Studies on the Origin of Milk Fat

1. THE LOCATION OF TRITIUM IN STEARIC ACID PRODUCED BY THE CATALYTIC ADDITION OF TRITIUM TO ELAIDIC ACID

BY R. F. GLASCOCK AND L. R. REINIUS*

The National Institute for Research in Dairying, University of Reading

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Schoenheimer & Rittenberg (1935) assumed that a specifically labelled stearic acid would result from the catalytic addition of deuterium to linoleic acid over a catalyst made from platinum oxide. It was therefore hoped that the catalytic addition of tritium to elaidic acid under somewhat milder conditions (room temperature, over a palladium-black catalyst at less than atmospheric pressure) would result in stearic acid labelled in the 9:10-position. This material was required primarily for work on the contribution of long-chain fatty acids to milk constituents. In a preliminary experiment on a goat (Duncombe & Glascock, 1954), carried out with tritiostearic acid prepared from oleic acid, it was found that steam-volatile fatty acids, glyceride glycerol, lactose and cholesterol isolated from the milk had low but significant specific activities. If the stearic acid administered was specifically labelled these results therefore implied extensive degradation.

It was also found that the specific activities of the saturated and unsaturated fractions of the glyceride fatty acids were about the same. This might have meant a marked isotope effect, hydrogen being removed in preference to tritium during the biological conversion of stearic acid into oleic acid. Before proceeding with further work it thus became necessary to ascertain whether or not the stearic acid used was specifically labelled.

problem. Thus Hunter & Popják (1951) describe a method for the splitting from long-chain fatty acids of two carbon atoms at a time as acetic acid. The whole procedure involves five steps, and even if applicable to the determination of the location of hydrogen isotopes would obviously present an enormous task if applied to stearic acid. Presumably some such method would have to be used, however, if an exact map of the isotopic distribution in stearic acid were required. For our work, however, it was necessary only to know whether all the tritium resided on the C₍₉₎ and C₍₁₀₎ atoms and, if not, how much resided elsewhere on the chain.

In the preliminary biological work already referred to it had been found that after feeding the labelled stearic acid to a lactating goat, the unsaturated fatty acid fraction of the milk had a high specific activity suggesting the biological conversion of stearic acid into oleic acid. If the tritium in this oleic acid were attached to the C₍₉₎ and C₍₁₀₎ atoms thus:

then the following series of reactions should yield non-radioactive pelargonic and azelaic acids by labilization at stage (3) of the hydrogen originally on the C₍₉₎ and C₍₁₀₎ atoms. This paper deals with the results obtained on submitting the milk oleic acid to this degradation scheme.

The degradation of stearic acid in such a manner as to determine whether or not the tritium in it is attached to the C₍₉₎ and C₍₁₀₎ atoms is a matter of considerable technical difficulty. Methods are available for the degradation of ¹⁴C-labelled fatty acids, but they are not readily applicable to this

* Present address: Department of Physiology, The Veterinary College, Helsinki.

EXPERIMENTAL

Preparation of tritium-labelled stearic acid. Elaidic acid, the *trans*-isomer of oleic acid, was used as the starting material in this preparation because it is available in pure crystalline form. The technique for adding tritium to the elaidic acid molecule was that already used for the preparation of tritiohexoestrol (Glascock & Reinius, 1954; Glascock & Pope, unpublished work). It was found that 96% of the

theoretical volume of tritium-hydrogen (nominal specific activity, 15.5 mc/ml.) was taken up by 14.4 mg. of elaidic acid in 68 hr. The product was extracted from the reaction vessel with ether, converted into the sodium salt and reprecipitated to displace tritium that might have entered the carboxyl group. It was pure white, with m.p. 71°; (Heilbron & Bunbury (1943) give 71.5–72°). The yield was 13.4 mg. (93%).

Assay. The whole of the product was dissolved in arachis oil, giving a total weight of solution of 1.027 g., and an 8.5 mg. sample was submitted to wet combustion and assayed for tritium as described by Glascock (1954). The oil solution was found to have a specific activity of 5.047×10^6 counts/min./mg., corresponding to 3.845×10^8 counts/min./mg. for the stearic acid dissolved in it. From this result it is calculated that the tritium-hydrogen used must have had a specific activity of 6.6 mc/ml. instead of the nominal 15.5 mc/ml.

Administration to goat. The goat was a British Saanen, 3 years old, weight 61 kg., in her second lactation and yielding 2.5 kg. of milk/day. She was fed on stock cereal diet with hay *ad lib.* and kept in a pen during the period of the experiment. A portion of the oil solution of [^3H]stearic acid described above, weighing 0.78 g. and containing 3.94×10^9 counts/min., was emulsified in 50 ml. of 5% (w/v) glucose solution together with 0.5 g. of glyceryl monostearate. This emulsion was administered quantitatively by mouth followed by 50 ml. of a similar but non-radioactive emulsion.

Milking. The goat was milked at intervals and milk samples were fractionated for the determination of the distribution of radioactivity in various constituents. This work is the subject of part of the next paper (Glascock, Duncombe & Reinius, 1956). Two milk samples, *A* and *B* (vol. 1370 and 820 ml. respectively), collected at 23 and 31 hr. after dosing, were used for the present work.

Extraction of total fat. The method used was that of McClymont (1951). The milk was centrifuged at 200 rev./min. for 1 hr., cooled in ice, and the cream removed into a beaker. To the cream was added an equal volume of ethanol containing 10% (v/v) of concentrated NH_3 solution (sp.gr., 0.880); the mixture was heated to boiling and then cooled. It was extracted with 3×25 ml. of a mixture of equal vol. of light petroleum (b.p. 40–60°) and ether. The combined extracts were dried overnight with anhydrous Na_2SO_4 and filtered and the solvent was distilled off.

The residue of mixed lipids was transferred to centrifuge tubes with the help of a small amount of light petroleum and the phospholipids were precipitated, according to the method of Bloor (1929), with acetone and MgCl_2 . The precipitate was centrifuged off and the supernatant solution removed and evaporated to dryness.

Ethanolic KOH (1 vol. of 40% w/v KOH to 10 vol. of absolute ethanol) was added to the fat in the proportions of 50 ml. to 1 g. of fat, and the mixture was refluxed for 3 hr. The solution was then extracted with 5×10 ml. of light petroleum to remove unsaponifiable material, and the extracted solution was heated on the steam bath to remove organic solvents, water being added towards the end to keep the soaps in solution. It was allowed to cool, and the fatty acids were liberated by the addition of a 10% excess of sulphuric acid (40%, v/v). Volatile fatty acids were then removed by steam distillation until more than the initial volume of solution had been distilled over.

Separation of unsaturated fatty acids. The method of Twitchell (1921) was used. The liquid in the flask was allowed to cool and the aqueous lower layer removed. The mixture of fatty acids was dissolved in 5 times its weight of 95% (v/v) ethanol containing 1.5% (v/v) glacial acetic acid. The mixture was heated to boiling, and an equal volume of boiling lead acetate (14% w/v in 95% ethanol containing 1.5% glacial acetic acid) was added. The mixture was allowed to cool to room temperature and then filtered. The filtrate, which contained the unsaturated fatty acids, was tested for an excess of lead acetate by the addition of an ethanolic solution of H_2SO_4 .

The ethanol was then removed by distillation under reduced pressure and the residue dissolved in ether. The ethereal solution was washed first with water and then with dilute HCl, and again with water until free from mineral acid. The solution was dried over Na_2SO_4 and the solvent removed. The residue of unsaturated fatty acids was dried to constant weight by heating gently under vacuum. Yield, 4.7 g. from sample *A*, and 2.7 g. from sample *B*.

Dilution with carrier. Both samples of mixed unsaturated fatty acids had specific activities of the order of 10^4 counts/min./mg. of combustion water. Since specific activities of the order of 100 counts/min./mg. of combustion water can be conveniently measured by the methods of assay used (the gas counters used will hold butane equivalent to 10 mg. of water) it was decided to use carrier oleic acid. The oleic acid used was a laboratory reagent labelled 'oleic acid, redistilled'. Its iodine value was measured and found to be 88.4, which is close both to the theoretical figure (91.5) and to the value on the bottle. The results obtained (see below), however, gave reason to believe that it was not pure, and hence the exact amount of carrier oleic acid used is not known.

Sample *A* was diluted by a factor of 5.14, and sample *B* by a factor of 9.92.

Preparation of dihydroxystearic acid

The method used was that of Swern, Billen, Findley & Scanlan (1945), which is based upon the oxidation of oleic acid with performic acid.

Sample A. To 6.578 g. of diluted unsaturated fatty acids were added 50 ml. of formic acid (B.D.H., 98–100%) and 3 ml. of H_2O_2 (B.D.H., 98–100 vol.). The mixture, cooled in a cold-water bath, was stirred for 30 min. after the addition of the H_2O_2 , and then transferred to a bath at 40°. The mixture was continually stirred over a period of 6 hr., 4×3 ml. portions of H_2O_2 being added at 1 hr. intervals. The reaction mixture was poured into 4 vol. of water, and the formoxy derivatives of dihydroxystearic acid were extracted with ether. The ethereal solution was washed with water and the ether was removed by distillation.

To the residue was added 3N-KOH until the mixture was alkaline. It was then boiled under reflux for 1 hr. to hydrolyse the formoxy derivatives, poured into an excess of 3N-HCl, cooled, and the crude dihydroxystearic acid, a white solid, separated from the aqueous layer, which was discarded.

The dihydroxystearic acid was recrystallized once from hot ethanol (5 ml. of ethanol to 1 g. of dihydroxystearic acid), yielding white crystals (2.331 g.), m.p. 88°. Recrystallization of a portion gave a product with the same m.p. [Swern *et al.* (1945) found m.p. 92–94°].

Sample B. The procedure was exactly as described for sample *A* except that 4.047 g. of diluted unsaturated fatty acids dissolved in 36 ml. of formic acid was used and oxidized with a total of 10 ml. of H_2O_2 added in 2 ml. portions. The product, twice recrystallized, melted at 89–90°, and the yield was 1.468 g.

Degradation of dihydroxystearic acid

Dilution. The specific activities of the dihydroxystearic acid samples were expected to be of the order of 2500 counts/min./mg. Only relatively small quantities were available (2.3 g. of sample *A* and 1.47 g. of sample *B*) and the proposed degradation was a two-stage one, giving rise to four different products: pelargonic aldehyde, pelargonic acid, azelaic semialdehyde and azelaic acid. It was therefore decided to dilute the samples about fivefold with carrier. For this purpose dihydroxystearic acid (obtained from L. Light and Co.) was recrystallized once from 95% (v/v) ethanol (m.p. 90°), and used to dilute the radioactive dihydroxystearic acid. The dilutions were: sample *A*: 2.276 g. was diluted to 9.688 g. (dilution factor = 4.26); sample *B*: 1.430 g. was diluted to 5.731 g. (dilution factor = 4.01).

The methods of degradation used were those of Scanlan & Swern (1940).

Fission of dihydroxystearic acid molecule at the 9:10-position. Using sample *A*, 9.688 g. of diluted dihydroxystearic acid was melted and 76.0 g. of glacial acetic acid added with stirring, the mixture being maintained at 55–60°. Pb_3O_4 (23.20 g.) was then added in about 15 small portions of equal size, each portion being added only when the colour from the previous portion had disappeared. The whole reaction time was 3 hr. When all the Pb_3O_4 had been added, the solution was tested for excess of lead tetraacetate with leucomalachite green. The mixture now contained pelargonic aldehyde and azelaic semialdehyde, only the pelargonic aldehyde being volatile in steam. The mixture was therefore distilled in steam until no more oil came over. This was separated from the water and part was converted into the dimedone derivative and part set aside for oxidation to pelargonic acid.

Pelargonic aldehyde dimedone derivative. Pelargonic aldehyde (0.250 mg.) and dimedone (600 mg.) were dissolved in 30 ml. of 50% (v/v) ethanol, and the solution was warmed and left standing overnight. White needles which separated on recrystallization from ethanol had m.p. 86° [Staff of the Research Laboratory of Hopkin and Williams Ltd. (1944) give 86°].

Pelargonic acid. The remaining pelargonic aldehyde was taken up in 50 ml. of ether and the solution was washed with NaHCO_3 until neutral. It was diluted to 100 ml. in a 100 ml. measuring cylinder and oxygen was bubbled through it for 2 hr. The solution was dried over Na_2SO_4 and filtered, and the ether removed by distillation. The solution of crude pelargonic acid was made alkaline with *N*-KOH to displace the hydrogen attached to the carboxyl group and reprecipitated with 2*N*-HCl. The pelargonic acid was taken up in ether, the ether solution washed with water and the ether then removed by distillation.

Pelargonic acid p-toluidide. To 509.4 mg. of crude pelargonic acid was added 0.34 ml. of thionyl chloride and the mixture was heated to 110–120° for 30 min. Then 0.345 g. of *p*-toluidine was added, and the mixture was heated at 140° for 30 min. The product was cooled and

recrystallized twice from benzene–ethanol mixture (50%, v/v). It had m.p. 83–84° [Heilbron & Bunbury (1943) give 84°].

Azelaic semialdehyde. The liquid remaining in the flask after the removal of the pelargonic aldehyde by steam distillation was cooled and extracted with ether. The ether was washed thoroughly with water, filtered and dried over Na_2SO_4 . The solution was filtered and the ether removed by distillation under reduced pressure. The crude azelaic semialdehyde remained as a yellow oil (yield 2.5 g.). It was not purified but converted into the following derivative.

Azelaic aldehyde semicarbazone. A solution of 4.05 g. of semicarbazide hydrochloride and 5 g. of hydrated sodium acetate in 10 ml. of water was prepared, and 1.5 g. of crude azelaic semialdehyde was added to 3 ml. of this solution. The derivative began to crystallize within a few minutes. After standing overnight the crystals were filtered off and recrystallized from aqueous ethanol. They had m.p. 161–162° [Heilbron & Bunbury (1943) give 163°].

Azelaic acid. The remainder of the azelaic semialdehyde, amounting to about 1 g., was refluxed with 25 ml. of 0.05*N*-NaOH and KMnO_4 solution (620 mg. of KMnO_4 in 5 ml. of water) was added a little at a time. Refluxing was continued until the colour of the permanganate was discharged. The solution was filtered, the combined filtrate and washings were concentrated to about 20 ml., and 2*N*-HCl was added until the solution was acid. The white precipitate of azelaic acid which formed was filtered off, washed with water and recrystallized from water. It had m.p. 104° [Heilbron & Bunbury (1943) give 106.5°].

The dihydroxystearic acid prepared from sample *B* was degraded in exactly the same way as that from sample *A* except that a smaller quantity (5.73 g.) was used, and the amounts of reagents required were proportionately less. The same derivatives were prepared, and found to have the same melting points.

Determination of tritium

Tritium was determined by combustion of 5–10 mg. samples of the derivatives and conversion of the combustion water into butane, followed by gas counting as described by Glascock (1951, 1952, 1954).

RESULTS

The original unsaturated fatty acid fractions from milk samples *A* and *B* were found to have specific activities of 10 800 and 10 200 counts/min./mg. of combustion water, and were diluted with carrier oleic acid by factors of 5.14 and 9.92 respectively. If both the milk unsaturated fatty acids and the carrier were pure oleic acid, the mixtures should thus have had specific activities of 2100 and 1030 counts/min./mg. of combustion water. The specific activities of the dihydroxystearic acid samples prepared from these oleic acid dilutions (after correction for the further dilution carried out at the dihydroxystearic acid stage) were, however, 3250 and 1470 counts/min./mg. of water. Corrected to the hydrogen content of oleic acid itself, these figures correspond to 3440 and 1555 counts/min./mg. of oleic acid combustion water. They are about

50 % higher than would have been expected if both carrier and milk unsaturated fatty acids were pure oleic acid, but they are in about the same ratio to each other as would then have been expected.

These higher values could not arise only from the milk unsaturated fatty acids being composed partly of acids other than oleic: the carrier oleic acid must also have been impure. The specific activity data obtained and the dilutions actually used would fit if the carrier was about 60 % oleic acid and the milk unsaturated acids about 65 % oleic acid. It is obvious, however, that the proportion of oleic acid in the milk acids cannot be calculated exactly if the purity of the carrier is not known, and this value is neither known nor easily ascertainable.

contained in the pelargonic aldehyde as in the azelaic semialdehyde. Since no loss of tritium had occurred during the fission of the molecule it seems unlikely that this is an analytical artifact; it indicates that about 68 % more tritium was attached to the carbon atoms on the methyl side than on the carboxyl side of the double bond of the original oleic acid.

Table 2 shows the specific activities of the two aldehydes and of the corresponding acids produced from them on oxidation. The loss of activity from the azelaic semialdehyde represents the tritium originally attached to the $C_{(9)}$ atom of the original oleic acid, and the loss of activity from the pelargonic aldehyde represents the tritium originally attached to the $C_{(10)}$ atom. The agreement between

Table 1. *Specific activity of dihydroxystearic acid and its decomposition products*

Substance	Specific activity (counts/min./ μ mole)	
	Sample A	Sample B
Dihydroxystearic acid	248.0	119.0
Pelargonic aldehyde	152.0	245.0
Azelaic semialdehyde	93.0	
Ratio: $\frac{\text{Specific activity of pelargonic aldehyde}}{\text{Specific activity of azelaic semialdehyde}}$	1.64	1.72

Table 2. *Diminution in specific activity on oxidation of pelargonic aldehyde and azelaic semialdehyde to corresponding acids*

Substance	Specific activity (counts/min./ μ mole)	
	Sample A	Sample B
Pelargonic aldehyde	152.0	81.0
Pelargonic acid	131.0	61.0
Difference (%)	14	25
Mean difference (%)	20	
Azelaic semialdehyde	93.0	47.5
Azelaic acid	83.0	45.0
Difference (%)	11	5
Mean difference (%)	8	

The specific activity of each sample of dihydroxystearic acid, and the specific activities of the two aldehydes to which each gives rise on fission, are given in Table 1, the results being expressed in counts/min./ μ mole for better comparison. It will be seen that, within the limits of experimental error, the activity originally contained in the dihydroxystearic acid was recovered in the decomposition products. It is evident, therefore, that the tritium was stably bound to the dihydroxystearic acid, none being lost by exchange even during treatment with a vigorous oxidizing agent rich in hydrogen ions. It is also to be noted that on the average there was 1.68 times as much activity

the duplicates is poor, as is to be expected when figures are obtained by difference. However, taking the values found, the loss of activity corresponds to an average of 3 % from the $C_{(9)}$ atom of the original oleic acid and 12 % from the $C_{(10)}$ atom. Although it is clear that these figures are only approximate, the conclusion can justifiably be drawn that more tritium resides on the $C_{(10)}$ atom than on the $C_{(9)}$ atom.

DISCUSSION

The fact that the dihydroxystearic acid samples were radioactive formally proves the biological conversion of stearic acid into oleic acid in the goat. This is in agreement with what has been found for body fat in the rat using deuterium (Stetten & Schoenheimer, 1940).

It is evident from the specific activities of the degradation products that the major part of the tritium, probably as much as 85 %, contained in the dihydroxystearic acid resided on carbon atoms other than the $C_{(10)}$ and $C_{(9)}$. The two main questions that arise are therefore: (1) to what extent does this distribution of tritium reflect that in the oleic acid extracted from the milk fat; (2) to what extent does it reflect the isotope distribution in the original stearic acid administered to the goat?

The distribution of tritium in the dihydroxystearic acid is almost certainly the same as that in the oleic acid from which it was prepared. If it were not, then the labilization that must accompany redistribution would also have resulted in loss of tritium into the reagent, performic acid, which is a substance rich in exchangeable hydrogen. Although, for reasons already given, an exact radioactivity balance was not possible because of the impurity of the carrier oleic acid, the specific activity of the dihydroxystearic acid was higher, not lower, than expected. Furthermore, in the next stage of degradation (dihydroxystearic acid to pelargonic and azelaic aldehydes) a radioactivity balance was achieved after the use of an equally vigorous and somewhat similar oxidizing agent (lead tetraacetate in glacial acetic acid).

Granted, therefore, that the tritium in the oleic acid was stably bound, it is virtually certain that a redistribution of tritium in the molecule had not occurred biologically because the operation of dehydrogenating enzyme systems would result in loss of tritium into coenzyme pools or body water, and not in reattachment to the original molecule in a different position. It therefore follows that the tritium in the original stearic acid was not confined to the $C_{(9)}$ and $C_{(10)}$ atoms and distribution down the chain must have occurred during its preparation.

That this is not only possible but probable is indicated by the findings of other workers investigating the hydrogenation of fats and fatty acids generally. This work, ably surveyed by Markley (1947), indicates that there is an appearance of double bonds in positions other than the original position during the catalytic hydrogenation of ethenoid acids or their esters. Hilditch & Vidyarthi (1929) found that the partial hydrogenation of methyl oleate gives rise to methyl esters of oleic acids with the double bond in both the 8:9- and the 10:11-positions. Moore (1919) hydrogenated ethyl oleate with palladium and nickel catalysts and found esters of monoethenoid acids in the product with the double bond in the 10:11- and in the 11:12-positions. It thus seems highly probable that in the stearic acid used in the work described in this paper the tritium resided also on these positions.

One would, however, still expect more tritium to be located in the 9:10-positions than was actually found. As already noted, the total amount of tritium in these positions was approximately 15 % of the total in the molecule, corresponding to 28 % in the original stearic acid if no isotope effect occurred during biological conversion into oleic acid. Neglecting the fact that the 9:10-position is most rapidly hydrogenated (Pigulevskii & Artamonov, 1942; quoted by Markley, 1947), and supposing

that the isotope were distributed evenly over the 8, 9, 10, 11 and 12 positions, one would expect the 9:10-positions together to account for 40 % of the tritium in the molecule. The difference between this figure and ours (28 %) is too great to be due to experimental error and must be due to some other cause. One possibility is that the stearic acid dehydrogenating system may have labilized all the tritium atoms on the $C_{(9)}$ and $C_{(10)}$ atoms, resulting in the loss of more than half as would be expected: i.e. half is lost by dehydrogenation followed by a further loss through exchange with unlabelled hydrogen in the body water. Fisher, Conn, Vennesland & Westheimer (1953), however, have shown that dehydrogenating enzymes are highly stereo-specific, and it is therefore unlikely that the stearic acid dehydrogenase could affect all the hydrogen atoms on the $C_{(9)}$ and $C_{(10)}$ atoms. Furthermore, Fisher *et al.* (1953) found that whereas ethanol dehydrogenase could remove all the deuterium from deuterio-diphosphopyridine nucleotide which had been prepared enzymically, it could remove only about half from deuterio-diphosphopyridine nucleotide prepared chemically. The residual deuterium in the chemically reduced diphosphopyridine nucleotide was presumably in positions not accessible to the dehydrogenase. It therefore seems highly improbable that the stearic acid dehydrogenase of the goat could labilize tritium atoms not directly involved in the dehydrogenation.

Another possible explanation for our observations is that the tritium introduced on the $C_{(9)}$ and $C_{(10)}$ atoms of the stearic acid was more accessible to the dehydrogenase than the hydrogen already attached to these carbon atoms in the original elaidic acid. A consideration of the configurations of all the possible isomers of [9:10- ^3H]stearic acid shows that, averaged over the whole molecular population, tritium and hydrogen are stereochemically equivalent, whether *cis*- or *trans*-oleic acid is used in the tritiation. This means that not more than half the tritium present could be removed during the formation of oleic acid unless the tritiation had been asymmetric; an asymmetric synthesis from the catalytic hydrogenation of non-optically active compounds has never been observed.

The only other possible explanation appears to be that the catalytically introduced tritium was not confined to the 8, 9, 10, 11 and 12 positions, as suggested above, but was distributed over more of the molecule than previous work would lead one to expect.

The fact that 1.68 times as much tritium was found in the 'methyl half' as in the 'carboxyl half' of the molecule might be accounted for by the finding of Moore (1919) that new double bonds can

form in both the 10:11- and 11:12-positions in one half of the molecule but only in the 8:9-positions in the other half. More tritium might thus enter the 'methyl half' of the molecule. Alternatively, the asymmetry of labelling of the oleic acid may be due to biological loss of tritium from the 'carboxyl half' of the molecule by, for example, β -oxidation and resynthesis. Neither of these mechanisms, however, would be expected to give rise to such a large difference between the tritium contents of the two halves of the molecule. Moreover, in this case, the ratio 1.68 is reliably known, there being good agreement between the values obtained from the duplicate samples. The most reasonable conclusion to be drawn from the work as a whole, therefore, is that the stearic acid produced by the catalytic addition of tritium to elaidic acid contains the isotope asymmetrically distributed over the molecule and not confined to the 9:10-positions.

SUMMARY

1. Stearic acid labelled with tritium by catalytic addition, with a palladium-black catalyst, was fed to a goat, and the unsaturated fatty acids of the fat from two milk samples were separated by the lead-salt method. These were found to be radioactive.

2. From these unsaturated acids, after dilution with carrier oleic acid, dihydroxystearic acid was prepared and obtained in pure crystalline form. This was found to be radioactive, thus formally proving the biological conversion of stearic into oleic acid in the goat.

3. The dihydroxystearic acid from each sample was decomposed into pelargonic aldehyde and azelaic semialdehyde. Complete recovery of the tritium originally in the dihydroxystearic acid was obtained within the limits of experimental error. The conclusion is drawn that the tritium in the dihydroxystearic acid was stably bound. It was found further that 1.68 times as much tritium was contained in the pelargonic aldehyde as in the azelaic semialdehyde.

4. The two aldehydes were oxidized to the corresponding acids, thus labilizing the tritium originally attached to the C_9 and C_{10} atoms. The loss of tritium was such that about 3% of the total tritium in the dihydroxystearic acid molecule must have been attached to the C_9 atom and 12% to the C_{10} atom.

5. The significance of these findings is discussed

in the light of possible artifacts, and the conclusion is drawn that the original stearic acid was asymmetrically labelled and that only about 28% of the total tritium in the molecule was attached to the carbon atoms which were reduced.

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Note added in proof. We are indebted to Dr W. G. Duncombe for showing, by means of paper chromatography and autoradiography, that the labelled stearic acid, prepared from elaidic acid by the technique described, contains no chemical or radiochemical impurities.

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