decrease at 1.0 mM, followed by a very sharp increase at 5.0 mM, where there was little difference between the quantities incorporated. The neutral lipids were separated on a column of Florisil so that the distribution of the lauric acid could be determined. The effect of lauric acid concentration on its incorporation into triglycerides, diglycerides, monoglycerides and cholesteryl esters in the presence of 5.0 mM-glucose is given in Table 1. The pattern of incorporation into triglycerides followed the incorporation into total lipids at concentrations below 2.5 mM, reaching a maximum at 0.6 mM and then declining. However, there was no secondary increase at 5.0 mM. The incorporation into the diglyceride fraction increased from 2.3 to 106 $\mu\text{moles}/500\text{mg. wet wt. of tissue}/90\text{min.}$ as the concentration was raised from 0.01 to 0.6 mM, but there was no further increase when the concentration was raised to 10.0 mM. The quantity of lauric acid incorporated into monoglycerides increased throughout the whole range, from 0.01 to 10.0 mM, from 0.7 to 13.8 $\mu\text{moles}/500\text{mg. wet wt. of tissue}/90\text{min.}$ The variability in the results was partly caused by the small quantity incorporated. The quantity of lauric acid incorporated into the cholesteryl ester fraction also increased continuously as the concentration was raised. The quantity incorporated at 10.0 mM (91.3 $\mu\text{moles}/500\text{mg. wet wt. of tissue}/90\text{min.}$) was responsible for the increased incorporation above 2.5 mM shown by the total lipid extract. At 0.6 mM the distribution of lauric acid between the neutral lipids was: triglycerides, 90.8%; diglycerides, 6.9%; monoglycerides, 0.8%; cholesteryl esters, 1.5%. At 10.0 mM, however, the distribution was: triglycerides, 38.6%; diglycerides, 28.5%; monoglycerides, 4.2%; cholesteryl esters, 28.7%.

Time-course of the incorporation of lauric acid.

Table 2. *Time-course of the oxidation and incorporation into lipids of lauric acid by intestinal rings*

The incubation was at 37° in phosphate-buffered saline, pH 6.8, containing glucose (5 mM) and $[1\text{-}^{14}\text{C}]$ lauric acid as label.

Incubation time (min.)	Concn. of lauric acid (mM)	Lipid ^{14}C (counts/min./mg. wet wt. of tissue)					
		...	0	10	20	40	60 90
	0.4		69	100	101	95	105 109
	2.5		46	54	40	39	42 30
		$^{14}\text{CO}_2$ (counts/min./mg. wet wt. of tissue)					
	0.4		1.2	4	5.9	8.3	10.9 12.9
	2.5		0.9	1.6	2.1	2.7	3.0 2.9

Since concentrations of lauric acid above 0.4 mM inhibited the respiration of intestinal rings, a study of the time-course of incorporation of lauric acid into lipids was made to determine whether the decreased incorporation at high concentration was the result of continuous incorporation, or of a rapid initial incorporation possibly followed by degradation of the synthesized lipids. Table 2 gives the radioactivity from $[1\text{-}^{14}\text{C}]$ lauric acid incorporated into the total lipids of intestinal rings at different time-intervals during incubation with 0.4 mM- and 2.5 mM-lauric acid. After 10 min. for equilibration of the manometers the incorporation at 2.5 mM was only 67% of that at 0.4 mM. It increased slightly for the next 10 min. and then decreased until at 90 min. it was only 55% of that at 10 min. The incorporation at 0.4 mM increased by 45% during the first 10 min. but there was little further incorporation during the next 80 min.

Time-course of oxidation of lauric acid. ATP is necessary for the oxidation of fatty acids and the synthesis of glycerides. The cessation of the incorporation of lauric acid at 2.5 mM into lipids after 10 min. might be expected from the inhibition of respiration by this concentration. However, at 0.4 mM respiration was only slightly decreased although the incorporation of lauric acid into lipids had almost ceased after 10 min. The time-course of the oxidation of lauric acid was therefore studied to see whether it correlated with the incorporation into lipids. After 10 min. for equilibration of the manometers the quantity of lauric acid oxidized was very similar at both concentrations, but at 2.5 mM it increased very slowly for 60 min. and then ceased (Table 2). At 0.4 mM the oxidation of lauric acid continued rapidly and after 90 min. over four times as much had been oxidized as at 2.5 mM. This suggests that the apparent cessation of the incor-

Table 3. *Effect of incubation time on the quantities of lauric acid incorporated into neutral lipids by intestinal rings*

Intestinal rings were incubated in phosphate-buffered saline, pH 6.8, containing [$1\text{-}^{14}\text{C}$]lauric acid and glucose (5 mM) at 37°.

Incubation time (min.) ...	Lauric acid incorporated (m μ moles/500 mg. wet wt. of tissue)			
	10	90	10	90
Concn. of lauric acid (mM) ...	0.4	0.4	2.5	2.5
Lipid fraction				
Triglycerides	1149	1240	498	230
Diglycerides	74	103	98	82
Monoglycerides	11	11	22	34
Cholesteryl esters	14	12	197	107

poration of lauric acid into lipids after 10 min. was caused by competition between the synthesis of glycerides and their degradation by a lipase, the activity of which was indicated by the loss of incorporated acid at 2.5 mM.

Analysis of the different neutral lipids for radioactivity after 10 min. and 90 min. incubation (Table 3) revealed that the loss of radioactivity incorporated at 2.5 mM after 10 min. incubation was caused by loss of half of the activity of the triglyceride and cholesteryl ester fractions. At 0.4 mM the quantity of lauric acid incorporated and its distribution between the neutral lipids were very similar after 10 and 90 min. incubation.

Effect of lauric acid on the incorporation of glucose carbon into lipids. The incorporation of radioactive fatty acids into glycerides could occur by a lipase-catalysed exchange reaction or by synthesis. To demonstrate that synthesis was taking place under the conditions used in this study the incorporation of ^{14}C from [$\text{U-}^{14}\text{C}$]glucose into lipids was followed at different lauric acid concentrations. Table 4 shows that the incorporation of glucose carbon into lipids followed the pattern of lauric acid incorporation into triglycerides. As the lauric acid concentration was increased from 0.01 to 0.5 mM the incorporation of label from glucose rose to a peak, and then fell when the lauric acid concentration reached 1.0 mM. After 1 hr. incubation in the presence of 0.5 mM-lauric acid, 556 m μ moles of glycerol had been incorporated/500 mg. wet wt. of tissue, assuming that all the label was in the glycerol moiety of the glycerides. This corresponds to the incorporation of 1.667 μ moles of fatty acid (calculated as triglycerides)/500 mg./hr., and is in good agreement with the quantity of lauric acid incorporated.

The time-course of the incorporation of glucose into lipids was also similar to that of lauric acid

Table 4. *Metabolism of [^{14}C]glucose by intestinal rings in the presence of lauric acid*

The incubation was at 37° in phosphate-buffered saline, pH 6.8, containing glucose (5 mM) labelled with [$\text{U-}^{14}\text{C}$]glucose (final specific activity 2.96×10^4 counts/min./ μ mole). Lauric acid was tipped from the side arm at 0 min.

Expt. 1: Time-course

Incubation time (min.) ...	^{14}C incorporated into total lipid (counts/min./mg. wet wt. of tissue)				
	0	5	10	35	60
Concn. of lauric acid (mM)					
0.1	0.56	0.95	1.15	1.75	2.05
1.0	0.56	1.01	1.15	1.33	0.89

Expt. 2: Effect of lauric acid concentration

Concn. of lauric acid (mM) ...	^{14}C incorporated with total lipid (counts/min./mg. wet wt. of tissue/hr.)				
	0.01	0.05	0.1	0.5	1.0
Lipid ^{14}C	1.6	1.6	2.1	3.2	0.9

Table 5. *Comparison of the effects of linoleic acid, oleic acid and stearic acid on intestinal rings*

Intestinal rings were incubated in phosphate-buffered saline, pH 6.8, containing glucose (5 mM) labelled with [$\text{U-}^{14}\text{C}$]glucose at 37° for 1 hr. in manometer vessels.

Fatty acid	Activity (% of control activity in the absence of added fatty acid)		
	O_2 uptake	$^{14}\text{CO}_2$ produced	^{14}C incorporated into lipids
Stearic acid (0.6 mM)	76.6	56.9	75.8
Stearic acid (6.0 mM)	102.7	89.3	129.2
Oleic acid (0.6 mM)	76.6	67.7	83.4
Oleic acid (6.0 mM)	100.0	93.4	120.7
Linoleic acid (0.6 mM)	53.3	55.7	—
Linoleic acid (6.0 mM)	83.5	102.5	—

(Table 4; cf. Table 2). After 10 min. incubation the incorporation of 1.0 mM-lauric acid had almost ceased, and after 35 min. label was lost from the lipid. With 0.1 mM-lauric acid incorporation of glucose label continued for 60 min. but the rate declined with time. Fractionation of the neutral lipids from tissues incubated with 1.0 mM-lauric acid for 5 min. showed that 71% of the label incorporated from glucose was in triglycerides, 14% in diglycerides and 10% in monoglycerides.

Metabolism of linoleic acid. A comparison of the effects of stearic acid, oleic acid and linoleic acid on

the metabolism of intestinal rings is shown in Table 5. In spite of differences in solubility the effects of these three acids were similar. At 0.6 mM only linoleic acid inhibited respiration, and in other experiments it was often found that no inhibition was caused by linoleic acid concentrations below 5.0 mM. The oxidation of linoleic acid measured by $^{14}\text{CO}_2$ production from $[1-^{14}\text{C}]$ linoleic acid in the presence of 5.0 mM-glucose increased as the concentration of linoleic acid was raised from 0.01 to 2.5 mM (Table 6). There was no peak like that occurring in the oxidation of lauric acid.

Incorporation of linoleic acid into lipids. The effect of concentrations of linoleic acid in the range 0.01–5.0 mM on its incorporation into lipids is shown in Table 6. Concentrations from 0.01 to 0.8 mM caused a linear increase in the incorporation into lipids. Increasing the concentration to 2.5 mM brought about a smaller increase and raising it to 5.0 mM had no further effect.

The quantities of linoleic acid incorporated into the different neutral lipids are shown in Table 7. The quantity of linoleic acid incorporated into triglycerides increased from 1.7 to 59.5 $\mu\text{moles}/500\text{ mg. wet wt. of tissue/hr.}$ as the concentration was raised from

0.01 to 2.5 mM, but fell to 31.7 $\mu\text{moles}/500\text{ mg./hr.}$ when the concentration was raised to 5.0 mM. Since the oxygen uptake in the presence of 5.0 mM-linoleic acid was depressed by 14% below the control incubated without fatty acid, some inhibition of lipid synthesis might be expected. However, the total quantity of linoleic acid incorporated into the neutral lipids was 2.1 $\mu\text{moles}/500\text{ mg./hr.}$ greater than at 2.5 mM. The incorporation of linoleic acid into diglycerides increased with increasing linoleic acid concentration from 0.01 to 2.5 mM, but no further increase occurred when the concentration was raised to 5.0 mM. At concentrations between 0.01 and 0.4 mM the quantity of linoleic acid incorporated into triglycerides exceeded that in diglycerides, but above 0.4 mM more was incorporated into diglycerides. At 5.0 mM three times as much had been incorporated into diglycerides compared with triglycerides: 103 compared with 31.7 $\mu\text{moles}/500\text{ mg. wet wt. of tissue/hr.}$

The incorporation of linoleic acid into monoglycerides increased almost linearly as the linoleic acid concentration was raised from 0.01 to 5.0 mM. At 0.4 mM the quantity incorporated into monoglycerides (13.6 $\mu\text{moles}/500\text{ mg. wet wt. of tissue/hr.}$) was less than that in triglycerides (22.9 $\mu\text{moles}/500\text{ mg./hr.}$) and diglycerides (20.5 $\mu\text{moles}/500\text{ mg./hr.}$). At 1.0 mM, however, the quantity in monoglycerides (67.2 $\mu\text{moles}/500\text{ mg./hr.}$) exceeded that in triglycerides (36.6 $\mu\text{moles}/500\text{ mg./hr.}$), but not that in diglycerides (75.8 $\mu\text{moles}/500\text{ mg./hr.}$). At 2.5 and 5.0 mM the monoglycerides fraction contained the greatest quantity.

The incorporation of linoleic acid into cholesteryl esters presented a different pattern from the incorporation into glycerides (Table 7). The quantity incorporated increased from 0.5 to 19.1 $\mu\text{moles}/500\text{ mg. wet wt. of tissue/hr.}$ as the linoleic acid concentration was raised from 0.01 to 0.4 mM. Further increase in the linoleic acid concentration resulted in a decreased incorporation, and at 5.0 mM only 12.7 μmoles were incorporated/500 mg. wet wt. of tissue/hr.

Time-course of the metabolism of linoleic acid. Intestinal rings were incubated without linoleic acid

Table 6. *Effect of concentration on the oxidation and incorporation into lipids of linoleic acid by intestinal rings*

Intestinal rings were incubated in phosphate-buffered saline, pH 6.8, containing $[1-^{14}\text{C}]$ linoleic acid and glucose (5 mM) at 37° for 1 hr.

Concn. of fatty acid (mM)	$^{14}\text{CO}_2$ produced (counts/min./mg. wet wt. of tissue/hr.)	Linoleic acid incorporated into lipid ($\mu\text{moles}/500\text{ mg. wet wt. of tissue}$)
0.01	26.9	3.3
0.05	133	13.9
0.10	254	26.4
0.40	812	80.3
0.80	1410	192.5
1.00	1160	196.0
2.50	1690	332.0
5.00	1453	338.0

Table 7. *Effect of concentration on the incorporation of linoleic acid into neutral lipids*

Intestinal rings were incubated in phosphate-buffered saline, pH 6.8, containing $[1-^{14}\text{C}]$ linoleic acid and glucose (5 mM) at 37° for 1 hr.

Lipid fraction	Concn. of linoleic acid (mM) ...	Linoleic acid incorporated ($\mu\text{moles}/500\text{ mg. wet wt. of tissue/hr.}$)						
		0.01	0.05	0.1	0.4	1.0	2.5	5.0
Triglycerides		1.7	6.4	12.2	22.9	35.2	59.5	31.7
Diglycerides		0.8	3.3	7.3	20.5	75.8	103	103
Monoglycerides		0.2	1.1	2.8	13.6	67.2	148	181
Cholesteryl esters		0.5	3.2	4.1	19.1	17.4	14.6	12.7

Table 8. *Time-course of the metabolism of linoleic acid by intestinal rings*

Intestinal rings were incubated in phosphate-buffered saline, pH 6.8, containing [1-¹⁴C]linoleic acid and glucose (5mm) at 37°.

Incubation time (min.) ...	Linoleic acid incorporated (mμmoles/500mg. wet wt. of tissue)		
	10	20	60
Concn. of linoleic acid (mm)	O ₂ uptake (μl./mg. wet wt. of tissue)		
0 (control)	0.23	0.48	1.29
0.4	0.24	0.58	1.29
2.5	0.25	0.50	1.25
14CO ₂ produced (counts/min./mg. wet wt. of tissue)			
0.4	366	551	812
2.5	461	797	1690
Linoleic acid incorporated into lipids (mμmoles/500mg. wet wt. of tissue)			
0.4	103	113	80.3
2.5	257	269	332

or with 0.4mm- or 2.5mm-linoleic acid for 10, 20 and 60min. Respiration was almost the same under all conditions (Table 8). It was linear for the first 20min. at a rate of 25μl. of oxygen/g. wet wt. of tissue/min. but after 60min. it had fallen by 19%.

The production of ¹⁴CO₂ from [1-¹⁴C]linoleic acid was fastest in the presence of 2.5mm-linoleic acid at all times studied (Table 8). After 10min. the production of ¹⁴CO₂ from 0.4mm-linoleic acid was 80% of that with 2.5mm, but after 60min. it was only 48% of the value for 2.5mm. When the incorporation of linoleic acid into lipids was studied the differences were even more marked (Table 8). At both concentrations there was a rapid initial incorporation but after 10min. only 40% as much had been incorporated at 0.4mm as at 2.5mm: 103 compared with 257mμmoles/500mg. wet wt. of tissue. From 10min. to 60min. at 2.5mm there was a slow but linear increase in the incorporation, but at 0.4mm there was a slight increase up to 20min., but after that there was a loss of label until at 60min. it was 80.3mμmoles/500mg. wet wt. of tissue, which was less than at 20min. This decrease in incorporation cannot readily be explained by a decrease in the linoleic acid in the medium during the incubation. Of the 2μmoles present initially 1.8μmoles remained after 20min., since 109mμmoles had been incorporated into lipids and 92.8mμmoles had been oxidized. This would decrease the concentration in the medium from 0.4 to 0.36mm.

The distribution of the incorporated linoleic acid between the neutral lipids after 10 and 60min.

Table 9. *Effect of incubation time on the incorporation of linoleic acid into neutral lipids by intestinal rings*

Intestinal rings were incubated in phosphate-buffered saline, pH 6.8, containing [1-¹⁴C]linoleic acid and glucose (5mm) at 37°.

		Linoleic acid incorporated (mμmoles/500 mg. wet wt. of tissue)			
Incubation time (min.) ...		10	60	10	60
Concn. of linoleic acid (mm) ...	0.4	0.4	2.5	2.5	
Lipid fraction					
Triglycerides		23.8	22.9	37.3	61.1
Diglycerides		34.5	20.5	87.1	103.1
Monoglycerides		20.1	13.6	135.7	149.5
Cholesteryl esters		10.0	19.1	7.8	14.6

incubation is shown in Table 9. The quantity of linoleic acid present in the tissue incubated with 0.4mm-linoleic acid was 12.3mμmoles/500mg. wet wt. of tissue less after 60min. incubation than after 10min. Although the quantity in cholesteryl esters was higher by 9.1mμmoles/500mg. wet wt. of tissue (91%) the amounts in tri-, di- and mono-glycerides were less by 0.9mμmole (3.5%), 14.5mμmoles (40.7%) and 6.5mμmoles/500mg. wet wt. of tissue (35.1%) respectively. In the presence of 2.5mm-linoleic acid, however, the quantity incorporated into lipid was 60.8mμmoles/500mg. wet wt. of tissue greater at 60min. than at 10min. This was composed of increases in linoleic acid of 88% in cholesteryl esters, and 59.8, 18.7 and 10.1% in tri-, di- and mono-glycerides respectively.

Effect of emulsification on the metabolism of linoleic acid and stearic acid. The absence of any inhibition of respiration or of the metabolism of linoleic acid at concentrations of linoleic acid below 5.0mm might have been caused by an intrinsic difference in its inhibitory activity compared with lauric acid. Alternatively, high concentrations might fail to accumulate within the tissue because of a low rate of entry caused by its low solubility. Intestinal rings were therefore incubated with emulsions of linoleic acid or stearic acid, stabilized with monostearin and lecithin. At 1.0mm stearic acid and linoleic acid emulsions inhibited respiration by 6%, but the non-emulsified acids inhibited by 12 and 9% respectively. A control emulsion prepared without fatty acid had no effect on respiration (Table 10).

The oxidation of linoleic acid determined by ¹⁴CO₂ production from [1-¹⁴C]linoleic acid was greater in the presence of emulsified linoleic acid. At 0.5mm 36% more linoleic acid was oxidized when it was emulsified and at 1.0mm 82% more was oxidized. The effect of emulsification on the incorporation of linoleic acid into lipids was the reverse

Table 10. *Effect of emulsification on the metabolism of linoleic acid and stearic acid by intestinal rings*

Intestinal rings were incubated in phosphate-buffered saline, pH 6.8, containing glucose (5 mM).

Conditions	Flask no	1	2	3	4	5	6	7	8	9	10
Emulsified stearic acid (mM)		1.0	—	—	—	—	0.5	—	—	—	—
Stearic acid (mM)		—	1.0	—	—	—	—	—	—	—	—
Emulsified linoleic acid (mM)		—	—	1.0	—	—	—	0.5	—	—	—
Linoleic acid (mM)		—	—	—	1.0	—	—	—	—	0.5	—
Emulsifier alone		—	—	—	—	+	—	—	—	—	—
Results											
O ₂ uptake (μ l./mg. wet wt. of tissue/hr.)	1.63	1.52	1.62	1.58	1.71	1.76	1.79	1.77	1.66	1.69	
¹⁴ CO ₂ produced (counts/min./mg. wet wt. of tissue)	—	—	147	81	—	—	87	—	64	—	
Lipid ¹⁴ C (counts/min./mg. wet wt. of tissue)	—	—	631	741	—	—	219	—	341	—	

of its effect on oxidation. At 0.5 mM intestinal rings incorporated 36% less linoleic acid into lipids when it was emulsified, and at 1.0 mM 15% less was incorporated.

DISCUSSION

Effect of concentration on the uptake of fatty acids by intestine. The determination of the quantities of fatty acids that have entered intestinal mucosal cells is made inaccurate by the adsorption of fatty acids to the cell surface (Dawson & Isselbacher, 1960b; Johnston & Borgström, 1964). If the extent of the metabolism of the fatty acids is taken as an index of the extent of entry of the fatty acids into the cells, the problem of adsorption is avoided. The increase in the oxidation and incorporation into lipids of lauric acid and linoleic acid as their concentration in the medium increased indicates that their rate of entry into the tissue depended on the concentration in the medium. The decrease in the metabolism of lauric acid at high concentrations is not contrary to this idea, since it is probable that the inhibition of metabolic processes by high concentrations of fatty acids depends on the accumulation of the fatty acids intracellularly. The incorporation of the fatty acids into glycerides can increase the time required for the accumulation of inhibitory concentrations, and this is presumably why glucose potentiates the oxidation of lauric acid at concentrations above 0.5 mM, since it acts as a source of α -glycerophosphate for glyceride synthesis.

State in which fatty acids are absorbed. The state in which the fatty acids enter the tissue is not known. The solubility of lauric acid in water at 37° is approx. 70 μ g./ml. (Ralston & Hoerr, 1942), which would give a 0.35 mM solution. Since at pH 6.8 not more than 10% of the acid would be dissociated the apparent solubility is presumably increased by the formation of micelles or emulsions. Since the uptake

of fatty acids at concentrations that exceed their solubility occurs faster than at lower concentrations, as indicated by their more rapid metabolism or inhibitory action, micelles or particulate fat must be absorbed. It is possible that the incomplete removal of bile salts during the washing of the intestine aids the formation of micelles. Emulsification of the linoleic acid apparently increases its uptake, since more was oxidized and the incorporation into glycerides decreased.

Incorporation of fatty acids into glycerides. The incorporation of lauric acid and linoleic acid into the tissue lipids occurred mainly into the neutral-lipid fraction. Jedeikin & Weinhouse (1954) observed that most of the palmitic acid in their experiments was incorporated into phospholipids. However, their incubation was for 3 hr. and it is probable that the lipase activity observed in the present studies could have destroyed the neutral lipid by that time.

The distribution of lauric acid at concentrations below 1.0 mM between the different neutral lipids was similar to the distribution of palmitic acid at 0.25 mM found by Dawson & Isselbacher (1960b) and of oleic acid at 0.6 mM found by Johnston & Borgström (1964). The change in the distribution at 1.0 mM-lauric acid and above was the result of the cessation of the incorporation early during the incubation followed by degradation of the synthesized triglyceride.

The presence of a lipase in intestinal mucosa, which could cause the loss of incorporated fatty acid, has been described by Dawson & Isselbacher (1960a) and by Tidwell, Pope, Askins & McPherson (1963). The absence of large accumulations of lower glycerides containing lauric acid may be due to the presence of aliesterases, which were found by DiNella, Meng & Park (1960) to be very active in hog mucosa on esters of short- and medium-chain fatty acids but not of long-chain fatty acids. The

distribution of linoleic acid within the glycerides, however, was different from that of lauric acid, and differed from the results of Kronld *et al.* (1963), who found little incorporation of linoleic acid into monoglycerides by everted sacs of rat intestine. However, it is possible that their methods were not sensitive enough to detect the small quantity of monoglycerides present. The observed distribution of linoleic acid is not readily explained. The effect of concentration on the incorporation of linoleic acid indicates that the large incorporation into monoglycerides and diglycerides at 2.5 and 5.0 mm is the result of degradation of triglycerides. However, the time-course of the incorporation at 2.5 mm shows that the synthesis of triglycerides containing linoleic acid occurs faster than the formation of monoglycerides and diglycerides between 10 and 60 min. incubation. The activity of the monoglyceride deacylase described by Tidwell *et al.* (1963) does not appear to be very significant under the conditions used in this investigation, and it is also difficult to attribute the results to the lipase described by DiNella *et al.* (1960) since this was equally active on mono-, di- and triglyceride mixtures containing the same number of ester equivalents. The small proportion of linoleic acid in triglycerides even at 0.01 mm suggests that the distribution might depend on factors other than lipase activity. Johnston & Borgström (1964) found that the duodenum and terminal ileum incorporated a smaller percentage of oleic acid into triglycerides than the proximal jejunum. It is also possible that the synthesis of di- and tri-glycerides containing two or three molecules of linoleic acid is relatively slow. Mattson & Lutton (1958) observed that there was a tendency towards the location of linoleic acid in the β -position of rat glycerides, and Savary, Constantin & Desnuelle (1961) found a preference for placing linoleic acid in the β -position of lymph glycerides by rats fed on a mixture of free fatty acids.

Incorporation of fatty acids into cholesteryl esters. The incorporation of lauric acid into the cholesteryl ester fraction was not inhibited by lauric acid concentrations that inhibited respiration. This is in agreement with the findings of Swell, Boiter, Field & Treadwell (1955) and of Murthy, Mahadevan, Sastry & Ganguly (1961) that mucosa could esterify cholesterol in the absence of ATP. The decrease in the lauric acid in cholesteryl esters with time of incubation would indicate that the incorporation occurred by an exchange reaction and that the labelled cholesteryl esters were destroyed by the cholesterol esterase demonstrated by Swell *et al.* (1955). Although Murthy *et al.* (1961) found linoleic

acid to be a tenfold better substrate than lauric acid for cholesteryl ester synthesis, the slow entry of linoleic acid into the tissue is probably responsible for the poor incorporation. The presence of high concentrations of linoleic acid inhibit the synthesis of cholesteryl linoleate.

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