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Route of Absorption and Distribution of Oleic Acid and Triolein in the Rat

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During the last few years a better understanding of the process of intestinal fat absorption has been gained, largely with the aid of isotopically labelled material, in combination with the technique of Bollman, Cain & Grindlay (1948) for collecting intestinal or thoracic lymph from unanaesthetized rats. The results of these investigations have shown that after absorption long-chain saturated fatty acids are mainly transported via the intestinal lymph as triglycerides both in rats and cats (Bloom, Chaikoff, Reinhardt, Entenman & Dauben, 1950; Bergström, Borgström, Carlsten & Rottenberg, 1950; Bloom, Chaikoff, Reinhardt & Dauben, 1951; Chaikoff, Bloom, Stevens, Reinhardt & Dauben, 1951; Bloom, Chaikoff & Reinhardt, 1951; Borgström, 1951, 1952*a, b, c*; Bergström, Borgström & Carlsten, 1954). Fatty acids with 10 or less carbon atoms are, however, mainly transported from the intestine via the portal blood vessels (Bloom, Chaikoff & Reinhardt, 1951; Kiyasu, Bloom & Chaikoff, 1952) largely in free form (Borgström, to be published). For summaries see Bergström & Borgström (1953, 1954).

The higher saturated fatty acids are thus not absorbed via different routes according to the state

in which they enter the intestinal mucosa, i.e. as free acids or esterified with glycerol, as postulated in the original fat-fatty acid partition theory of Frazer (Frazer, 1938, 1943, 1946, 1948*a, b*, 1950, 1951, 1952).

There is also much experimental evidence that the unsaturated long-chain fatty acids are mainly absorbed via the lymphatics irrespective of whether they are fed as free acids or glycerides.

Munk (1880) fed oleic acid to dogs with a cannulated thoracic duct and found that there was a considerable increase in the neutral fat of the lymph. Munk & Rosenstein (1891) fed olive oil, oleic and erucic acid to a patient having a lymphatic fistula and obtained a high recovery of the ingested fat in the lymph. Freeman & Ivy (1935) obtained the same results after feeding olive oil to dogs. Bollman, Flock, Cain & Grindlay (1950) observed a marked increase of glycerides in the lymph after feeding oleic acid to dogs. Reiser & Bryson (1951) and Reiser, Bryson, Carr & Kuiken (1952) fed linoleic acid with conjugated double bonds to rats having a thoracic duct fistula and recovered part of the labelled acid in the lymph fat both after free acid and glyceride ingestion.

On the other hand, Frazer (1943), after feeding olive oil and oleic acid to rats and assessing the results with a technique mainly based on chylomicron counts and histological observations, concluded that 'fatty acid passes by the portal vein to the liver while neutral fat goes by the lymphatic route...' and this opinion has been reiterated in many subsequent publications (see above). Tidwell (1950), however, obtained a similar chylomicronographic response after feeding, respectively, olive oil and oleic acid to rats.

We now wish to report the results of some experiments in which [^{14}C]oleic acid has been fed either in free form or as triolein to rats with a thoracic-duct fistula. Some observations on the distribution of the labelled acid in these two forms to rats without a fistula have also been made.

EXPERIMENTAL

Methyl [^{14}C]oleate and [^{14}C]oleic acid were prepared according to Bergström, Pääbo & Rottenberg (1952), and triolein was prepared from oleic acid via the acid chloride prepared with oxalyl chloride.

The labelled substances were fed to rats with a thoracic-duct fistula (A) and also to animals without a fistula (B). The specific activity of the [^{14}C]oleic acid was 2300 counts/min./mg. ($10\ \mu\text{C}/\text{mg.}$).

A. Animals with a thoracic-duct fistula

Male rats weighing about 250 g. and starved for 12 hr. before operation were used. The procedure used for cannulating the thoracic duct was, with a slight modification, that described by Bollman *et al.* (1948). Instead of a straight cannula, a heated polythene cannula was bent at right angles about 0.5 cm. from the tip and was inserted through the left lateral body wall into the thoracic duct. This bend permits a cannula of larger calibre to be used, as the tension on the tube after insertion is reduced. After operation the rats were placed in restraining cages and had free access to 1% (w/v) NaCl solution until an adequate lymph flow was obtained. Thereafter, 0.64% (w/v) NaCl solution with 0.04% (w/v) KCl was fed *ad lib.* during the whole experiment. Three hours before the labelled substance was administered the rat had free access to white, fat-free bread. These rats were given either 0.5 ml. of labelled oleic acid or triolein by stomach tube when under light ether anaesthesia.

The lymph was collected for 24 hr., after which the animals were killed. In most of these experiments the rat in its cage was placed in a metabolic chamber and the expired CO_2 was recovered in NaOH. The lymph fat was extracted with ethanol-ether (3:1, v/v) and after the evaporation of this extract *in vacuo* the residue was extracted with light petroleum (b.p. 40–60°)– CHCl_3 (2:1, v/v). The total fat from the lymph samples was separated into neutral fat and phospholipids on columns of silicic acid and these fractions were saponified as described by Borgström (1952c).

The whole gastrointestinal tract was removed in one piece and digested, together with the faeces collected during the experiment, with a solution of 30% (w/v) KOH at 100° for 12 hr. The mixture was then acidified and the fatty acids were extracted with light petroleum (b.p. 40–60°). The

amount of fat absorbed in these experiments was calculated by taking the difference between the amount of isotope administered and the amount recovered from the intestinal tract and faeces.

The small activity present in the intestinal wall has thus been included in the unabsorbed amount in these cases, but this amount is negligible in fistula animals 24 hr. after feeding.

B. Non-cannulated rats

The labelled material was administered by gastric intubation in amounts corresponding to 0.1 ml./sq.dm. body surface (Lee, 1929). For comparison with the cannulated animals the activity of the expired CO_2 was measured on some rats which had received 0.5 ml. labelled material through a stomach tube. At different intervals after the feeding the rats were bled to death under ether anaesthesia. The gastrointestinal tract was removed and the contents of the small intestine were washed out with saline.

The small intestine was finely dispersed in ethanol in a Waring Blendor and the total fat extracted with ethanol-ether (3:1, v/v), and after the evaporation of this extract *in vacuo* the residue was extracted with light petroleum (b.p. 40–60°)– CHCl_3 (2:1, v/v). The total fat was then fractionated into neutral fat and phospholipids on silicic acid columns and saponified (Borgström, 1952c).

Measurement of radioactivity

The determination of ^{14}C in fatty acids was done with a thin end-window Geiger-Müller tube on samples of 'infinite thinness'. Samples of an ethanolic solution were evaporated on aluminium planchets to leave less than 0.2 mg./cm².

In order to relate the activity of the fatty acids and the activity of the expired CO_2 that was counted as BaCO_3 at 'infinite thickness', suitably diluted samples were plated as described above and similar samples were oxidized to CO_2 and counted as BaCO_3 at 'infinite thickness' (Bergström, Borgström & Rottenberg, 1951).

RESULTS

The results of the lymph experiments (A) are given in Table 1. When labelled oleic acid was fed as the free acid, 66–92% (mean 78.2%) of the fed activity was absorbed in 24 hr. Rats receiving labelled triolein absorbed 57–92% (mean 76.2%) of the activity. It is seen that the fed free oleic acid was absorbed just as well as triolein in 24 hr. After feeding free [^{14}C]oleic acid, 38–96% of the absorbed activity was recovered in the lymph fat from the thoracic duct (mean 63.0%). The corresponding figures obtained when labelled triolein was fed were 51–83% (mean 65.5%). Feeding the labelled oleic acid as the free acid or as the glyceride made no significant difference to the recovery of the activity in the lymph lipids.

About 2% of the activity found in the lymph lipids was in the form of phospholipids, the remainder in the form of neutral fat in both groups. It was found that the labelled fatty acid in the lymph lipids followed the mono-unsaturated fatty acid within the limits of the fractionation method of

Table 1. Recovery of [^{14}C]oleic acid and [^{14}C]triolein in thoracic lymph after oral administration to rats

Rat no.	Substance fed	Amount of administered activity absorbed (%)	Amount of absorbed activity recovered in lymph as fatty acids (%)	Amount of lymph fat activity found in		Amount of absorbed activity in expired CO_2 (%)
				Glyceride fatty acids (%)	Phospholipid fatty acids (%)	
1	Oleic acid	91.5	64.0	98.2	1.8	4.8
2	Oleic acid	65.5	38.1	98.8	1.2	3.7
3	Oleic acid	73.0	96.4	97.4	2.6	3.5
4	Oleic acid	91.8	62.5	97.9	2.1	—
5	Oleic acid	69.0	53.8	97.5	2.5	4.5
Mean		78.2	63.0	—	—	—
6	Triolein	92.1	59.3	97.5	2.5	4.4
7	Triolein	56.5	50.5	97.7	2.3	5.0
8	Triolein	85.0	69.2	97.4	2.6	4.0
9	Triolein	71.0	82.9	98.8	1.2	—
Mean		76.2	65.5	—	—	—

Fig. 1. Percentage of absorbed isotope in expired CO_2 after feeding [^{14}C]oleic acid as triolein (●), methyl oleate (Δ) or free oleic acid (○). A, rats with lymph fistula; every point represents the mean of three animals. B, rats without fistula; each point represents a single animal.

Bergström & Pääbo (1954). This method is based on chromatographic separation of the products of hydroxylation of the mixture of saturated and unsaturated fatty acid with performic acid.

While non-cannulated rats fed [^{14}C]oleic acid or methyl [^{14}C]oleate expired about 16% of the

Fig. 2. Relative specific activity of phospholipid fatty acids of small intestine after feeding [^{14}C]oleic acid (○), or [^{14}C]triolein (●).

absorbed activity as carbon dioxide within 24 hr., rats with the thoracic duct cannulated expired in the same time about 4% irrespective of whether the oleic acid had been administered as glyceride or as free acid (Fig. 1).

In Fig. 2 are given the curves for the specific activities of the fatty acids of the phospholipids of the intestinal wall as percentage of the specific activity of the fed fatty acids. The specific activities of the phospholipid fatty acids increased to a maximum of about 10% of that of the fed fatty acids about 4 hr. after administration. However, only a part of these phospholipids represents material that had been present in the epithelial cells of the mucosa.

DISCUSSION

After feeding labelled free oleic acid or triolein to rats with the thoracic duct cannulated, means of 63.0 and 65.5% of the absorbed activity were

recovered in the lymph lipids during the following 24 hr.

These figures give for the first time quantitative information about the route of transport of oleic acid after absorption from the small intestine.

In corresponding experiments with [^{14}C]palmitic acid dissolved in olive oil (Blomstrand, 1954), in which the same procedures were used as in the experiments reported here, a mean of 67.4 % of the absorbed activity was recovered in the thoracic duct lymph as [^{14}C]fatty acid (range 58.5–75.0 in five experiments). If these figures obtained for labelled palmitic acid are compared with those obtained for oleic acid in this investigation no significant differences are found.

The figures obtained for palmitic acid recovered in the lymph in these experiments are not as high as those given by Bloom *et al.* (1950). These authors found a mean of 75.9 % of the absorbed activity in the thoracic duct lymph as [^{14}C]fatty acid (range 58.5–91.8 in ten experiments). The differences of the means in these two series of experiments may be due to differences in the analytical methods used. Bloom *et al.* (1950) determined the radioactivity directly from the total fatty acids of the lymph obtained after saponification, while in experiments from this laboratory the neutral and phospholipid fatty acids were isolated by a chromatographic procedure before the activity determinations. In this way small losses of activity probably occurred.

Probably there are several causes for the large variation between different animals. There are certainly considerable anatomical differences between different animals in the arrangement of lymph vessels, some of which might by-pass the fistula. Possibly anastomoses exist between lymph and blood vessels which make possible an increased by-passing of the thoracic duct if the lymph flow is partly blocked by, for instance, clots that form in the lymph vessels of some cannulated animals. Also the lymph flow is probably not entirely normal with an open fistula. It is not unlikely that the rats with the highest recoveries are those most approaching normal.

It is seen in Fig. 1, however, that the amount of isotope expired during 24 hr. by a cannulated rat is roughly one-fourth to one-third of the amount expired by the non-cannulated rat. This corresponds roughly to the loss of labelled material via the fistula and shows that the absorbed labelled compounds are metabolized at a normal rate. It must be emphasized that the extent of lymphatic transport of oleic acid was found to be the same as palmitic acid when studied with the same technique (Blomstrand, 1954). No difference was observed in the recovery of labelled oleic acid in the thoracic-duct lymph lipids during the 24 hr. following the administration of labelled oleic acid as free acid or

as triolein. The capacity of the intestinal cells to synthesize triglycerides is thus apparently very great. These findings are in general in agreement with the results earlier obtained on the absorption of long-chain unsaturated fatty acids in humans, dogs and rats by Munk (1880), Munk & Rosenstein (1891), Freeman & Ivy (1935), Bollman *et al.* (1950) and Fernandes (1953).

Frazer (1943) followed the absorption of oleic acid and olive oil in the rat with chylomicron counting and reached other conclusions. In the case of olive oil he observed a quicker and stronger response, which led him to the interpretation that the free oleic acid fed was absorbed directly via the portal route and rapidly metabolized in the liver, whereas the olive oil was mainly absorbed via the lymphatics and deposited in the fat depot. These interpretations formed part of the basis for the original 'fat-fatty acid partition theory'. Chylomicron counting—a method of doubtful quantitative significance—seems unsuitable for studies of this kind and has led to erroneous interpretations of this phase of the absorption process (for further discussion cf. Bergström & Borgström, 1954).

That fatty acids of different structure are incorporated into the phospholipids of the small intestinal wall to different extents has been found in earlier work with elaidic acid (Sinclair & Smith, 1937), conjugated linoleic acid (Barnes, Miller & Burr, 1941), and with different saturated fatty acids (Bergström *et al.* 1952; Borgström, 1952*d*).

The values obtained for the incorporation of labelled oleic acid in this work correspond to those found with double-bond conjugated linoleic and myristic acids.

In conclusion it must be emphasized that a very great variability in the rate of the absorption of fat and fatty acids is to be expected. Not only is the general condition of the animal of importance but the presence and nature of other food constituents greatly affect the absorption rate. The influence of these factors on the route of absorption and distribution in the body seems to be of less importance.

SUMMARY

1. The intestinal absorption of [^{14}C]oleic acid has been studied in rats with cannulated thoracic ducts.

2. It was found that oleic acid was transported via the thoracic-duct lymph and incorporated into triglycerides and phospholipids to the same extent as found for palmitic acid in earlier work. The same results were obtained whether the oleic acid was fed as free acid or as triolein.

3. The incorporation of the labelled acid into the lipids of the intestinal wall and the appearance of the isotope in the expired carbon dioxide has been followed.

4. The results indicate clearly that there is no detectable difference between the routes followed during absorption of fed free oleic or palmitic acids, or of glycerides containing these acids.

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Intestinal Absorption and Metabolism of 2:2-Dimethylstearic Acid in the Rat

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The intestinal absorption of different straight-chain carboxylic acids has been extensively studied during recent years (for a summary see Bergström & Borgström, 1954).

In order to investigate the influence of steric hindrance at the carboxyl group we have prepared [*carboxy*-¹⁴C]2:2-dimethylstearic acid and studied its absorption and metabolism in the rat.

EXPERIMENTAL AND RESULTS

A survey of the methods previously used for the preparation of 2:2-dimethylstearic acid is given by Cason, Sumrell & Mitchell (1950). In the present work it has been prepared by the following reactions.

2:2-Dimethylsuccinic acid. 2:2-Dimethylsuccinic acid was prepared by a slight modification of the method of Vogel (1928). Solutions of ethyl 2-cyano-3:3-dimethylacrylate (20 g.) in ethanol (100 ml.) and KCN (11 g.) in water (25 ml.) were mixed and kept at room temperature for 24 hr. The ethanol was removed by distillation. After cooling, a very large excess of concentrated HCl was added and the solution refluxed for 3 hr. After dilution with water and addition of (NH₄)₂SO₄, it was extracted five times with ether. The ether solution was dried with Na₂SO₄ and evaporated to dryness. Impurities were removed by boiling the product twice with small amounts of benzene, cooling to room temperature and collecting the crystals by filtration. Yield 13.2 g. (69%); m.p. 142°. (Found: equiv. 73.1. Calc. for C₈H₁₀O₄, 73.1.)