II. A STUDY OF THE PASSAGE OF FATTY ACIDS OF FOOD INTO LIPINS AND GLYCERIDES OF THE BODY USING DEUTERIUM AS AN INDICATOR

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It was shown by Schoenheimer & Rittenberg [1935] that fat containing deuterium when fed to mice may subsequently be found in both the "organ fat" and the "body fat" of the animals. The present authors [1936], after administering an oil containing D to a rat for 7 days, also showed that the lipins of the liver and kidney contained fatty acids in which D was present. This suggests that the lipins may play an active part in fat metabolism and not merely a passive role as constituents of protoplasm which are important because of their physical properties.

Sinclair [1935] has come to a similar conclusion as a result of experiments in which the administration of a fat containing elaidic acid led to the appearance of this acid in the lipins of liver and muscle. In Sinclair's experiments one day was the shortest period that was allowed to elapse after the fat feeding before the organs of the animals were taken for examination. It appeared to us that a shorter period than this might yield results of interest and, further, the greater ease with which D can be detected as compared with elaidic acid made its use especially advantageous for this type of work.

EXPERIMENTAL

The "deutero-fat" was obtained by partially "deuterating" pure unboiled linseed oil with 100 % deuterium (electrolytic) using palladium black as catalyst. About 100 g. oil were treated at a time, the gas (in glass bulbs) was initially at atmospheric pressure, and the apparatus was entirely of glass with the exception of one short connexion of flexible metal tubing joined to the glass by piceined cones. The oil was heated electrically to about 70° and shaken mechanically during the reaction, the progress of which was observed manometrically. It was found possible to introduce D to the extent of 4-5 atoms % of the H present without solidifying the fat or making it unpalatable to the rats.

Groups of 3 or 4 rats were used according to the size of the animals. The total weight of the group was usually about 1 kg. They had been kept on the ordinary laboratory diet before the experiment. Each group was starved for a day before receiving the food containing "deutero-fat". This consisted of 4 g. crushed dog biscuit, moistened with 10 ml. water, and in this 1.9 g. of the deutero-fat were incorporated. The food was usually well taken and was eaten within 3 hr. At the appropriate time (6, 10 or 24 hr.) after giving the food the animals were stunned and killed by bleeding. The blood was collected with addition of oxalate and centrifuged to separate corpuscles and plasma. The liver, kidneys and brain were

removed and a sample of adipose tissue from the abdomen was taken. The tissues or organs of the whole group were worked up together. The adipose tissue was heated with about its own wt. of 50 % KOH and 10 ml. alcohol until saponification was complete. The alcohol was then removed on the water bath, the soaps taken up in water and acidified with dilute H_2SO_4 . The liberated fatty acids were taken up in light petroleum and a portion of the solution evaporated. The last traces of the solvent were removed by heating to 100° under reduced pressure in an atmosphere of H_2 for $\frac{1}{2}$ hr. The liver, kidneys, brain, blood corpuscles and blood plasma were dealt with as follows. The organs after chopping with a razor were ground up in a mortar and extracted in the cold for 24 hr. with 4–5 times their wt. of alcohol—the blood corpuscles and blood plasma were added directly to the alcohol. The extraction was repeated with absolute alcohol for at least another 24 hr. The tissue substance after removal of the 2nd extract by filtration was dried in a vacuum desiccator over H_2SO_4 and then extracted with ether for 8 hr. in a Soxhlet apparatus. The two alcohol extracts were evaporated to dryness under reduced pressure in a current of H_2 and the residue extracted with dry ether. This extract was added to the Soxhlet extract and the whole distilled to remove the ether. With the liver it was found convenient at this stage to continue with only one-fourth of the extract. The residue containing all the ether-soluble substances of the organ (or blood fraction) was dissolved in 4 ml. dry ether and precipitated with 20 ml. acetone. The precipitate was filtered off, washed with acetone-ether and dried in a vacuum desiccator. It was then taken up in dry ether and the solution evaporated in a weighed flask. The acetone-soluble material was recovered in a similar manner by distillation from a tared flask. In these two fractions the D content was estimated as described below. The acetone-insoluble fraction consists essentially of the lipins of the tissue, and the acetone-soluble fraction of the glycerides and sterols.

Combustion technique. The commercial O_2 used in the combustions was known to be liable to contain traces of oil, and for this reason, as well as on general grounds, the dried gas was purified by submitting it to exactly the same treatment as it would subsequently encounter in the combustion tube, i.e. it was passed through a duplicate tube similarly packed and heated and was then dried before entering the combustion tube proper. In the earlier combustions Pregl's "universal filling" was used in supramax glass tubes. The too hygroscopic lead peroxide was omitted since interchange of water from one combustion to another was not permissible, but otherwise, as regards temp., rate of O_2 -flow etc., the essential conditions of the Pregl technique were observed, though the heating was electrical. Later it was found convenient to use quartz combustion tubes, omitting the lead chromate from the filling and raising the temperature somewhat (750 – 800°). The tubes (Fig. 1) were wound directly with nichrome ribbon and then boxed in with thick alundum cement. The current (A.C.) was controlled by rheostat and ammeter and the temperature inside the lagging observed by means of a thermocouple.

Since, of the products of combustion, only the water was required and this in the free form, the emergent gases were passed through a glass spiral (*H*) immersed in a "slush" of solid carbon dioxide and alcohol. (A similar cooled spiral followed by two U-tubes packed with $\text{Mg}(\text{ClO}_4)_2$ was used for drying the O_2 stream between the two combustion tubes.) There were no rubber connexions, a pieceined ground stopper (*G*) permitted the introduction of the Pt boat containing the sample for analysis, and on completion of the combustion, glass taps served to cut off the O_2 stream (at *A*) and its exit (at *B*) and to connect the combustion tube (at *C*) to the Hg vapour pump. Moderate evacuation then sufficed to enable the water

sample to be rapidly distilled through a large tap (*D*) into a separate compartment of the apparatus where it was condensed in a detachable "water vessel" of the type shown (*E*), and then resublimed from -15° to -30° into a second vessel (*F*) under a better vacuum.

Fig. 1.

Purification of water from combustion. About half of the samples dealt with were known to contain N and the rest might contain small amounts, but since this was in the form of substituted NH_3 it was not at first thought necessary to supplement the filling by Cu for the decomposition of oxides of N. However, a peculiarity in the behaviour of the collected water during sublimation led to tests which revealed the presence of HNO_3 in appreciable quantities in the collected water. It was therefore decided to use the Cu catalyst, but to do so in a separate apparatus, so as to avoid the necessity of frequent reduction of the Cu. This apparatus was essentially similar to the main combustion apparatus (lower part of Fig. 1) without the provision of the O_2 stream, with a ground joint for the attachment of a "water vessel" in place of *G*, a narrow U-tube in place of *H*, and the Cu catalyst alone as filling. The latter was maintained (electrically) at a mild red heat and the whole apparatus thoroughly evacuated before each experiment. The water sample from a combustion attached at *G* was first cooled to -80° while the evacuation was completed, and then (taps *C* and *D* being closed) allowed to warm up in stages to room temp. while the U-tube was cooled. The controlled distillation over the heated catalyst was accelerated in its later stages by pumping off permanent gases through *D* and *K*. The purification of the water sample was then completed by a double vacuum sublimation (between -15° and -30°) to the receivers *E* and *F*. As a check the water samples were always tested for neutrality after the densities had been determined.

Density determination. The purified water samples were generally about 10 mg. but in several cases as small as 4–5 mg. Their densities were determined by the method of Gilfillan & Polanyi [1933] to a precision of one part in 100,000, and the atomic % of D calculated to 0.01 %. As a check on the combustion technique samples of ox-liver lecithin free from D were similarly treated, one such control being inserted after every two of the ordinary combustions. Similar controls were used initially in perfecting the technique and afterwards—throughout the recorded experiments—they yielded water samples of normal density within the precision of measurement.

RESULTS

The % of the "glyceride" and "lipin" fractions obtained from the organs or tissues and the D contents of these fractions are given in Tables I and II respectively. The % figures for both fractions are reckoned on the wt. of the fresh tissue except with the blood corpuscles and plasma, which are based on 100 ml. The D figures represent atoms % D in the crude "glyceride" and "lipin" fractions. The fat administered contained 4.87 atoms % D.

Table I. *Glyceride fractions*

Organ or tissue	Glyceride %			Atoms % D in glycerides		
	6 hr.	10 hr.	24 hr.	6 hr.	10 hr.	24 hr.
Liver	1.26	1.41	1.55	0.86	0.67	0.30
Kidney	1.27	1.43	—	0.12	0.22	0.20
Brain	2.98	2.87	2.50	0.01	0.05	0.025
Blood corpuscles	0.32	0.43	0.50	0.67	0.12	0.17
Blood plasma	0.23	0.28	0.19	1.26	0.64	—
Adipose tissue	—	—	—	0.06	0.03	0.13

Table II. *Lipin fractions*

Organ or tissue	Lipin %			Atoms % D in lipins		
	6 hr.	10 hr.	24 hr.	6 hr.	10 hr.	24 hr.
Liver	3.97	4.42	4.11	0.47	0.52	0.42
Kidney	—	2.85	2.79	0.16	0.14	0.17
Brain	4.0	—	4.39	0.004	0.08	0.03
Blood corpuscles	0.59	0.40	0.26	0.12	0.10	—
Blood plasma	0.07	—	0.08	0.12	0.47	—

DISCUSSION

Acetone precipitation does not produce a sharp separation of lipins from glycerides so that the experiments give comparative rather than strictly quantitative indications of the distribution of the deuterio-fatty acids between glycerides and lipins after absorption. Perhaps the figures of greatest interest are those obtained 6 hr. after administration of the fat. At this time the fatty substances of all the tissues examined showed the presence of D. As would be expected the highest % D was found in the glycerides of the blood plasma and following these in descending order come those of liver, blood corpuscles and kidney. In the brain and adipose tissue D was only just detectable. The % D in the liver lipins and glycerides was very much greater than in those of the kidney, which accords with the generally accepted views on the special part played by the liver in fat metabolism. A comparison of the % D in the various organ fats with that in the fat administered shows that at the end of 6 hr. about 26 % of the plasma glyceride, 18 % of the liver glyceride and 2.5 % of the kidney glyceride was derived from the deuterio-fat administered. This indicates a selective intake of fat from the blood by the liver soon after its absorption. The lipins of the liver also participate in this phenomenon. Although the % D in the liver lipins is lower than that in of the glycerides, if one takes into account the smaller proportion of fatty acids which they contain (65 %) it is seen that about 14 % of the lipin fatty acids had come from the deuterio-fat of the food.

The figures obtained after 10 hr. show a decline in the D content of the blood fat. This might be expected from previous observations on fat absorption. Owing to technical difficulties the 24 hr. figures for the plasma and corpuscles were not

obtained. In the liver there is a gradual decline in the % D in the glycerides during 24 hr. but much less change in that of the lipins. In the kidney the glyceride figures at 10 and 24 hr. are about the same and higher than the 6 hr. value, whereas the lipin D does not change significantly. The kidney, therefore, does not appear to respond to the changes in blood fat either as rapidly or to the same extent as the liver. On the whole these results suggest a specially active participation in fat metabolism by the lipins of the liver. Artom *et al.* [1937] have reached a similar conclusion from experiments in which radioactive P was used to trace the entry of H_3PO_4 into lipins. The lower % D in the plasma lipins in comparison with those of the liver at the end of 6 hr., renders it unlikely that the liver lipins were not synthesized in the liver but merely taken up from the blood.

The D content of the adipose tissue was never very marked and this was to be expected because of the diluent effect of the comparatively large amount of adipose tissue fat in the animal compared with that (1.9 g.) which was administered.

The brain in all the experiments contained only traces of D in its lipin and glyceride fractions. This does not suggest any rapid exchange in this organ between its lipins and the blood fat. It confirms observations made by Hahn & Hevesy [1937], who found a definite but small uptake of labelled P by brain lipins an hour after the injection into rats of Na phosphate containing the radioactive isotope of phosphorus.

SUMMARY

1. Rats were fed with a fat containing 4-5 atoms % deuterium and the distribution of the "deutero-fatty acids" in "lipin" and "glyceride" fractions of liver, kidney, brain and blood determined 6, 10 and 24 hr. after.

2. After 6 hr. D was present in considerable amount in plasma glycerides, liver glycerides and liver lipins. There was much less in the lipid fractions of the kidney and plasma and the D was only present in traces in brain and adipose tissue.

3. The D in the liver glycerides decreased more rapidly in 24 hr. than that of the liver lipins. In the lipid fractions of kidney and brain there were no notable changes in % D between 6 and 24 hr.

4. The results suggest that liver lipins may play a very active part in fat metabolism.

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REFERENCES

- Artom, Perrier, Santangelo, Sarzana & Segrè (1937). *Nature, Lond.*, **139**, 836
Cavanagh & Raper (1936). *Nature, Lond.*, **137**, 233.
Gilfillan & Polanyi (1933). *Z. phys. Chem.* **166**, 254.
Hahn & Hevesy (1937). *Skand. Arch. Physiol.* **77**, 148.
Schoenheimer & Rittenberg (1935). *J. biol. Chem.* **111**, 163.
Sinclair (1935). *J. biol. Chem.* **111**, 515.