XXXVII. THE BIOCHEMICAL SYNTHESIS OF THE FATTY ACIDS.

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The methods by which fat is formed in the living organism remain at present completely unknown to us. Even the chemical reactions by which fatty acids are built up, comparatively simple though these must be, have hitherto found no satisfactory explanation. The one fact which appears to be completely established, by a large mass of experimental evidence, is that the carbohydrate of the food may be converted into fat inside the living organism, although neither the place where this change takes place nor the method by which it is accomplished is known with any degree of certainty.

The evidence as to the formation of fat from protein is less convincing, but it is at any rate possible that a conversion of protein into fat may also take place.

But little may be learnt from an attempt to correlate the composition of the fat stored up in the organism with the nature of the food supplied. There is abundant evidence that fatty acids taken in the food may be merely stored up unchanged in the body. The glycerides of palmitic, stearic and oleic acids are the constituents which most generally occur. Acids belonging to more highly unsaturated series than oleic acid have been demonstrated but these are more probably connected with further changes in the building up of fat into complex molecules, possibly of the nature of lecithin, than with the synthesis of the fatty acids themselves.

Two of the most prolific factories of fat are perhaps to be found (1) in plants, in such nuts as that of the cocoa-nut tree (*Cocos Nucifera*), where an abundant transformation of carbohydrate into fat must take place, and (2) in animals in the active mammary gland.

In both these instances, where a comparatively rapid conversion of

carbohydrate into fat is probably taking place the resultant fats are characterised by the presence of considerable quantities of the lower fatty acids. In cocoa-nut oil, the acids containing the even numbers of carbon atoms from six to eighteen, in butter from four to twenty, have been described. In these acids the carbon atoms are linked in straight chains and there is no evidence that any acid with a branched structure exists.

The question now arises whether the normal fatty acids present in butter are products of synthesis or of degradation. Knoop [1904] and Dakin [1908, 1909] have shown that the fatty acids are broken down by oxidation of the β-carbon atom; all the lower fatty acids present in butter may therefore be derived by oxidation from the arachidic or stearic acids present.

Some evidence on this point may be obtained from agricultural experiments; the problem has been directly investigated in an attempt to determine the reason of the variations which occur in the proportion of volatile fatty acids present in butter fat. Swaving (1906) carried out feeding experiments in the North of Holland to determine the cause of the low percentage of volatile soluble acids. Van der Zande and Siegfeld showed that a diet rich in carbohydrate, e.g. turnips, increased the proportion of the lower fatty acids and more recently Siegfeld [1907] and Amberger [1907] have shown that the increase is more especially in the insoluble volatile acids (i.e. caprylic, capric and lauric). Amberger, in a series of experiments carried out on the same set of cows showed that whereas food rich in protein such as malt germs diminishes the proportion of lower fatty acids, food rich in carbohydrate such as turnips increases this proportion. If the percentage of the lower fatty acids increases with the amount of the carbohydrate in the food, it would appear more probable that they exist as intermediate synthetic products on their way to the higher fatty acids, than as degradation products. Such evidence as exists is therefore in favour of a synthesis in which all fatty acids containing even numbers of carbon atoms from four to twenty linked together in straight chains are formed from carbohydrate in some way through the agency of the mammary gland.

Previous Hypotheses as to the Nature of the Reactions by which Fatty Acids are formed from Carbohydrate in the Animal Organism.

Emil Fischer suggested that stearic and oleic acids are formed by the condensation of three hexose molecules or of six triose (glycerose) molecules in such a way that a straight chain containing 18 carbon atoms

is formed. From this, by further processes of oxidation and reduction, stearic and oleic acids are formed. Palmitic acid with its chain of 16 carbon atoms would be compounded from two pentose and one hexose molecules. Glucose, gluconic and glucuronic acids are suggested as the precursors of the pentose molecules. In favour of this hypothesis it is difficult to find any evidence either of a chemical or biological nature. Against it the following considerations may be urged:

(*a*) No laboratory method is known by which two hexose molecules may be made to condense in such a way that a straight chain of twelve carbon atoms is produced.

(*b*) Pentoses are known to exist in the organism in combination in the nucleoproteins but there is no indication that the pentoses are in any way connected with the formation of fat or with normal carbohydrate metabolism.

(*c*) If it be granted that the fatty acids of butter are products formed synthetically from carbohydrate, the hypothesis presents insuperable difficulties. No combination of hexose and pentose molecules will produce myristic acid ($C_{14}H_{28}O_2$) by direct addition, yet this acid occurs commonly in fats, e.g. lard, butter, cod-liver oil and many vegetable fats. The existence of an intermediate tetrose sugar would have to be assumed as a normal constituent.

3 hexose molecules	give	stearic acid.
2 pentose and one hexose molecules	,,	palmitic acid.
2 pentose and one tetrose ,,	,,	myristic acid.
2 hexose molecules	,,	lauric acid.
2 pentose ,,	,,	capric acid.
2 tetrose ,,	,,	caprylic acid.

There is no evidence of the formation of a tetrose molecule in the organism and it is exceedingly unlikely that a regular series of fatty acids should be formed in this way.

(*d*) In the case of stearic acid the reduction of seventeen hydroxyl groups would be assumed.

This hypothesis has therefore no evidence in its favour and involves reactions which are not analogous with any of those known to us. It does not therefore furnish us with a satisfactory explanation of the problem under consideration.

The second hypothesis, which is perhaps the more generally accepted, is that the fatty acids are built up by repeated condensations of a compound containing two carbon atoms. This was first suggested by Nencki and afterwards developed by Magnus-Levy [1902], Leathes and others who regarded acetaldehyde as the substance from which by a series of aldol condensations

the straight chains containing even numbers of carbon atoms were formed. The reactions involved would be represented by the following equations:

$$\text{(1)}\quad CH_3.CHO + CH_3.CHO = CH_3.CHOH.CH_2.CHO$$
$$\text{(2)}\quad CH_3.CHOH.CH_2.CHO = CH_3.CH_2.CH_2.COOH + H_2O$$
$$+\begin{matrix}H\\H\end{matrix}\!\!>\!O.$$

If the aldol on the other hand were reduced to butyl aldehyde, it would be available again to take part in a similar condensation:

$$CH_3.CHOH.CH_2.CHO + H_2 = CH_3.CH_2.CH_2.CHO + H_2O$$
$$CH_3.CH_2.CH_2.CHO + CH_3.CHO = CH_3.CH_2.CH_2.CHOH.CH_2.CHO.$$

a normal six-carbon-atom chain being thus produced.

In favour of this hypothesis, it may be urged:

(*a*) It does account for the production only of those fatty acids containing even numbers of carbon atoms, since only multiples of two will exist.

(*b*) Hoppe-Seyler showed that by the action of caustic alkali on lactic acid at from 200°–300°, acetic, butyric and caproic acids were formed. Pasteur had previously shown that butyric and caproic acids were formed by the bacterial fermentation of sugar. Acetic aldehyde may be obtained from lactic acid and may therefore be a degradation product of sugar.

On the other hand it is open to the following criticisms:

(*a*) Lieben [1883, 1901] and his pupils have shown that when the higher aldehydes condense with acetaldehyde under the influence of dilute alkalies, the resulting aldehydes possess a branched and not an open-chain structure:

$$CH_3.CH_2.CH_2.CHO + CHO.CH_3 \longrightarrow CH_3.CH_2.\underset{\substack{|\\ CHOH\\ |\\ CH_3.}}{CH}.CHO$$

It has since been shown that both aldol and crotonaldehyde will undergo auto-condensation with the formation of a normal eight-carbon-atom chain [Raper, 1907; Smedley, 1911]; but the difficulty of adding on acetic aldehyde to a higher aldehyde so as to build up chains increasing by the addition of two carbon atoms has not been surmounted. One must therefore assume that the condensation of aldehydes in the body does not take place in the same manner as it does when brought about by the action of condensing agents in the laboratory.

(*b*) No free aldehydes other than the sugars have been detected in the body. If present in quantity they would probably be injurious to the life of the cell. Parnas [1910] has shown that an enzyme is present in the liver by which free aldehydes are at once removed.

(*c*) There is no biological evidence that acetaldehyde is formed as an intermediate substance in the body metabolism.

The aldol condensation does not therefore furnish us with a satisfactory analogy for the method by which the fatty acids are built up.

A survey of the general methods of producing fatty acids in the laboratory shows that the most satisfactory method by which fatty acids may be built up by increments of two carbon atoms is by means of Reformatski's reaction in which aldehydes are condensed with bromoacetic ester in the presence of zinc;

$$R.CHO + BrCH_2.COOEt + Zn + H_2O = R.CHOH.CH_2.COOEt + Zn\begin{matrix}OH\\Br\end{matrix}.$$

As however neither zinc nor bromoacetic ester occurs in the body, this does not furnish us with any helpful analogy for biochemical synthesis.

The Degradation-products of Carbohydrates: their suitability as Units in the biochemical synthesis of Fatty Acids.

But little is known as to the manner in which carbohydrate breaks down within the body. It has been repeatedly established that when a solution of glucose in Ringer's fluid is perfused through the isolated heart sugar disappears [Locke and Rosenheim, 1907; MacLean and Smedley, 1913]. This is the only instance in which it has been established beyond the region of controversy that sugar disappears when subjected to the action of an isolated organ. But even here the decomposition products of the sugar molecules are unknown. The controversy as to whether glycogen is a storage product or a stage in the normal metabolism of sugar throws little light on the problem under consideration. The discussion as to whether glucose is the source of the lactic acid in the animal organism has more bearing on the subject of fat formation. Embden has shown that the transfusion of blood rich in sugar through a glycogen-free liver resulted in the abundant formation of lactic acid: blood poor in sugar similarly transfused gave rise to lactic acid in inconsiderable amount. The formation of lactic acid from carbohydrate is also indicated by the experiments of Mandel and Lusk on phlorizin diabetes. It seems probable that both carbohydrate and protein may give rise to lactic acid in the body. The occurrence of lactic acid as a possible cleavage product of carbohydrate suggests that the breaking down of sugar takes place in such a way as to give rise to compounds containing three carbon atoms. Lactic acid itself is not a very reactive substance nor does

it appear a hopeful starting material for the synthesis of fatty acids. It is however closely related to pyruvic acid.

$CH_3.CHOH.COOH$	$CH_3.CO.COOH$
Lactic Acid	Pyruvic Acid

There is also evidence that pyruvic acid itself is probably of considerable importance in animal metabolism. It has been demonstrated that a close connection exists in the organism between the *a*-amino- and the *a*-keto-acids. Embden and Schmitz [1910, 1912] have shown that if a solution of ammonium pyruvate be perfused through a liver, alanine is formed. Fellner [1912] further showed that if a liver rich in glycogen be perfused with blood containing ammonia, alanine is formed, and from a consideration of Embden's experiments pyruvic acid is indicated as the intermediate substance. Neubauer and Knoop and Kertess [1911] have also suggested the formation of alanine from pyruvic acid in the body.

Knoop [1910] and Knoop and Kertess [1911] have shown that if γ-phenyl-α-amino-butyric acid be fed to a dog, a considerable proportion of the acid appears in the urine as the acetyl derivative; the same phenomenon was observed by Neubauer and Warburg [1910] in their perfusion experiments. There is some reason to believe that the acetylating agent may be pyruvic acid, since de Jong [1900, 1904] showed that ammonium carbonate and pyruvic acid react with formation of acetyl-alanine. It seems therefore probable that pyruvic acid may be an intermediate substance formed in the body from carbohydrate.

Pyruvic acid is a reactive substance, readily losing carbon dioxide under the influence of oxidising agents and forming acetic acid. A study therefore of its chemical properties and of its powers of condensation seemed of especial interest.

The condensation of Pyruvic Acid with Fatty Aldehydes and the oxidation of the products formed.

It had already been shown that if anhydrous hydrochloric acid be passed into a mixture of benzaldehyde and pyruvic acid, cinnamoyl-formic acid results [Erlenmeyer, 1901];

$$C_6H_5.CHO + CH_3.CO.COOH = C_6H_5.CH:CH.CO.COOH + H_2O.$$

Later both benzaldehyde and cinnamyl aldehyde were condensed with pyruvic acid by adding a small amount of 10% caustic soda to the mixture [Erlenmeyer, 1903].

In order to make use as far as possible only of reagents which may be considered to bring about reactions somewhat similar to those brought about

by enzymes within the body, the condensation of the fatty aldehydes with pyruvic acid was attempted in very dilute alkaline solution. The intermediate unsaturated α-keto acid which was expected to result was not isolated, but the product was at once oxidised by silver oxide in alkaline solution or by hydrogen peroxide in neutral solution.

The behaviour of crotonaldehyde was first investigated.

Experimental.

Condensation of crotonaldehyde with pyruvic acid and oxidation of the product formed.

5 grams pyruvic acid, 5 grams crotonaldehyde, 75 cc. n. NaOH and 1 litre of water were added together and left for three days at the room temperature, the solution being approximately 1/50 normal. The liquid became deep yellow but no insoluble oil separated as in the condensation of crotonaldehyde alone. The solution was neutralised by the addition of 12·5 cc. n. H_2SO_4 and steam distilled to remove any free aldehyde.

Oxidation of Reaction-product.

Silver oxide was precipitated from 30 grams silver nitrate and added to the solution of the condensation product of crotonaldehyde with pyruvic acid after it had been steam distilled. 200 cc. of a 1/3 normal solution of baryta were gradually added and the whole allowed to stand over night. Next morning the silver oxide had been largely converted to silver. The precipitate was filtered off, washed and concentrated under reduced pressure until 50 cc. remained. In order to convert any hydroxy-acid that might conceivably be present to the corresponding unsaturated acid, 10 grams of baryta were added and the mixture boiled for 30 minutes.

Excess of sulphuric acid was then added and the whole steam-distilled. 1500 cc. of the steam distillate required 28·5 cc. normal potash for neutralisation. The neutral distillate was evaporated almost to dryness and to the potassium salt so obtained, 10 % H_2SO_4 was added. Crystals separated which melted at 132° after once recrystallising from dilute alcohol. The melting point was unchanged on mixing with a specimen of sorbic acid prepared by the condensation of crotonaldehyde and bromoacetic ester, and hydrolysis of the ester formed. The crystals were therefore satisfactorily identified as sorbic acid.

In subsequent experiments the oxidation of the neutral condensation product was carried out by means of hydrogen peroxide. An amount of hydrogen peroxide exactly equivalent to the pyruvic acid originally taken

was used and the neutral mixture of condensation product and peroxide left to stand over night at the ordinary temperature; the product was concentrated under reduced pressure and steam distilled as before, and the final product consisted of a mixture of acetic and sorbic acids, the yield being somewhat improved by this means. From 5 grams of crotonaldehyde 0·5 g. sorbic acid was thus obtained.

The reaction must therefore have proceeded as follows:

$$CH_3.CH:CH.CHO + CH_3.CO.COOH = CH_3.CH:CH.CH:CH.CO.COOH$$
$$CH_3.CH:CH.CH:CH.CO.COOH + H_2O_2 = CH_3.CH:CH.CH:CH.COOH + H_2O + CO_2$$

Sorbic Acid.

The condensation of Butyl Aldehyde with Pyruvic Acid.

10 grams of butyl aldehyde, 10 grams of pyruvic acid and 150 cc. normal potash were shaken up with 2 litres of water and at the end of 12 days the mixture was neutralised and concentrated under diminished pressure. To the concentrated residue silver oxide from 43 grams of silver nitrate and 200 cc. n/3 baryta were added. After standing over night, the silver precipitate was filtered off and the filtrate concentrated to 250 cc., 50 grams of baryta added and boiled for 30 minutes. The whole was then acidified with dilute sulphuric acid and distilled in steam. 1500 cc. of distillate were neutralised by 59·2 cc. normal potash and evaporated to dryness. The residue was acidified and extracted with ether. After evaporating off the ether, the residue was distilled under a pressure of 20 mm. About 3 grams boiling from 130°–140° were obtained. The liquid rapidly decolourised bromine water and gave on analysis the numbers required for the compound $C_6H_{10}O_2$.

0·1409 g.; 0·3262 g. CO_2; 0·1134 g. H_2O.
C 63·14 % H 8·94 %.
Calc. for $C_6H_{10}O_2$ C 63·15 % H 8·77 %.

In subsequent experiments, the product similarly prepared appeared to consist of a mixture of octylenic acid (probably obtained by the self-condensation of the butyl aldehyde) and of hexylenic acid obtained from butyl aldehyde and pyruvic acid. The difficulty of separating these in a small quantity of a liquid mixture is considerable.

In another experiment where hydrogen peroxide was used as the oxidising agent as described under crotonaldehyde, the product obtained distilled under reduced pressure (15 to 20 mm.) from 120°–128° and gave on analysis the following numbers.

0·1220 g.; 0·2858 g. CO_2: 0·1074 g. H_2O.
C 63·85 %: H 9·75 %.

In investigating the condensation of iso-valeraldehyde and oenanthol with pyruvic acid, chiefly the products of condensation of the aldehydes with themselves were isolated. From the condensation product of iso-valeraldehyde and pyruvic acid, a small amount of the barium salt of an acid was obtained, the percentage of barium in which agreed with that required for the barium salt of the corresponding unsaturated keto-acid.

Condensation of these higher fatty aldehydes with pyruvic acid under varying conditions is now being investigated. The condensation of croton and butyl aldehydes with pyruvic acid and the oxidation of the product formed with hydrogen peroxide in neutral solution furnishes a method by which an unsaturated fatty acid may be built up containing two more carbon atoms than the aldehyde from which it is derived. These condensations have also been investigated under similar conditions in the aromatic series [Smedley and Lubrzynska, 1913].

Conclusions.

The hypothesis now brought forward [Smedley, 1912] suggests that pyruvic acid, formed in the body as a decomposition product of carbohydrate, is the starting-point for the synthesis of the fatty acids. The stages which are assumed to occur are represented by the following equations:

(1) $CH_3.CO.COOH = CH_3.CHO + CO_2$.
Pyruvic Acid → Acetaldehyde.

(2) $CH_3.CHO + CH_3.CO.COOH = CH_3.CHOH.CH_2.CO.COOH$
$CH_3.CH:CH.CO.COOH + H_2O$.
Pentylenic α-keto-acid.

(3a) $CH_3.CH:CH.CO.COOH + O = CH_3.CH:CH.COOH + CO_2$.

(4a) $CH_3.CH:CH.COOH + 2H = CH_3.CH_2.CH_2.COOH$.
Butyric Acid.

(3b) $CH_3.CH:CH.CO.COOH = CH_3.CH:CH.CHO + CO_2$.
Pentylenic α-keto acid.

(4b) $CH_3.CH:CH.CHO + CH_3.CO.COOH = CH_3.CH:CH.CHOH.CH_2.CO.COOH$.

and by reactions similar to 3a and 4a

$CH_3.CH_2.CH_2.CH_2.CH_2.COOH$.
Caproic acid.

The evidence supporting this hypothesis may be briefly summarised as follows.

1. Pyruvic acid is probably a degradation product of carbohydrate in the body.

The perfusion experiments of Embden, Knoop and Neubauer show that pyruvic acid is converted into alanine through the agency of the liver cells

and that a close connection exists between the α-amino- and α-keto-acids. Pyruvic acid may probably be an intermediate stage in the transformation from glycogen to alanine (Fellner).

There is some reason to believe that in the acetylation of certain amino-acids which has been observed both in perfusion and in feeding experiments, pyruvic acid is the acetylating agent.

The close connection between alanine and pyruvic acid suggests that the alanine group of the protein molecule may furnish an additional source of the pyruvic acid available for the synthesis of fatty acids.

2. The decomposition of pyruvic acid into acetaldehyde and carbonic acid, which constitutes the first stage of this process, has been shown by Neuberg to be readily brought about by an enzyme present in yeast, termed "carboxylase."

3. The present hypothesis postulates that free acetaldehyde is not liberated but that the decomposition of the keto-acid is in some way regulated by the pyruvic acid with which the "nascent" aldehyde combines.

The condensation of fatty aldehydes with pyruvic acid has now been shown to take place in the laboratory under the influence of dilute alkalis at ordinary temperature.

4. Oxidation of the α-keto acid according to the equation

$$R.CO.COOH + O = R.COOH + CO_2$$

may be brought about in the laboratory by hydrogen peroxide at the ordinary temperature in neutral solution (p. 370).

5. The reduction of the unsaturated acid is the final stage; there is abundant evidence that reduction can take place in the body although very little is known as to the mechanism by which it is accomplished.

6. The α-keto-acid, synthesised as above, may be split into CO_2 and aldehyde, and a further condensation with pyruvic acid may then be effected. An acid with two more carbon atoms than the original aldehyde would thus be synthesised.

As yet no α-keto-acids have been detected within the body: it may be that they occur only within the cell and that reduction or oxidation always accompanies their liberation. The above hypothesis accounts for the formation of a series of straight chain acids beginning with four carbon atoms and increasing by increments of two carbon atoms: it involves only reactions which are analogous with those which are known to occur in the laboratory and there is reasonable evidence for believing that the starting material, pyruvic acid, can be formed from carbohydrate in the body.

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