Studies on the Metabolism of Glycerol by the Small Intestine *In Vitro* and *In Vivo*

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Current schemes for the digestion and absorption of long-chain triglycerides (Bergström & Borgström, 1955; Frazer, 1958) suggest that a portion is partly hydrolysed to free fatty acids and lower glycerides which, after absorption, are resynthesized to triglyceride in the mucosal cell. Another portion is completely hydrolysed, and the free fatty acids are esterified in the mucosa with an endogenous glyceride-glycerol precursor which is thought to be intracellular glucose (French & Popják, 1951; Buell & Reiser, 1959; Dawson & Isselbacher, 1960b). The glycerol, liberated in the gut lumen, is said to be a poor glyceride-glycerol precursor in the gut mucosa, for when rats were given labelled glycerol with either triglyceride or free fatty acid there was negligible incorporation of the label into either small-gut mucosal lipids (Karnovsky & Gidez, 1951; Buensod, Favarger & Collet, 1953; Buchs & Favarger, 1959) or triglycerides of thoracic-duct lymph (Bernhard, Wagner & Ritzel, 1952).

Buell & Reiser (1959) also showed that L- α -glycerophosphate, but not free glycerol, could serve as a glyceride-glycerol precursor in an homogenate system from pig small intestinal mucosa. Similar results were obtained by Clark & Hübscher (1961), using a mitochondrial preparation of rabbit small intestinal mucosa. As both systems *in vitro* contained adenosine triphosphate, it may be supposed that free glycerol is metabolically inert in the small intestinal mucosa owing to the apparent lack of a glycerokinase, or the presence of a very active phosphatase which would rapidly hydrolyse any L- α -glycerophosphate formed in an homogenate system. These observations support Kennedy's (1957) contention that L- α -glycerophosphate, but not free glycerol, is the immediate precursor of glyceride glycerol.

Buchs & Favarger (1959) gave labelled glycerol intravenously to mice after a meal of free fatty acids, and found that the specific activity of glyceride glycerol was higher in small intestine than in liver or carcass. Because of the discrepancy between these results and those of feeding experiments, we have investigated the metabolism of glycerol by small gut *in vitro* and *in vivo*.

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METHODS

Materials

Radioactive materials were obtained from The Radiochemical Centre, Amersham, Bucks. [$1\text{-}^{14}\text{C}$]Glycerol and uniformly (U) labelled [^{14}C]glucose were chromatographically pure in a one-way system with butanol-acetic acid-water (4:1:1.7, by vol.).

Sodium taurocholate, prepared according to Norman (1955), contained less than 5% of free cholic acid.

Human serum albumin was obtained from The Lister Institute, Elstree, Herts. A palmitate-albumin complex was made containing palmitate (3.91 $\mu\text{moles/ml.}$) in 10% (w/v) albumin (Fillerup, Migliore & Mead, 1958).

Hyamine 10X [$N\text{-[2-methyl-4-(1,1,3,3-tetramethylbutyl)-phenoxyethoxyethyl]-}N,N\text{-dimethyl-}N\text{-benzylammonium chloride}$] (Nuclear Enterprises, Edinburgh) was converted into the free base by the method of Eisenberg (1958).

Female albino rats, 150–250 g., fed on Purino chow, were obtained from A. Tuck and Son, Rayleigh, Essex.

Incubations were made in phosphate buffer, pH 7.4, containing (m-moles/l.): NaCl, 123; KCl, 4.94; MgSO_4 , 1.24; Na_2HPO_4 , 20.2; HCl, 0.003.

Techniques used *in vitro*

Small-intestine segments. The procedure of Dawson & Isselbacher (1960b) was modified so that $^{14}\text{CO}_2$ could be trapped in Hyamine in an inner well (capacity 2 ml.) of a 25 ml. Erlenmeyer incubation flask. Two weighed jejunal segments from non-fasting rats were placed in the outer well of the flask with either [$1\text{-}^{14}\text{C}$]glycerol (0.304 μmole , 0.5 μC) or [$\text{U-}^{14}\text{C}$]glucose (0.146 μmole , 0.5 μC) in 4 ml. of phosphate buffer-salts medium. The flask was gassed with O_2 , closed with a vaccine cap and shaken in a water bath (A. Gallenkamp and Co. Ltd.) for 1 hr. at 25°.

After incubation the flasks were packed in ice and 1 ml. of Hyamine was introduced, by tuberculin syringe, through the vaccine cap into the inner well (Siperstein & Fagan, 1958). A portion (1 ml.) of 25% trichloroacetic acid was added to the outer well through the vaccine cap. After shaking for 30 min. the Hyamine- $^{14}\text{CO}_2$ was transferred quantitatively with xylene to a 5 ml. volumetric flask; a portion (1 ml.) was taken for assay of ^{14}C . Preliminary experiments confirmed that 90–95% of the CO_2 from $\text{NaH}^{14}\text{CO}_3$ (620 μmoles , 0.01 μC) could be trapped by Hyamine under these conditions.

The contents of the outer well were homogenized and, after centrifuging (3000 rev./min. for 15 min. at 4°), the precipitate was washed twice with 5% trichloroacetic acid at 4°. Lipid was extracted from the precipitate by CHCl_3 –

methanol (2:1) (Folch, Lees & Sloane-Stanley, 1957). Water (one-fifth of the volume of CHCl_3 -methanol) was added, the mixture shaken and separated into two phases by centrifuging (2500 rev./min. for 5 min.). A portion of the CHCl_3 phase was evaporated to dryness in an atmosphere of N_2 and made up to volume with light petroleum (b.p. 67–70°). After washing with 0.15M-KCl, samples of the light petroleum were taken for further analysis.

A sample of lipid was placed on a chromatography column (1 cm. diam.) with silicic acid (1 g.) (Mallinckrodt Chemical Works; 100 mesh), and 0.5 g. of Hyflow Supercel (Hopkin & Williams Ltd.). The 'glyceride fraction' was eluted with 50 ml. of diethyl ether, and the phospholipids with 50 ml. of CHCl_3 -methanol (1:9).

On three occasions mono-, di- and tri-glycerides were separated according to the method of Barron & Hanahan (1958), as modified by Dawson & Isselbacher (1960a).

A sample of the 'glyceride fraction' was dried under N_2 and saponified in ethanolic 0.5M-KOH for 30 min. at 60–70°. After addition of an equal volume of water and acidifying, the fatty acids, cholesterol, and non-saponifiable material were extracted with light petroleum (b.p. 60–80°). After the addition of unlabelled glycerol, and treatment with periodic acid, the formaldehyde from the glycerol C-1 and C-3 atoms was isolated as the formal-dimedone (the formaldehyde compound of 5,5-dimethyl-cyclohexane-1,3-dione) (Cahill, Leboeuf & Renold, 1959). This precipitate was washed and dried, dissolved in xylene and a portion counted in the liquid scintillator.

On three occasions the glycerol in the 'glyceride fraction' was isolated as glycerol tribenzoate (Wood, Joffe, Gillespie, Hansen & Hardenbrook, 1958) and recrystallized to constant specific activity before the formal-dimedone was prepared. In another three experiments, after separation of the formal-dimedone the formic acid from the glycerol C-2 atom was oxidized with Hg^{2+} ions (Topper & Hastings, 1949) and the CO_2 trapped in Hyamine.

Radioactive materials in xylene or light petroleum (b.p. 67–70°) were counted in an Ecko liquid-scintillation counter. The liquid scintillator consisted of 10 ml. of xylene containing 0.3% (w/v) of 2,5-diphenyloxazole, and 0.01% of *p*-bis-2-(5-phenyloxazolyl)-benzene (Nuclear Enterprises, Edinburgh). [^{14}C] or [^3H]-Hexadecane was used as reference material to determine the efficiency of the scintillation counter, as well as the phosphorescence quenching of the individual samples. For ^{14}C the efficiency was 45%, background 12 counts/min.; for ^3H the efficiency was 12%, background 120 counts/min. Usually the activity of radioactive samples was at least 100 times the background.

The results are expressed as μm -moles of radioactive substrate metabolized to CO_2 or lipid and are the mean of duplicate determinations which usually did not vary more than 10–15%.

Small intestinal mucosa. Rat small-gut mucosa was separated from the underlying tissue with a scalpel blade. There was virtually no muscle in the mucosal preparation, as shown by light-microscopy. The mucosa was incubated, and $^{14}\text{CO}_2$ and [^{14}C]lipid were isolated in a manner similar to that described for the small-gut segments.

In four experiments, after incubation with [^{14}C]glycerol or [^{14}C]glucose, rat small-gut mucosa was washed with 0.9% NaCl and homogenized in CHCl_3 -methanol (2:1). After the addition of water and centri-

fuging to separate the two phases, a portion of the water-methanol phase was concentrated under N_2 and chromatographed on 18.25 in. $\times$ 22.5 in. acid-washed Whatman no.1 filter paper, aq. 72% (w/v) phenol and butanol-propionic acid-water (45:9:23:4:30:6, by vol.) being used as the two solvent systems (Benson *et al.* 1950). The radioactive substances on the chromatogram were located by radioautography with Ilfex X-ray film (Ilford Ltd.). The radioactivity in each spot was counted directly on the chromatogram by means of 3 cm. windowless gas-flow counter (20th Century Electronics Ltd.) flushed continuously with helium passed through ice-cold ethanol. The radioactive spots on the chromatogram were identified presumptively by their chromatographic positions and then by co-chromatography with authentic materials.

Mucosa was also obtained from the small intestine of two guinea pigs and a rabbit. Human jejunum was obtained at operation (partial gastrectomy for duodenal ulcer), and was placed immediately in oxygenated phosphate buffer at room temperature. About 30 min. elapsed between receipt of the tissue and beginning incubation.

Techniques used in vivo

Feeding experiments. Four starved rats were given a suspension of arachis oil, 100 mg. (expressed as triolein about 100 μmoles), with [^{14}C]palmitate (0.084 μmole , 2 μC) by stomach tube. One hour later they received, by stomach tube, either [^{14}C]glycerol (1.22 μmoles , 2 μC) or [^{14}C]glucose (0.58 μmole , 2 μC). After another hour the animals were killed and the stomach and small intestine washed thoroughly with 0.9% NaCl. Lipid was extracted from the small-gut wall, and the stomach and small-gut washings. The non-lipid radioactivity remaining in the washings was counted in dioxan (Langham, Eversole, Hayes & Trujillo, 1956).

For the calculation of the percentage of radioactivity recovered it was assumed that [^{14}C]palmitate was not significantly metabolized to glyceride glycerol, or [^{14}C]glycerol and [^{14}C]glucose to fatty acids. After saponification of the lipid the [^{14}C]palmitate was recovered in the light-petroleum fraction, whereas the glycerol and glucose radioactivity was isolated as the formal-dimedone from the aqueous fractions.

Small-intestine segments. Under ether anaesthesia, rat jejunal segments were isolated by ligature (Schmidt-Nielsen, 1947) and injected with palmitate (1.2 μmoles) as the albumin complex. Alternate segments also received [^{14}C]glycerol (0.0146 μmole , 0.024 μC) or [^{14}C]glucose (0.007 μmole , 0.024 μC). The abdomen was closed and the rat allowed to recover. One hour later the animal was killed and the isolated segments were removed. After the segments had been homogenized in 5% trichloroacetic acid, lipid was extracted and the glycerol and glucose radioactivity recovered as the formal-dimedone.

Cannulation of the rat thoracic duct. This was performed as described by Bollman, Cain & Grindley (1948) and at the same time a duodenostomy was made. After an overnight period, 2 ml. of a water-clear solution containing ($\mu\text{moles/ml.}$) taurocholate (16), oleic acid (17.7), [^{14}C]glycerol (5.9; 0.18 μC) and [^3H]palmitate (0.00083; 0.19 μC) were injected through the duodenostomy. Thoracic-duct lymph was collected for the following 4–16 hr. Trichloroacetic acid at 4° was added to the lymph so that the final

concentration was 5% (w/v). After centrifuging, lipid was extracted from the precipitate; the glycerol radioactivity was recovered as the formaldimedone from the 'glyceride fraction'.

RESULTS

Experiments in vitro

[1-¹⁴C]Glycerol is metabolized by rat small-gut segments *in vitro*. In the experiment illustrated in Fig. 1 the production of radioactive lipid and CO₂ was linear at 25° for 1 hr., when it accounted for approx. 3% of substrate radioactivity. In some experiments this production was diminished in the second 30 min. of incubation. The metabolism of glycerol could not be due to bacteria contaminating the system *in vitro*, for the number of bacteria present after an incubation, as determined by colony counting, was less than 10⁵/ml. Forty times this number, obtained by diluting a bacteriological culture from the rat small gut, did not metabolize any measurable amount of [1-¹⁴C]glycerol when incubated for 1 hr. at 25°.

It has been previously demonstrated that the incorporation of [U-¹⁴C]glucose into rat small-gut lipid (presumably glyceride glycerol) is stimulated

when free fatty acids enter the mucosal cell and are esterified (Dawson & Isselbacher, 1960b). These experiments were repeated, and the incorporation of [1-¹⁴C]glycerol into lipid compared with that of an approximately equal number of triose units as [U-¹⁴C]glucose (Table 1). In the presence of palmitate, as the albumin complex, there was a fivefold increase in the incorporation of [1-¹⁴C]glycerol into lipid, and a threefold increase when [U-¹⁴C]glucose was the tracer substrate.

Presumably, absorption of fat and synthesis of triglyceride occur in the small-gut mucosa and not the underlying tissue. In two experiments, when these tissues were incubated separately, the mean incorporation of [1-¹⁴C]glycerol into lipid was 3.09 μ m-moles/100 mg. of mucosa, as compared with 0.13 μ m-mole/100 mg. of underlying tissue; 1.46 μ m-moles were metabolized to CO₂/100 mg. of mucosa, as compared with 0.29 μ m-mole/100 mg. of underlying tissue. A mucosal preparation was not regularly used in this study as its reproducibility was poor, possibly due to variable damage during its preparation.

After incubation *in vitro* with [1-¹⁴C]glycerol, about 80% of the lipid radioactivity was in the triglyceride fraction, 10% in the diglyceride, 1% in the monoglyceride and 10% in the phospholipid fraction.

After saponification of the 'glyceride fraction' only 1-2% of the glyceride radioactivity was soluble in light petroleum (60-80°), and this was presumed to be fatty acids and sterols. Nearly all the radioactivity was in the aqueous fraction (Table 2). This was recovered as the formaldimedone (a procedure in which the glycerol C-1 and C-3 atoms are isolated). If the carbon chain of [1-¹⁴C]glycerol were incorporated intact into glyceride glycerol, there would be complete recovery of radioactivity by this method, and this was found to obtain in both control experiments, and those with added free fatty acids. In three experiments

Fig. 1. Time curve of [1-¹⁴C]glycerol metabolism by rat small-gut segments *in vitro*. Each flask contained [1-¹⁴C]glycerol (0.304 μ mole, 0.5 μ c) in 4 ml. of phosphate buffer. Incubation was at 25°. Each point is the mean of two determinations: O, CO₂; ●, lipid.

Table 1. Incorporation of [1-¹⁴C]glycerol and [U-¹⁴C]glucose into rat small-gut lipid, and the effect of added free fatty acid

In each flask there was [1-¹⁴C]glycerol (0.304 μ mole, 0.5 μ c) or [U-¹⁴C]glucose (0.146 μ mole, 0.5 μ c). Palmitate (3.91 μ mole) as an albumin complex was added in those experiments marked 'Free fatty acid'. Incubation was for 1 hr. at 25°. Results are expressed as mean $\pm$ s.e., with number of observations in parentheses.

Additions	[¹⁴ C]Substrate incorporated into lipid (μ m-moles/100 mg. of tissue)	
	From [1- ¹⁴ C]glycerol	From [U- ¹⁴ C]glucose
None	3.52 $\pm$ 0.30 (18)	2.00 $\pm$ 0.19 (12)
Free fatty acid	16.20 $\pm$ 2.21 (5)	5.40 $\pm$ 0.20 (4)

Table 2. *Distribution of radioactivity in small-gut 'glycerides' obtained from incubations with [1-¹⁴C]glycerol*

Recovery of radioactivity as the formal-dimmedone from aqueous fractions, after saponification, from control experiments and from experiments when the incorporation of [1-¹⁴C]glycerol was stimulated by 3.91 μ moles of palmitate as an albumin complex ('+Free fatty acid') compared with the recovery from a [1-¹⁴C]glycerol standard solution.

Experiment	Lipid fraction	Percentage of 'glyceride' radioactivity after saponification		
		Water fraction (as formal-dimmedone)		
		Mean $\pm$ s.e.	No. of experiments	Range
[1- ¹⁴ C]Glycerol standard		88 $\pm$ 2	7	86-96
Control	1-2	89 $\pm$ 4	6	86-97
+Free fatty acid	1-2	82 $\pm$ 3	4	79-88

glycerol tribenzoate was first isolated from the aqueous fraction; degradation of this compound gave similar results. This suggested that the aqueous radioactive material was glycerol, and that little randomization of the label had occurred. Further evidence that the C-2 atom of glycerol was not appreciably labelled was obtained in three experiments in which oxidation of the C-2 atom did not yield appreciable ¹⁴CO₂. In two similar experiments in which [U-¹⁴C]glucose was the glyceride glycerol precursor about two-thirds of the glyceride glycerol radioactivity was recovered as the formal-dimmedone, as theoretically expected. Therefore, in subsequent experiments, to calculate the recovery of [U-¹⁴C]glucose, the counts/min. as the formal-dimmedone were multiplied by 1.5.

Paper chromatography of the methanol-water extract of rat small-gut mucosa, after incubation with [1-¹⁴C]glycerol, showed that the main metabolites were lactic acid and alanine. Glutamic acid, some Krebs-cycle intermediates, and phosphorylated compounds were also labelled; no free labelled glucose was present. A similar chromatogram was obtained after incubation with [U-¹⁴C]glucose, but there was also evidence that some glucose had been converted into glycerol.

The ability of the small-gut mucosa to metabolize glycerol was not confined to the rat, for the metabolism of [1-¹⁴C]glycerol to CO₂ and lipid was demonstrated with preparations of mucosa *in vitro* from guinea pig, rabbit and man. Furthermore, in the presence of palmitate (as the albumin complex) in the incubation medium, the incorporation into lipid was increased (Table 3).

Experiments in vivo

The discrepancy between the ability of rat small-gut mucosa to metabolize glycerol to glyceride glycerol and the poor incorporation of labelled glycerol given by mouth (Buensod *et al.* 1953) was investigated. These feeding experiments were repeated with [U-¹⁴C]glucose as well as with [1-¹⁴C]glycerol (Table 4). 1.4% of the [1-¹⁴C]glycerol and 0.4% of the [U-¹⁴C]glucose were

Table 3. *Metabolism of [1-¹⁴C]glycerol by small intestinal mucosa from different species*

Each flask contained [1-¹⁴C]glycerol (0.304 μ mole, 0.5 μ C). In some flasks (control) there were no additions. Systems marked '+Free fatty acid' contained palmitate (3.91 μ moles) as an albumin complex. Incubation was for 1 hr. at 25°. Each value is the mean of two determinations on tissue obtained from one animal.

Product	...	...	[1- ¹⁴ C]Glycerol metabolized (μ m-moles/100 mg. of tissue)	
			CO ₂	Lipid
Rabbit				
Control			1.74	4.52
+Free fatty acid			0.40	27.90
Guinea pig				
Control			9.21	9.60
+Free fatty acid			2.09	101.00
Man				
Control			5.34	12.80
+Free fatty acid			2.20	26.60

recovered from gut-wall lipids despite the fact that 88-93% of these substances had been absorbed. However, although 60-78% of the [1-¹⁴C]palmitate had been absorbed, only 5% was isolated from the gut-wall lipids.

The poor incorporation of [1-¹⁴C]glycerol into mucosal lipids could not be explained by a rapid removal of glycerol by an intact blood supply to the intestine, for an appreciable incorporation was demonstrated in four experiments *in vivo* in which isolated gut segments were used (Table 5). The incorporation of [1-¹⁴C]glycerol into lipid was compared with an equal number of μ C and triose units of [U-¹⁴C]glucose. About 10% of the glycerol radioactivity was recovered as the formal-dimmedone, compared with less than 1% of the labelled glucose. The lipid of the control segments, in which only the palmitate in albumin solution was injected, contained 0.10-0.18% of the total radioactivity administered to the animal. Therefore the lipid radioactivity in the segments containing

Table 4. Feeding experiments with [1-¹⁴C]glycerol and [U-¹⁴C]glucose

Starved rats were given a suspension of 100 mg. of arachis oil (expressed as triolein, about 100 μmoles) and [1-¹⁴C]palmitate (0.084 μmole, 2 μc) by stomach tube. One hour later they received, by stomach tube, [1-¹⁴C]glycerol (1.22 μmoles, 2 μc) (Expt. A), or [U-¹⁴C]glucose (0.58 μmole, 2 μc) (Expt. B). After another hour, and extraction of the lipid from the stomach and small-gut lumina, the remaining aqueous radioactivity was counted. Glycerol and glucose radioactivity was recovered as the formaldimedone after saponification of the gut-wall lipid.

Expt.	Glyceride-glycerol precursor	Percentage of radioactivity recovered			
		Gut lumen		Gut wall	
		Lipid fraction	Aqueous fraction	Lipid fraction	Aqueous fraction
A	[1- ¹⁴ C]Glycerol	39.1	11.8	4.0	1.4
B	[U- ¹⁴ C]Glucose	21.5	6.6	5.7	0.4

Table 5. Metabolism of [1-¹⁴C]glycerol and [U-¹⁴C]-glucose by rat small-intestine segments in vivo

Palmitate (1.2 μmoles) as the albumin complex was injected into each segment. Segments, other than those marked 'control', received either [1-¹⁴C]glycerol (0.0146 μmole, 0.024 μc) or [U-¹⁴C]glucose (0.007 μmole, 0.024 μc). One hour later the lipid glycerol was isolated from the gut wall as the formaldimedone.

Segment	Injected radioactivity recovered from gut-wall lipid (%)
(1) Control	0.1*
(2) + [1- ¹⁴ C]Glycerol	10.8
(3) + [U- ¹⁴ C]Glucose	1.0
(4) Control	0.18*
(5) + [1- ¹⁴ C]Glycerol	9.5
(6) + [U- ¹⁴ C]Glucose	0.5

* In control segments a maximum of 0.18% of the total radioactivity administered to the animal was recovered as gut lipid that was not saponified.

Table 6. Recovery of administered radioactivity in lipids from thoracic-duct lymph

Each rat received, by duodenostomy, 2 ml. of a solution containing (μmoles/ml.): taurocholate, 16; oleic acid 17.7; [1-¹⁴C]glycerol, 5.9 (0.18 μc); [9,10-³H₂]palmitate, 0.00083 (0.19 μc).

Rat	Time of collection (hr.)	Vol. of lymph (ml.)	Radioactivity recovered after saponification (%)	
			[1- ¹⁴ C]-Glycerol	[9,10- ³ H ₂]-Palmitate
1	4	6	8.4	51
2	6	5	11.6	66
3	5	4	9.4	50
4	6	10	7.1	61
5	15	25	18.8	75
6	15	45	17.5	84
7	15	50	31.6	83
8	7	50	11.3	36
9	16	60	12.5	53
10	8	60	14.4	39

[1-¹⁴C]glycerol could not have arisen from re-circulated metabolites.

It seemed possible that the poor incorporation of labelled glycerol, fed in conjunction with free

fatty acids by stomach tube resulted from differences in the rate and site of absorption of these two substances. An attempt was made to present oleic acid and glycerol to the small-gut mucosa so that they might be absorbed simultaneously. Three moles of oleic acid to 1 mole of glycerol in an aqueous taurocholate solution with [1-¹⁴C]glycerol and [9,10-³H₂]palmitate as radioactive markers was given intraduodenally to rats, and the thoracic-duct lymph collected (Table 6). Although there was only a 36–84% recovery of [9,10-³H₂]-palmitate, 7–31% of the glycerol radioactivity was recovered as lymph glyceride glycerol.

DISCUSSION

Despite previous assumptions to the contrary, these experiments demonstrate that small-gut mucosa can incorporate glycerol into glyceride glycerol. As this incorporation was stimulated when fatty acids entered the mucosal cell, and as this metabolic activity was mainly in the mucosa compared with underlying tissue, it probably represents a pathway for the esterification of absorbed fatty acids *in vivo*. Furthermore, in these experiments, glycerol seemed to be as good a precursor of glyceride glycerol as glucose.

Although there was only a superficial investigation of the metabolites formed from [1-¹⁴C]glycerol *in vitro*, the finding of lactic acid, alanine and other unidentified compounds, in addition to the appreciable production of ¹⁴CO₂, further emphasizes the ability of the intestinal mucosa to metabolize glycerol. From the results of experiments on homogenate systems of small-gut mucosa (Buell & Reiser, 1959; Clark & Hübscher, 1961) it might be inferred that glycerol is metabolically inert in the intestinal mucosa because of the apparent lack of glycerokinase. The usual schemes of glyceride synthesis have L-α-glycerophosphate as the immediate precursor of glyceride glycerol (Kennedy, 1957). Although there was radioautographic evidence in the present experiments of the forma-

tion of some phosphorylated compounds from [^{14}C]glycerol, identification of these compounds and evidence of their importance in glyceride-glycerol formation was not obtained.

It is difficult to interpret the physiological significance of the results of the experiments *in vitro* in which such small amounts of substrate are metabolized, but the results of the experiments *in vivo* on rats with thoracic-duct fistulae demonstrated that under certain conditions an appreciable amount of glycerol is incorporated into glyceride glycerol by the rat small intestine. Previous evidence to the contrary was based on experiments in which glycerol and either fatty acid or triglyceride were given by stomach tube (Buensod *et al.* 1953; Buchs & Favarger, 1959). Using this technique we confirmed the observation that there is negligible incorporation of glycerol into glyceride glycerol of the small-gut wall. However, there are several objections to this type of experiment, as glycerol and fatty acid will not enter the mucosal cell at the same time. Water-soluble materials leave the stomach more quickly than does fat (Wiggins & Dawson, 1961), and fat is probably absorbed more slowly than is glycerol. In addition, measurement of the mucosal lipids is no estimate of the amount that has been absorbed, e.g. in our feeding experiment although 60–80% of the labelled palmitate had been absorbed, only 5% was recovered from the lipids of the small-gut wall. Therefore, in the experiments on rats with thoracic-duct fistulae the problem of gastric emptying was overcome by giving glycerol and fatty acid intraduodenally; to minimize differences in the rate of absorption of these substances they were given in an aqueous solution. Under these conditions, 7–31% of the [^{14}C]glycerol was recovered as glyceride glycerol in lipid of thoracic-duct lymph in spite of incomplete recovery of fatty acid. This is to be compared with a recovery of less than 1% of the label when labelled glycerol was given with oleic acid by stomach tube (Bernhard *et al.* 1952).

The previous evidence of negligible incorporation of free glycerol into the glyceride glycerol of absorbed fat has been used to calculate the extent of intraluminal hydrolysis of triglycerides given by mouth (Bernhard *et al.* 1952; Reiser, Bryson, Carr & Kuiken, 1952; Reiser, Williams & Sorrels, 1960). This is done by feeding with triglycerides labelled in the fatty acid and glycerol moieties, and comparing the specific activity of the glycerol and fatty acid of the triglycerides recovered from thoracic-duct lymph. As 25–50% of the given glyceride glycerol was not recovered, it has been assumed that this is the extent of complete triglyceride hydrolysis in the gut lumen. In the present investigation glycerol and fatty acid were pre-

sented to the mucosal cell in a manner possibly similar to that which may obtain when triglycerides are completely hydrolysed in the small-gut lumen, i.e. 1 mole of glycerol and 3 moles of fatty acid in a finely emulsified aqueous solution of bile salts. These results cast doubt on the validity of calculations of the extent of the intraluminal hydrolysis of triglycerides based on the assumption that free glycerol is not used again in mucosal glyceride-glycerol synthesis.

SUMMARY

1. [^{14}C]Glycerol is metabolized to glyceride glycerol by rat small-gut segments *in vitro*. Under conditions in which free fatty acid enters the mucosal cell and is esterified, there was a fivefold increase in the incorporation of [^{14}C]glycerol to glyceride glycerol. The carbon chain of glycerol appeared to be incorporated intact; no radomization of the label could be demonstrated.

2. The ability to metabolize [^{14}C]glycerol *in vitro* to carbon dioxide and lipid was also demonstrated with jejunal mucosa from guinea pig, rabbit and man.

3. When [^{14}C]glycerol and oleic acid in an aqueous solution were given intraduodenally to rats, 7–31% of the label was recovered in lymph glyceride glycerol. The discrepancy between these results and current concepts of the digestion and absorption of triglycerides in the small intestine is discussed.

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Microbial Growth on C₁ Compounds

3. DISTRIBUTION OF RADIOACTIVITY IN METABOLITES OF METHANOL-GROWN *PSEUDOMONAS* AM1 AFTER INCUBATION WITH [¹⁴C]METHANOL AND [¹⁴C]BICARBONATE*

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The pathway by which carbon is incorporated into cell constituents during growth of *Pseudomonas* AM1 on methanol or formate has been investigated by chromatographic analysis of the metabolites labelled during incubation of the organism with ¹⁴C-labelled substrates (Large, Peel & Quayle, 1961). The results of this study indicated that substantial quantities of carbon dioxide and carbon in a more reduced form are assimilated by a pathway which leads to rapid labelling of serine, glycine, malate and aspartate.

It has been suggested that growth of *Pseudomonas* AM1 on methanol or formate, as with growth of *Pseudomonas* PRL-W4 on methanol (Kaneda & Roxburgh, 1959), involved the condensation of glycine with a C₁ unit, derived from methanol or formate, to give serine by the action of serine hydroxymethylase. The mechanism of the prior synthesis of glycine was not known. A study has now been made of the distribution of radioactivity in glycine, serine, malic acid and phosphoglyceric acid, isolated from methanol-grown *Pseudomonas* AM1 after incubation with [¹⁴C]methanol or [¹⁴C]bicarbonate (hydrogen [¹⁴C]carbonate).

* Part 2: Large, Peel & Quayle (1961).

METHODS AND MATERIALS

Maintenance and growth of the organism. The growth conditions have been described by Peel & Quayle (1961).

Isotopic materials. Radioactive chemicals were purchased from The Radiochemical Centre, Amersham, Bucks., and handled as described by Large *et al.* (1961).

Brief incubation of cells with [¹⁴C]methanol or [¹⁴C]bicarbonate. Cultures of methanol-grown bacteria were harvested by centrifuging while in the logarithmic-growth phase. The cells were resuspended in a solution containing 10 mM-KH₂PO₄ adjusted to pH 7.0 with 2N-NaOH, 10 mM-ammonium chloride, and methanol (see below) to a cell density of 6 mg. dry wt. of bacteria/ml. The bacterial suspensions (50 ml. or 25 ml., see Tables 1-3) were placed in a sintered-glass funnel (G = 1; 8 cm. diameter × 5 cm. depth) through which was blown either air or air-carbon dioxide (99:1, v/v). After 5 min. incubation at 30°, [¹⁴C]methanol or [¹⁴C]bicarbonate was added to the suspensions and 1 min. later the entire contents of the funnel were tipped into 4 vol. of boiling ethanol. Each resulting extract was cooled and centrifuged, and the insoluble residue was further extracted with two 5 ml. portions of aq. 20% (v/v) ethanol. These extracts were combined and evaporated at 30° under nitrogen at reduced pressure to a volume of approx. one-fiftieth of the original. Samples (0.15 ml.) of the concentrated supernatant solutions were chromatographed two-dimensionally on paper (Whatman no. 4,