Mode of Formation of Milk Fatty Acids from Acetate in the Goat

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In a previous paper (Popják, French & Folley, 1951) some results of an experiment carried out on a lactating goat with the aid of $CH_3{}^{14}CO_2Na$ were described. It was found that, in the origin of milk fat, synthesis within the udder from acetate played an outstanding part. This conclusion was based on a comparison of the specific activities of four crude fatty-acid fractions obtained from milk glycerides with those of the plasma fatty acids after a single injection of $CH_3{}^{14}CO_2Na$.

During the first 12 hr. of the experiment the steam-volatile acids insoluble in water (consisting mainly of C_8–C_{12} acids) had the highest specific activities, the next in order being the water-soluble steam-volatile acids (C_4–C_8 acids). These were followed by the specific activity of the non-volatile solid and liquid acids, the latter having the lowest specific activities. From 12 hr. onwards, however, the specific activity-time curve of the non-volatile solid acids gradually crossed over the specific activity-time curves of the two steam-volatile fractions so that by the 23rd hour of the experiment the specific activities of the four fractions were in the following order: non-volatile solid acids > water-insoluble steam-volatile acids > water-soluble steam-volatile acids > non-volatile liquid acids. This same order was maintained until the end of the experiment (48 hr.) (cf. Popják *et al.* 1951, Fig. 3).

The specific activity-time curves of all four fractions, after reaching their maxima 3–4 hr. after the injection of labelled acetate, decayed smoothly with an average half-life of about 4 hr. The similarity of the half-lives suggested that the different specific activities of the fractions were in some way connected with the biochemical mechanism of fatty-acid synthesis rather than with any other factor (e.g. differences in turnover time, dilution with non-isotopic blood fat). Since the specific activities of these mixtures of fatty-acid samples bore no simple relationship to the average chain length of their component acids, it was pointed out that resolution into individual acids was required in order to interpret more fully the role of acetate in milk-fat synthesis.

In this paper the results of complete fractionations of the fatty-acid samples obtained in the previously described experiment are reported together with the chemical degradations of acetic, butyric and caproic acids, in which the position of the ^{14}C-label in individual carbon atoms of the fatty-acid chain has been established.

It would have been a gargantuan task to resolve into individual acids the four crude fatty-acid fractions from twenty-two milk samples. If the specific activities of the four fractions had maintained the same order relative to one another throughout the experiment, it would have been justifiable to pool the samples from the entire experiment for the isolation of the individual acids. However, because of the change in the relative position of the specific activity-time curves at 12 hr. after the injection, it was decided to divide the acids into two main groups, (1) those obtained up to 12 hr. and (2) those between 12 and 48 hr. of the experiment. Thus during the first period the fatty acids had their highest specific activities and during the second period the specific activities of all the fractions were already declining. In addition to these two main parts of the experiment, some results obtained on two small samples of the water-soluble volatile acids, one representing 0–7 hr. and the other approximately the entire experimental period, are also presented.

It is now apparent that, although acetate is a major source of carbon for milk fatty-acid synthesis, the results strongly suggest that another precursor, which we believe to be a C_4 compound, derived from the blood, must also be involved. As a result of the participation of this second precursor of low isotope content, it has been possible to show by chemical degradations that the synthesis of fatty acids must occur by the stepwise elongation of a shorter acid at the carboxyl end with a C_2 compound and not by the simultaneous condensation of C_2 units. Some of these results have been published in a preliminary form (French, Hunter, Martin & Popják, 1951).

RESULTS

Table 1 shows the extent of fractionation achieved. All the acids, with the exception of acetic, were chromatographically pure. The acetic acid was contaminated with probably not more than 10% of butyric acid, but as the quantities of this acid were rather small (5–10 mg.) no further purification was attempted.

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Table 1 shows that there is a definite trend in the specific activities of the pure acids with increasing chain length from butyric acid upwards. During the first 12 hr. of the experiment the specific activities increased up to and including capric (decanoic) acid, whereas during the second part of the experiment the specific activities increased up to myristic (tetradecanoic) and palmitic (hexadecanoic) acids.

Table 1. *Specific activities* (1×10^{-3} μc. ^{14}C/*mg.* C) *of pure fatty acids obtained from glycerides of milk during various intervals after the intravenous injection of* 5 *mc. of* ^{14}C *as* $CH_3{}^{14}CO_2Na$ *into a lactating goat*

	Period of experiment from which fatty acids were pooled			
Fatty acid	0–7 hr.	0–48 hr.	0–12 hr.	12–48 hr.
Acetic	—	11·80	14·68	—
Butyric	18·00	7·63	11·45	0·350
Caproic (hexanoic)	28·75	9·86	21·15	0·424
Caprylic (octanoic)	—	—	28·40	0·717
Capric (decanoic)	—	—	33·75	0·943
Lauric	—	—	23·80	1·080
Myristic	—	—	10·28	1·260
Palmitic	—	—	10·25	1·260
Stearic	—	—	0·49	0·063
Oleic	—	—	0·31	0·063

This difference between the two parts of the experiment explains the crossing over of the specific activity-time curves of the steam-volatile acids and the solid non-volatile acids (Popják *et al.* 1951, Fig. 3).

The meaning of these results will be discussed below under individual acids, together with the information obtained from the chemical degradation of acetic, butyric and caproic acids, which are given in Table 2.

Acetic, propionic and valeric acids

By using standard methods of fat extraction, saponification, extraction of the unsaponifiable material from the soaps followed by steam distillation, we consistently demonstrated the presence of acetic acid among the water-soluble steam-volatile fatty acids by paper chromatography. It amounted to 1–2% of the total water-soluble steam-volatile acids. Two of these samples were isolated as the silver salt for radioactive assay. It is impossible to say at present whether the acetic acid was present originally as glyceride or combined with another fat-soluble compound. Its relationship to the metabolically active acetate used in the synthesis of the higher fatty acids is therefore not clear and its relatively low specific activity cannot be accounted for. The possibility has to be considered also that the acetic acid might have been derived partly by bacterial decomposition of milk constituents during the period between milking and extraction of the fat. The further possibility that the presence of acetate in the milk fat was an artifact due to temporary raising of the blood acetate level by the injection of $CH_3{}^{14}CO_2Na$ may be dismissed, since acetate was found in the milk at all times of the experiment up to 48 hr. after the injection. Whatever the origin of this acetate, its occurrence nevertheless served as a useful guide showing that redistribution of the label from the injected acetate did not occur in the animal (cf. Table 2) since ^{14}C was found only in the carboxyl carbon. In the subsequent discussion of fatty-acid synthesis from the injected acetate, we shall refer to 'acetate' to denote the metabolically active C_2 derivative of acetic acid. There is no evidence available from our experiment to show what this active derivative might be, nevertheless it appears justifiable to speak of the methyl and carboxyl carbon atoms of this metabolite, since from evidence discussed below it is clear that the polarity of the molecule was maintained.

In addition to acetic acid, propionic and valeric acids were also found among the water-soluble steam-volatile fatty acids by paper chromatography. No radioactivity could be detected over the spots of these acids on the paper although the activities of acetic, butyric and caproic acids were definitely measurable on the paper. The considerations as to the origin of acetic acid apply also to the origin of propionic and valeric acids. Both of these acids, however, are known to occur in the rumen and therefore may find their way into the milk via the blood. In this connexion it might be mentioned that in an as yet unpublished experiment, in collaboration with Prof. G. Peeters and Dr S. J. Folley, in which an isolated cow udder was perfused with blood containing acetate, no propionic or valeric acid was found in the milk fat.

Butyric acid

The butyric acid had a lower specific activity in both periods of the experiment than any longer acid up to and including lauric (dodecanoic); later its ^{14}C content was the lowest of all acids up to palmitic (hexadecanoic) (Table 1). The degradation of butyric acid revealed the presence of equal amounts of ^{14}C in carbon atoms 1(COOH) and 3, carbons 2 and 4 being totally inactive. This finding indicates that butyric acid has been synthesized in the udder by the condensation of two acetate units of equal isotope content, the methyl carbon of one having been linked with the carboxyl carbon of the other. It has been mentioned in the introduction that we do not consider acetate to be the only source of butyric acid in the milk. The necessity for this assumption should become clear from the information obtained by the degradation of caproic acid.

Table 2. *Specific activity* (1×10^{-3} $\mu c.$ $^{14}C/mg.$ C) *of individual carbon atoms in acetic, butyric and caproic acids from milk glycerides after intravenous injection of* 5 *mc. of* $CH_3{}^{14}CO_2Na$

Period of experiment (hr.)	Fatty acid	C atom numbers 6	5	4	3	2	1(COOH)	Average of all C atoms
0– 7	Butyric	—	—	0·00	36·00	0·00	36·00	18·00
	Caproic	0·00	36·20	0·00	36·20	0·00	96·70	28·75
0–48	Acetic	—	—	—	—	0·00	23·60	11·80
	Butyric	—	—	0·00	15·26	0·00	15·26	7·63
	Caproic	0·00	13·20	0·00	13·20	0·00	32·60	9·86
12–48	Butyric	—	—	0·00	0·700	0·00	0·700	0·350
	Caproic	0·00	0·556	0·00	0·556	0·00	1·504	0·424

Caproic (hexanoic) acid

The results of the degradation of caproic acid from the early part of the experiment (cf. Table 2) should be considered first. Only carbon atoms 1(COOH), 3 and 5 contained ^{14}C. The specific activity of the COOH carbon was about 2·5 times that of carbons 3 and 5 whose specific activities were equal, just as in carbons 1 and 3 of butyric acid. Further, the specific activities of carbons 3–6 of caproic acid were sufficiently close to the activities of the butyric acid carbons as to leave very little doubt that in fact these carbons were derived from the carbon chain of butyric acid. Therefore the synthesis of caproic acid must have occurred by the elongation of this shorter chain, at what corresponds to the carboxyl carbon of butyric acid, by the addition of acetate. It may be inferred from the similarity of the radioactivities of carbons 3–6 of caproic to those of butyric acid that the newly synthesized caproic acid has not been diluted in the udder to a significant extent by pre-acid itself or another C_4 compound which is converted into the acid. If this second explanation is the right one, the 'butyric' part of caproic acid in the milk should have a lower isotope content than the 'acetic' part at all times of the experiment.

The degradation of caproic acid from the later part of the experiment showed that even here the specific activity of its 'acetic' part was about 2·5 times greater than that of its 'butyric' part. Therefore the contribution of the first mechanism to explain the observed differences must be negligible. There is a good deal of evidence to show that the pools of the volatile acids up to capric (decanoic) (see later) within the cells of the mammary gland are very small.

We cannot avoid, therefore, the conclusion that the butyric acid synthesized from acetate in the udder is continuously being diluted about 2·5 times by a non-isotopic C_4 compound. Thus butyrate in the udder seems to have a dual origin which may be represented as follows:

$$\left.\begin{array}{r} 2CH_3C^{**}OOH \rightarrow CH_3C^{**}H_2CH_2C^{**}OOH \\ C_4 \text{ compound (non-isotopic} \rightarrow CH_3CH_2CH_2COOH \end{array}\right\} = CH_3C^{*}H_2CH_2C^{*}OOH.$$

The synthesis of caproic acid may then be visualized by

$$CH_3C^{*}H_2CH_2C^{*}OOH + CH_3C^{**}OOH \rightarrow CH_3C^{*}H_2CH_2C^{*}H_2CH_2C^{**}OOH.$$

existing non-isotopic caproic acid, i.e. the metabolic 'pool' of caproic acid in the gland must be very small.

The difference between the ^{14}C content of the 'acetic' and 'butyric' parts of caproic acid would admit at first sight two possible explanations. The first one is that the butyric acid synthesized from acetate was diluted 2·5 times by pre-existing non-isotopic butyrate. This would imply a fairly large pool of this acid. If this were the case, then during the second part of the experiment, when the specific activity of acetate must have been declining rapidly, the 'acetic' part of caproic acid should have a lower specific activity than the 'butyric' part. The second possible explanation is that the butyric acid synthesized from acetate is being continuously diluted 2·5 times by a non-isotopic C_4 compound derived from the blood. This C_4 compound might be butyric

Caprylic (octanoic) and capric (decanoic) acids

The increase of specific activity with increasing chain length is also observed in the case of caprylic (octanoic) and capric (decanoic) acids at all times of the experiment (cf. Table 1). These acids have not been degraded yet, but the results are consistent with the view that they are being synthesized by the same mechanism as outlined for caproic acid, i.e. caprylic by the elongation of caproic and capric by the lengthening of caprylic acid. Also the pools of these acids presumably must be small, otherwise the newly synthesized acids of high ^{14}C content would have been diluted by pre-existing non-isotopic acids and the increasing specific activities with increasing chain lengths could not have been observed during the entire experimental period.

Lauric (dodecanoic) acid

During the first 12 hr. lauric (dodecanoic) acid had a lower activity than capric or caprylic. Later, however, its activity became higher than that of capric. That lauric acid is synthesized by the same mechanism as for the shorter acids appears probable from the position which it occupies during the second period. However, since in the earlier period lauric acid does not attain as high activity as capric acid, its turnover time must be more prolonged than that of the shorter acids. It is probable that the cells contain an appreciable pool of lauric acid.

Myristic (tetradecanoic) and palmitic (hexadecanoic) acids

These acids have equal activities in both the early and late periods, but relative to the other acids they change from a low level to become eventually the most active of the acids. The arguments adduced in the case of lauric acid could apply to these two also, since it is likely that these acids, which are regular components of tissue fat, are present in considerable quantity within the cells. The equal activity of myristic and palmitic acids may suggest that the two acids are in equilibrium, i.e. that palmitic is broken down to myristic as well as being formed from it.

Stearic (octadecanoic) and oleic (octadec-9-enoic) acids

The very low relative activity of these acids (Table 1) seems to suggest that they have a different mode of origin, being derived largely from preformed molecules in the blood, or synthesized chiefly from a precursor other than acetate. However, it is possible that relatively large pools of these acids in the cells play a part in producing the observed low activities, for by the later part of the experiment a relative increase in these activities has occurred.

In the first period stearic acid was slightly more active than oleic, but later the activities became equal. This suggests a possible equilibrium between the two acids.

DISCUSSION

Certain general principles of fat metabolism and milk secretion are implicit in the present results. It has been stated in the previous paper (Popják *et al.* 1951) that the formation of milk-fat results from the endogenous metabolism of the gland cells and that fatty acids taken up from the blood probably are broken down more or less to C_2 units which become mixed with exogenous acetate. From the results presented above the further points which have emerged are: (1) the stepwise elongation of fatty-acid chains at the carboxyl end by the addition of a C_2 unit derived from acetate; (2) the participation of a C_4 compound in the formation of butyric acid; and (3) the small size of the metabolic pools of the short-chain acids (butyric-capric) in the mammary gland cells.

It is possible on the basis of these results, to suggest the main features of the synthetic process, and to consider whether this is peculiar to the mammary gland or whether it is a general process in mammalian tissue. The question is, if body fat and milk fat are synthesized from the same precursor(s), why do the products differ in composition?

Acetate is known to be a building-stone available for all positions of the fatty-acid chain (see Bloch, 1947, 1948). The interconversion of palmitic and stearic acids by the addition or removal of C_2 units was demonstrated by the experiments of Schoenheimer & Rittenberg (1937) and of Stetten & Schoenheimer (1940) with deuterium-labelled fatty acids. Zabin (1950), in Dr K. Bloch's laboratory, has recently shown in rats, under conditions in which fatty-acid synthesis is depressed, that after administration of $CH_3{}^{14}CO_2Na$, almost all the radioactivity of stearic acid could be accounted for by the radioactivity of the carboxyl carbon. The elongation of lauric (dodecanoic) and myristic (tetradecanoic) acids to palmitic and stearic acids has been shown to occur also (Klem, 1943). Thus the elongation of fatty acids by the addition of a C_2 unit is well established for the longer acids which are constituents of body fats.

The short-chain acids, which were shown in this investigation to be formed likewise by the lengthening of a still shorter acid by the addition of an acetate molecule and which might be expected as intermediates in the total synthesis of the longer ones, are, however, thought to be absent from cells of internal organs. Moreover, Rittenberg, Schoenheimer & Evans (1937) failed to find any evidence that deuterium-labelled butyric and caproic acids could be used for synthesis of higher acids. Therefore, a scheme of simple reversal of β-oxidation as a general mechanism of fatty-acid synthesis in the tissues (with the exception of the mammary gland as presented here) could not be supported by experimental evidence. An alternative hypothesis of simultaneous condensation of C_2 units, giving long-chain acids directly, has been considered (e.g. by Peters & Van Slyke, 1946). It should, however, be pointed out that the failure of the deuterium-labelled butyric and caproic acids to give rise to fatty acids labelled with deuterium does not constitute an absolute proof against the possibility of the carbon chain of these acids having been utilized in the synthesis of higher acids.

The question of the general applicability of the mechanism of fatty-acid synthesis as revealed in the mammary gland hinges on the relationship which exists between the fatty acids found in the milk triglycerides and the various precursors which are

undergoing metabolism within the cells. This relationship is a matter of conjecture as far as the chemical form in which the precursors react is concerned, but it can be concluded from our results that the carbon chains take part in the synthesis in the manner outlined above. The glyceride fatty acids of the milk are perhaps best regarded as metabolic products which have become stabilized by esterification with glycerol and secretion into the milk ducts. The process of secretion might be regarded as one which intervenes with great rapidity between the formation of these acids and their further metabolism in the cell, so as to trap in the milk glycerides substances which would otherwise be metabolized so rapidly as never to accumulate to any great extent. According to this argument then the difference between milk- and body-fat formation might be more apparent than real; the process of rapid secretion by the mammary gland having only the effect of 'preserving'—rather conveniently—the intermediary products of synthesis.

Stadtman, Stadtman & Barker (1949) have shown by the use of ^{14}C that *Clostridium kluyveri* synthesizes caproic acid by the addition of a C_2 unit derived from ethanol to the carboxyl carbon of butyric acid. Stadtman & Barker (1949*a–e*) have studied the reactions involved in this synthesis in considerable detail by means of a cell-free enzyme system. The anaerobic synthesis of fatty acids by this organism is essentially an energy-yielding mechanism, and it is not clear at present how far it is comparable to fat synthesis in animals. It is quite probable, however, that many of the intermediate steps are similar.

Smith & Dastur (1938) were the first to suggest that the short-chain fatty acids in the milk might be intermediates in the origin of the higher ones. Similar views, based on the *in vitro* utilization of specific substrates by mammary tissue with high respiratory quotients, have been expressed by Folley & French (1948, 1950). The results described in the present paper are, however, the first direct evidence on the question.

The participation of a C_4 precursor in the formation of milk fatty acids may be related to information available on the metabolism of lactating ruminants. Butyric acid itself is absorbed from the rumen, but its concentration in the peripheral blood is low (McClymont, 1949). The ketone bodies, however, circulate in considerable amount, and Shaw & Knodt (1941) have demonstrated the uptake of β-hydroxybutyrate by the udder of the lactating cow. They estimated the quantity taken up to be equivalent to all the milk acids up to and including tetradecanoic, i.e. more than one-third of the total acids. If this substance is used for milk-fat synthesis, it seems probable from our results, that it contributes to the formation of butyric acid and therefore also to the equivalent parts of the carbon chain of all acids up to palmitic. The quantitative extent of its contribution may thus be considerable, although it seems likely that some of it would be metabolized via a C_2 unit and thus become merged with the metabolic acetate.

The conception of milk-fat formation advanced here is somewhat at variance with that of Hilditch (1947) whose views have recently been re-affirmed (Achaya & Hilditch, 1950). It hardly need be stressed here that our results do not admit the possibility that the short-chain volatile acids in the milk were derived directly from the degradation of oleic acid, which according to Hilditch should be their primary source. We have not been able to isolate from our small material Δ^9 unsaturated acids of shorter chain length than oleic, which according to Hilditch should also be derived from oleic acid degradation, and therefore cannot offer direct evidence on their mode of origin. It should, however, be pointed out that the derivation of oleic acid from stearic and of palmitoleic by the dehydrogenation of palmitic acid have been proved (Stetten & Schoenheimer, 1940). It does not seem at all improbable, therefore, that tetradec-9-enoic, dodec-9-enoic and dec-9-enoic acids originate also from the dehydrogenation of the corresponding saturated acids.

EXPERIMENTAL

The crude fatty-acid fractions as described in the earlier publication (Popják *et al.* 1951) formed the starting material of the fractionations. These fractions were: (1) water-soluble and (2) water-insoluble steam-volatile acids; (3) non-volatile solid and (4) non-volatile liquid acids.

Methods of fractionation

Water-soluble steam-volatile fatty acids. These were resolved into individual acids by azeotropic distillation as described by Schicktanz, Steele & Blaisdell (1940). Benzene, toluene and xylene were used successively to separate acetic, butyric and caproic acids. The residual fatty acid, after the distillation with xylene was completed, was pure caprylic (octanoic) acid.

Water-insoluble steam-volatile fatty acids. These were separated by fractional distillation of the free acids at 6 mm. of Hg pressure in the distillation apparatus described by Schoenheimer & Rittenberg (1937). The capacity of our still was 5 g. Fractionation by partition chromatography (Howard & Martin, 1950) was also employed.

Non-volatile solid and liquid fatty acids. These were converted into methyl esters either by refluxing with dry methanol in the presence of H_2SO_4 or by treatment of the acids with diazomethane. The methyl esters were then distilled at 0·5 mm. of Hg pressure in the apparatus used for the fractionation of the water-insoluble volatile acids.

Identification of the fatty acids and criteria of their purity

Mostly chromatographic methods were used for this purpose: paper chromatography for acids up to and including caproic (hexanoic) (Martin, 1950) and partition

chromatography for acids of longer chain length (Howard & Martin, 1950). In addition, in the case of acids of C_{14} to C_{18} chain length (of which larger amounts were available), an essential criterion of purity was, before testing by chromatography, that the specific activity of the fraction should remain constant after redistillation.

The fatty acids, the specific activities and degradations of which are reported were pure chromatographically unless specified.

Methods of chemical degradations of fatty acids

Acetic acid was degraded by the pyrolysis of the Li salt *in vacuo* at 380°, essentially as described for barium acetate (see Calvin, Heidelberger, Reid, Tolbert & Yankwich, 1949). The acetone formed during the pyrolysis was collected in a trap cooled with liquid air and subjected to the iodoform reaction. The carbon atom of CHI_3 corresponds to the methyl carbon of acetate. The Li_2CO_3, the other product of pyrolysis, the carbon atom of which is derived from the carboxyl carbon of acetate, was converted into $BaCO_3$ and used for the assay of ^{14}C.

Butyric acid was degraded by oxidation of ammonium butyrate with H_2O_2 as described by Wood, Brown, Werkman & Stuckwisch (1944). This degradation can be carried out conveniently on 200 mg. of butyric acid. The only product of the oxidation, useful for the identification of the labels in the carbon chain of butyric acid, is acetone, the carbonyl carbon of which is derived from carbon atom 3 and the methyl carbons from carbons 2 and 4 of butyric acid. The following equations represent the main reactions in this degradation:

$$\overset{4}{CH_3}.\overset{3}{CH_2}.\overset{2}{CH_2}.\overset{1}{COOH} \xrightarrow{H_2O_2} \overset{4}{CH_3}.\overset{3}{CO}.\overset{2}{CH_3} + CO_2 \quad (1)$$

$$\overset{4}{CH_3}.\overset{3}{CO}.\overset{2}{CH_3} \xrightarrow{NaOI} \overset{2,4}{CHI_3} + \overset{2,4}{CH_3}.\overset{3}{COOH} \quad (2)$$

Unfortunately the CO_2 evolved in reaction (1) is almost equally derived from all four carbon atoms of butyric acid.

The acetic acid, resulting from the iodoform reaction (2), was separated from the reaction mixture by steam distillation after removal of the excess I_2 with Ag_2SO_4. It was finally obtained as the Ag salt and its radioactivity determined. The radioactivity of the carboxyl carbon of butyric acid was obtained by difference.

Caproic acid was degraded by a new method (Hunter & Popják, 1951), according to which carbon atoms 1(COOH) and 2 of caproic acid are split off as acetic acid and carbons 3, 4, 5 and 6 give butyric acid. The steps involved in this degradation are: α-bromination of the acid chloride, conversion into ethyl or higher ester (best yield with *neo*pentyl ester), dehydrobromination of the α-bromo ester, yielding the ester of hex-2-enoic acid. The fusion of this ester with KOH at 330° results in the fission of the double bond and gives acetic and butyric acids which were degraded further by the methods already mentioned.

Assay of ^{14}C was carried out mostly on solid samples of 'infinite' thickness >25 mg./sq.cm. as described previously (Popják, 1950), but in a few instances the measurements were made on samples of 1–3 mg./sq.cm. thickness. The radioactive counts were all corrected to infinite thickness by the use of an experimentally determined self-absorption curve. The specific activities expressed in this paper as $\mu c.$ ^{14}C/mg. C may be converted into counts/min. by the following factors: a substance containing 1×10^{-3} $\mu c.$/mg. gave 1300 counts/min. in samples of 1 sq.cm. area or 2470 counts/min. in samples of 2 sq.cm. area in infinite thickness. Because of the high activity of our material the assays could easily be carried out to an accuracy of ±1% or better.

SUMMARY

1. Milk fatty acids obtained from a lactating goat after the injection of $CH_3{}^{14}CO_2Na$ have been resolved into individual acids and their ^{14}C content determined. All the even numbered saturated acids from C_2 to C_{18} and also oleic acid have been isolated. Traces of propionic and valeric acids were also identified by paper chromatography.

2. Chemical degradations of acetic, butyric and caproic acids have been carried out. Only the carboxyl carbon of acetic acid contained ^{14}C.

3. Butyric acid is synthesized from two acetate molecules in such a way that the methyl carbon of one becomes linked to the carboxyl carbon of the other. It is inferred that only about 40% of the butyric acid is synthesized from acetate, the other 60% being derived from a non-isotopic C_4 compound, possibly β-hydroxybutyric acid. Caproic acid is synthesized by the elongation of the butyric acid chain at the carboxyl end by the addition of acetate.

4. The metabolic 'pools' of the short chain acids (up to C_{10}) in the mammary gland cells appear to be very small.

5. The results are consistent with the view that all the milk fatty acids up to and including palmitic are formed by the stepwise elongation of a shorter acid by the addition of a C_2 compound derived from acetate. Stearic and oleic acids might be derived mainly from the blood or from a precursor other than acetate.

6. The possible relationship between the mechanism of fatty-acid synthesis in the mammary gland and in other mammalian tissues is discussed.

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Formation of Cellulose by Certain Species of *Acetobacter*

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Brown (1886) showed that the surface membranes (often designated pellicles) formed by *Bacterium xylinum* when grown on suitable carbohydrate media consist of cellulose, and this material has been examined by Hibbert & Barsha (1931), Tarr & Hibbert (1934), Khouvine, Champetier & Sutra (1932), Sisson (1936), Franz & Schiebold (1943), Frey-Wyssling & Mühlethaler (1946) and by Hestrin, Ashner & Mager (1947). *Bact. xylinum* is now known as *Acetobacter xylinum*. Frey-Wyssling & Mühlethaler (1946) have shown also that the pellicle formed by *Acetobacter xylinoides* Henneberg from glucose consists of cellulose, but the surface covers which are formed from certain substrates by *A. pasteurianum* Hansen, *A. kützingianum* Hansen and *A. acetigenum* Henneberg, have not hitherto been subjected to detailed examination.

In this laboratory, while studying bacteria from an East African vinegar brewery, Dr D. Kulka isolated a strain of *A. acetigenum* (hereafter referred to as *A. acetigenum* E.A.) which produced very thick pellicles on glucose media. Chemical examination showed the pellicle material consisted of cellulose and this was confirmed by X-ray examination. The results of the X-ray examination are reserved for a future communication.

The production of cellulose by this organism, by *A. acetigenum* (type culture), by *A. pasteurianum* and by *A. kützingianum*, is now described.

EXPERIMENTAL AND RESULTS

Cultural conditions governing the formation of cellulose

Pellicles which gave reactions for cellulose were formed by *A. acetigenum* E.A. from soluble starch, dextrin, sucrose, maltose, lactose, glucose, fructose, galactose, α-methyl-D-glucoside, β-methyl-D-glucoside, salicin, arabinose, xylose, rhamnose, mannitol, erythritol, glycerol, and ethylene glycol, respectively. Tarr & Hibbert (1931) reported the inability of *A. xylinum* to form cellulose from the α- and β-methyl-D-glucosides, three pentoses, erythritol and ethylene glycol, respectively. They did not ascertain whether their organism could utilize salicin.

A. acetigenum E.A., *A. acetigenum* (Henneberg) Bergey *et al.*, National Collection of Type Cultures (N.C.T.C.) 5346, *A. pasteurianum* (an atypical strain) N.C.T.C. 613 and *A. kützingianum* (Hansen) Bergey *et al.*, N.C.T.C. 3924, produced cellulose in a medium (Henneberg, 1926) consisting of $(NH_4)_2SO_4$, 3 g.; KH_2PO_4, 3 g.; $MgSO_4.7H_2O$, 2 g.; glucose, 20 g. and ethanol (added after sterilization) 25 g., together with water to 1 l. Equally vigorous or more profuse growth could be obtained by substituting corn-steep liquor (C.S.L.), 1–2% (v/v), or Witte's peptone 0·2% (w/v) in place of or in addition to the ethanol. The same adjuncts also stimulated growth in yeast-water (made by boiling 75 g. of brewery pressed yeast for 30 min. in 1 l. of water) to which glucose or another suitable carbohydrate had been added. In some cases addition of sterile $CaCO_3$ (precipitated chalk) improved the yield of cellulose. In media containing