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DIFFERENTIATION IN THE ABSORPTION OF OLIVE OIL AND OLEIC ACID IN THE RAT

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In studies of fat absorption various triglycerides have been fed to animals, but the absorption of ingested fatty acids has never been systematically investigated. According to the current hypothesis fat is completely hydrolysed before it can be absorbed, and resynthesis of neutral fat occurs in the intestinal cell with the aid of intermediate phosphorylation and the adrenal cortical hormone [Verzár & McDougall, 1936]. The reconstituted triglyceride passes by the lacteals to the thoracic duct and so to the systemic circulation. No significant quantity of fat is thought to pass by the portal vein nor is the absorption of unhydrolysed fat considered a possibility. If this hypothesis is correct, one would expect fatty acid and glycerol to behave during and after absorption in a manner similar to neutral fat. The object of this paper is to show that such a correlation does not exist, and to offer a possible explanation of the differences observed.

Methods

Animals. Throughout the experiments described, adult male rats from our own stock were used.

Materials. The fats used were olive oil and redistilled oleic acid. When oleic acid was administered glycerol was given as well in amounts equivalent to the corresponding triglyceride. Except where stated elsewhere, the fatty materials fed to the animals were stained prior to use with Sudan IV to make a 0·1% solution of the stain in oil. By the use of fat or fatty acid labelled with Sudan IV, its passage to the depots or the liver can be determined.

Feeding. The animals were fed by stomach tube without anaesthesia when detailed measurement of the quantity of olive oil administered was required. This method was not found suitable for long-term experiments. In these the fatty material was fed mixed with the food and the amount consumed was checked against faecal analyses.

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Analyses. Estimation of fat administered and of the amount remaining in the intestine at the end of the experimental period was made by the gravimetric modification of Bloor's method previously described by Elkes, Frazer & Stewart [1939]. In some instances faecal analyses were used over a period determined by charcoal administration.

Chylomicrographs. Investigation of the passage of fat along the various pathways was made by simple observation in the case of lacteals and the chylomicrograph for changes in systemic or portal blood fat. This method for studying blood fat, originally described by Gage & Fish [1924], was fully investigated and standardized by Frazer & Stewart [1939]. Samples of blood for chylomicrographs were withdrawn from the tail vein for the systemic blood or direct from the portal vein.

Operation. For the simultaneous portal and systemic chylomicrographs, the animals were kept anaesthetized with urethane and the portal specimens obtained direct from the portal vein through a small abdominal incision.

Histology. Histological examination of the intestinal cells was made in frozen sections prepared by standard technique.

RESULTS

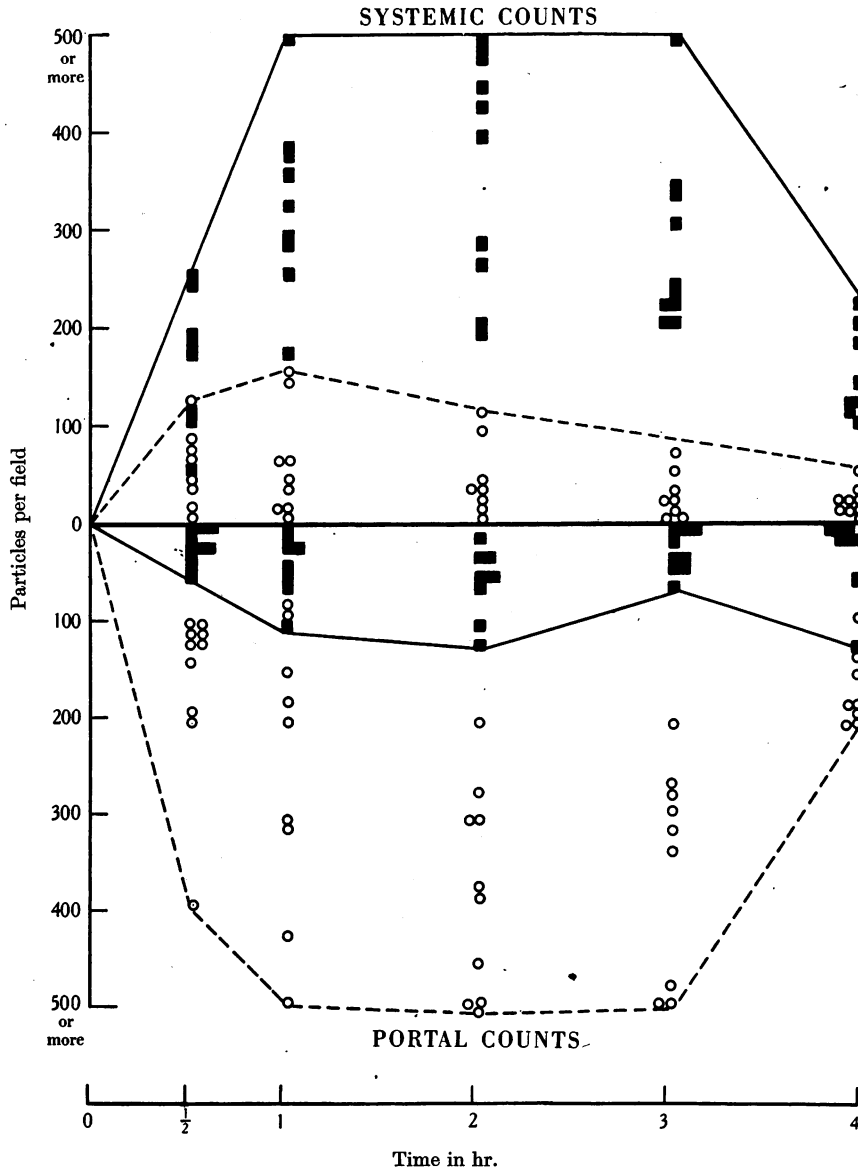
Amount of fatty material absorbed

Using thirty-six rats no significant difference was detected between the percentage absorption of olive oil or of equivalent amounts of oleic acid and glycerol. In all cases 80–90% of the fatty material reaching the intestine was absorbed and quantities up to 1 g. were taken up from the intestinal lumen during the experimental period. Similar results were obtained with faecal analyses in long-term experiments. Animals fed on fat or fatty acid over periods of several weeks showed normal growth and weight curves and no increase of fatty material in the faeces.

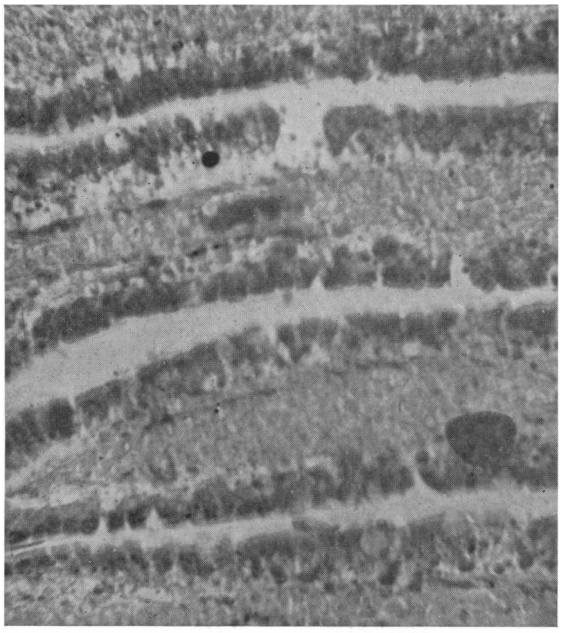
Appearance of the intestinal cells

Seventy rats were used in these experiments and sections were examined from animals killed at hourly intervals. After feeding with neutral fat, a characteristic picture is seen. Above the bile duct there is very little evidence of fat absorption. In the lower part of the duodenum and upper part of the jejunum the intestinal cells are filled with large Sudan-staining globules of fat which can be seen from 1 hr. onwards (Plate 1A). Only in that part of the duodenum, which is just below the bile duct, does the picture closely resemble that obtained with oleic acid.

When oleic acid and glycerol are fed on the other hand, the cells throughout the intestine are filled with a finely granular, fatty, Sudan-staining material (Plate 1B). These fine particles do not appear to unite to form the large globules which are seen after feeding with neutral fat.



Text-fig. 1. Simultaneous portal and systemic particle counts. Specimens prepared from blood samples taken simultaneously from the portal and systemic systems in rats following the ingestion of neutral fat or fatty acid and glycerol. The squares are from counts after feeding triglyceride, while the circles are from those following fatty acid. The continuous lines indicate the maximum counts after triglyceride and the dotted lines the maxima after an equivalent amount of fatty acid.



A

B

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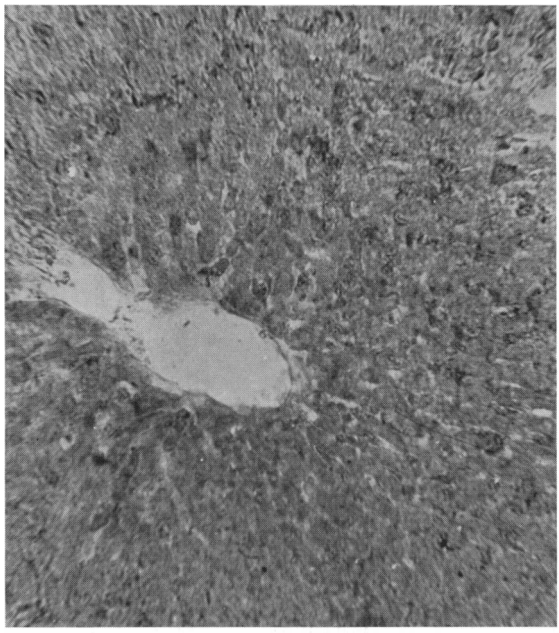
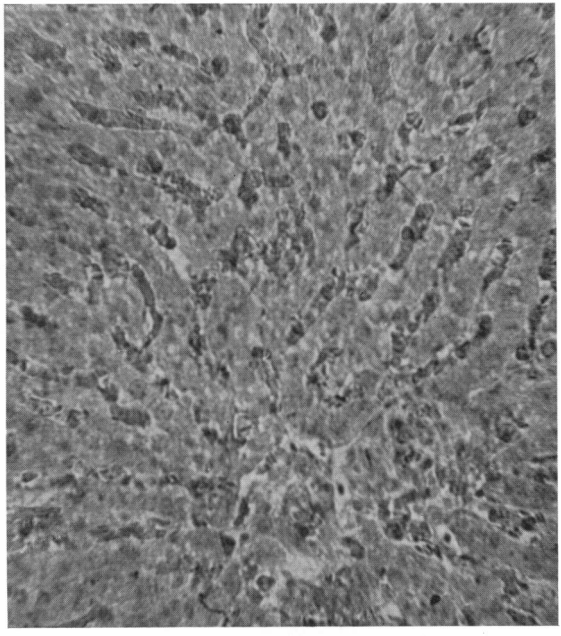


A



B

Pathways of absorption

In these experiments twenty rats were used. After feeding with neutral fat, sections of the villi show large quantities of fat in the areolar tissue corium and in the central lacteal. The lacteals on macroscopic examination appear milky. Simultaneous portal and systemic particle counts show a large increase of particles in the systemic blood but only a small rise in the portal blood (Text-fig. 1).

If an equivalent amount of oleic acid and glycerol is administered the areolar tissue and lacteals are relatively free from fatty material, and the lymphatic vessels in the root of the mesentery do not appear milky. Simultaneous portal and systemic counts reveal that the portal blood has a marked increase of particles, whereas the systemic blood shows very little increase (Text-fig. 1).

Destination of absorbed fat

The observations in this section are based on the examination of thirty-six rats. If groups of rats are given 2 c.c. of Sudanized olive oil mixed with their ordinary food, for a period of 1 week, the fat depots are found to be deeply stained. No such staining is apparent if equivalent amounts of stained oleic acid and glycerol are fed.

In the demonstration of liver fat, a surplus of fatty material must not be given as this leads to considerable accumulation of fat in the liver due to flooding of the fat depots. The 2 c.c. or more of fatty material, which is commonly used in absorption experiments in rats, is much in excess of the normal amounts ingested. If 1 c.c. of Sudanized olive oil is fed to a rat and the animal is killed after 5 hr. there is a relatively small amount of Sudan-stained fatty material visible in the liver (Plate 2A). If an exactly similar quantity of Sudanized oleic acid is fed, large quantities of stained material are found in the liver (Plate 2B). It should be noted that in these liver sections no other fat stain is used apart from Sudan IV which is in the original fatty material fed to the animal.

Discussion

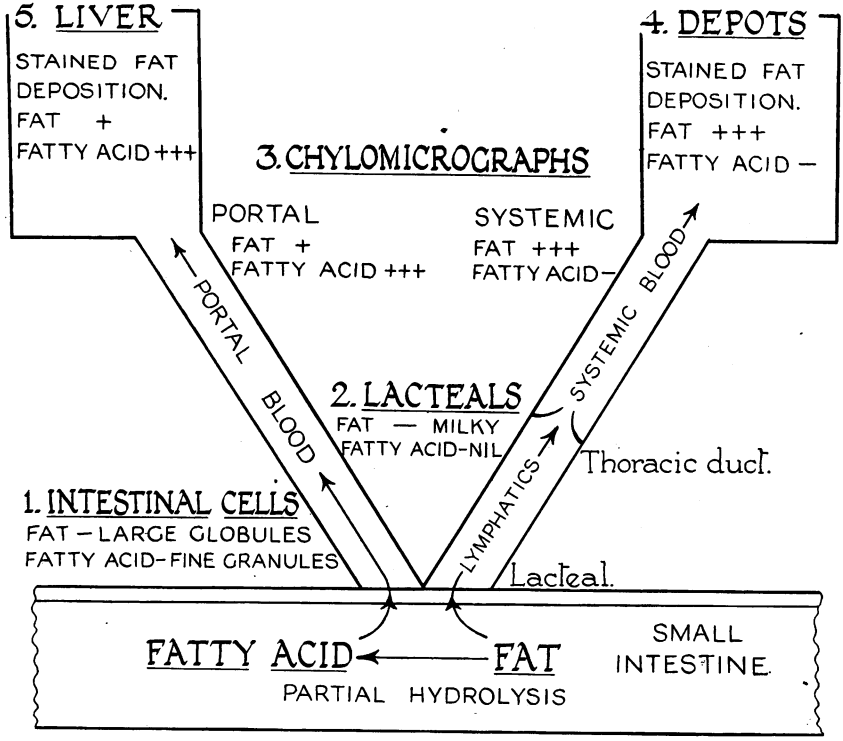
It is apparent from these observations that there are fundamental differences in the behaviour of neutral fat as compared with fatty acid and glycerol both during and after absorption. From analyses of the residual contents of the intestine or of the faeces, it is unquestionable that comparable amounts of the fat or fatty acid ingested disappear from the lumen. It must be assumed that these amounts have been absorbed, and indeed it can be demonstrated that absorption has occurred in each case. The picture obtained, however, with neutral fat is quite different from that seen with fatty acid. In the intestinal cell neutral fat gives large globules of oil; this appearance is very similar to that described by Jeker [1936] 6 hr. after ingestion of oil. Fatty acid gives a fine granular deposition, which stains more brownly with Sudan

and which resembles more closely in appearance that described by Jeker [1936] as occurring some 30 min. after ingestion of olive oil. In our experience this type of fatty acid-containing cell is found only in that part of the intestine which is just below the pancreatic duct after feeding with olive oil, but over a wide area if fatty acid itself is administered. It appears as though the fatty acid is in a much finer state of division than neutral fat when it is contained within the intestinal cell. Verzár & McDougall [1936] have cited Jeker's findings as evidence for resynthesis of neutral fat from fatty acid. There is, however, nothing to show that such a conversion actually occurs, and the two differing pictures are easily obtained by taking sections from two different parts of the intestinal tract, or by taking the sections at varying time intervals after the administration of olive oil. Sections of intestinal cells taken within an hour or so of ingestion of neutral fat show mainly a fatty acid picture—if they are taken 6 hr. later the cells show the characteristic appearance of neutral fat absorption.

From the study of the pathways leading from the intestine, the neutral fat globules seem to pass into the lacteals giving rise to a milky appearance of the intestinal lymphatics, thence the fat-laden chyle empties by way of the thoracic duct into the systemic blood. Within an hour of ingestion of neutral fat, there is always a striking increase of fat in the systemic blood. Fatty acid does not pass into the lymphatic system, but into the portal capillaries. It is perhaps significant that in other parts of the body particulate dyes when injected tend to pass into the lymphatics while water-soluble substances go direct into the blood stream. Fatty acid is certainly in a very fine state of division, and according to some authorities [Verzár & Kúthy, 1930] it is absorbed as a water-soluble complex with bile acid. The fatty acid, like other water-soluble absorbed substances, passes by the portal vein, and thus, after ingestion of fatty acid, there is no great increase of fat in the systemic blood. Using labelled fat the destination of absorbed fat can be determined. Gage & Fish [1924] found that stained fat fed to animals caused staining of the fat depots, but no investigators, so far as we are aware, have used stained fatty acid in following out the destination of absorbed fatty material. As would be expected if stained neutral fat is fed, its destination is found to be the main fat depots. Heavy staining is found not only in the subcutaneous depots but also in the omental and perirenal areas. Stained fatty acid fed over a period of 10 days results in no staining of these areas. On the other hand, feeding with stained, neutral fat, provided that it is not excessive in amount, does not cause much deposition of stained fat in the liver, but the feeding of stained fatty acid results in a striking accumulation of Sudanized fatty material. It may be suggested that labelling with Sudan is not a reliable method, but we have tried to upset the Sudan labelling of our samples by hydrolysis and other methods, and we have never succeeded in separating

the Sudan stain from the oily fraction. The temporary disappearance of Sudan coloration when the fat is very finely dispersed can be easily explained and readily demonstrated with Sudanized oil. It does not have any significant bearing upon the interpretation of our experiments.

It is not possible to correlate these findings with the lipolytic hypothesis. An explanation of our observations, which is also in accord with the experimental results of other workers, is afforded by the partition hypothesis [Frazer,



Text-fig. 2. The partition hypothesis of fat absorption. The diagram shows the partition of fat and fatty acid by partial hydrolysis in the intestine, and it indicates the main evidence upon which the hypothesis is based.

1938]. According to this view (Text-fig. 2) lipolysis is only partial, and hydrolysis of the triglyceride molecule is not regarded as an essential preliminary to its absorption. The split and unsplit fractions of neutral fat are absorbed by different mechanisms into the intestinal cell, where they give rise to different histological pictures, and from which they pass by different routes to different destinations. Fatty acid passes by the portal vein to the liver, while neutral fat goes by the lymphatic route to the systemic blood and thence to the depots to be stored for future use. The degree of lipolysis is, thus, a determining factor in the immediate fate of absorbed fat.

SUMMARY

1. Neutral fat absorption gives rise to large globules in the intestinal cell, whereas fatty acid absorption shows a fine brown granular deposit.

2. Neutral fat absorption is accompanied by milky lacteals; not to fatty acid absorption.

3. Neutral fat absorption gives a systemic lipaemia, but little change in the portal blood. Fatty acid causes a marked portal lipaemia with little change in the systemic blood.

4. Neutral fat can be traced to the fat depots, and provided it is administered in moderate doses, it fails to give marked deposition in the liver. Fatty acid, on the other hand, does not appear in the fat depots, but it gives rise to a marked deposition in the liver.

5. These findings cannot be correlated with the current hypothesis of complete lipolysis prior to absorption.

6. The partition hypothesis of fat absorption is put forward as a possible explanation of the observations reported.

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EXPLANATION OF PLATES 1 AND 2

PLATE 1

A. Section from the small intestine of the rat 6 hr. after feeding with olive oil. The fat is present in the intestinal cells in large Sudan-staining globules. Magnification ×300. B. Section from the small intestine of the rat 6 hr. after feeding with fatty acid and glycerol: the fatty material is in the form of fine brown granules in striking contrast to the fat in A. Magnification ×300.

PLATE 2

A. A section of liver from the rat killed 4 hr. after feeding with 1 c.c. Sudanized olive oil. A small amount of stained fatty material is found. Magnification ×300. B. A section of liver from a rat killed 4 hr. after feeding with 1 c.c. Sudanized oleic acid with an equivalent amount of glycerol. Marked accumulation of stained fatty material is found throughout the liver. Magnification ×300.