Chemistry. — *Researches on fat metabolism.* VII. *β-Oxidation of normal saturated dicarboxylic acids administered per os.* By P. E. VERKADE, J. VAN DER LEE, A. J. S. VAN ALPHEN and M. ELZAS. (From the Laboratory of the Dutch Commercial University and the State Serum Institute at Rotterdam). (Communicated by Prof. J. BÖESEKEN).

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§ 1. In a previous communication on fat metabolism VERKADE and VAN DER LEE [1]) considered it justifiable to give the following general scheme for the degradation of the normal saturated fatty acids in the living organism:

$$\begin{array}{ccc}
CH_3{-}(CH_2)_n{-}COOH & \xrightarrow{\omega} & HOOC{-}(CH_2)_n{-}COOH \\
\downarrow\beta & & \downarrow\beta \\
CH_3{-}(CH_2)_{n-2}{-}COOH & \xrightarrow{\omega} & HOOC{-}(CH_2)_{n-2}{-}COOH \\
\downarrow\beta & & \downarrow\beta \\
CH_3{-}(CH_2)_{n-4}{-}COOH & \xrightarrow{\omega} & HOOC{-}(CH_2)_{n-4}{-}COOH \\
\downarrow\beta & & \downarrow\beta \\
\text{etc.} & \xrightarrow{\omega} & \text{etc.}
\end{array}$$

It should be remarked here in passing that the possibility of the existence of still further mechanisms for the degradation of these acids was in no way excluded.

As far as the dicarboxylic acids are concerned, this scheme was then based on the following facts:

1. The phenomenon of the *ω-oxidation* of the fatty acids discovered in the laboratory of one of us (V.). The administration of certain simple triglycerides, particularly of tricaprin and triundecylin, preferably together with a liberal amount of carbohydrate, to healthy subjects, leads to accumulation in the blood of the dicarboxylic acid produced by methyl-group oxidation of the component fatty acid (*dioic acid-acidosis*) and excretion thereof in the urine (*diaciduria*) [2]). The same thing was later found with mixed glycerides, which contain a strongly *diacidogenic* fatty acid in sufficient quantity as a component acid [3]).

2. The presence in the urine of typical lower odd or even dicarboxylic

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[1]) VERKADE and VAN DER LEE, Z. physiol. Chem. **227**, 213 (1934).

[2]) VERKADE, ELZAS, VAN DER LEE, Miss DE WOLFF, Mrs. VERKADE—SANDBERGEN and VAN DER SANDE, ibid. **215**, 225 (1933); VERKADE and VAN DER LEE, Biochem. J. **28**, 31 (1934).

[3]) VERKADE and VAN DER LEE, Z. physiol. Chem. **225**, 230 (1934); VERKADE, VAN DER LEE and HOLWERDA, ibid. **234**, 21 (1935).

acids besides the odd or even dicarboxylic acid formed by ω-oxidation. After administration of tricaprin VERKADE and VAN DER LEE [1]) found in the urine of their subjects besides a large amount of sebacic acid also small amounts of suberic acid and adipic acid (C_{10}—C_8, C_6), while after administration of triundecylin the urine contained besides a large amount of undecanedioic acid also azelaic acid and pimelic acid (C_{11}—C_9, C_7) in small amounts.

Thus the occurrence of *β-oxidation of the normal saturated dicarboxylic acids* included in the above scheme was then not proved by means of *in vivo* experiments. In our opinion conclusive evidence can be supplied only by such experiments; *in vitro* experiments have little or no value. VERKADE and VAN DER LEE then contented themselves with considering and finally rejecting the possibility that dicarboxylic acids should be formed exclusively by ω-oxidation of fatty acids and should not be intermediate products, but by-products of fat catabolism, which further play no part. In such a case the scheme given below would represent our present knowledge of the degradation of saturated fatty acids in the living body.

$$\begin{array}{lll}
CH_3{-}(CH_2)_n{-}COOH & \xrightarrow{\omega} & HOOC{-}(CH_2)_n{-}COOH \\
\downarrow\beta & & \\
CH_3{-}(CH_2)_{n-2}{-}COOH & \xrightarrow{\omega} & HOOC{-}(CH_2)_{n-2}{-}COOH \\
\downarrow\beta & & \\
CH_3{-}(CH_2)_{n-4}{-}COOH & \xrightarrow{\omega} & HOOC{-}(CH_2)_{n-4}{-}COOH \\
\downarrow\beta & & \\
\text{etc.} & \xrightarrow{\omega} & \text{etc.}
\end{array}$$

Obviously it was necessary to attempt to detect this postulated occurrence of β-oxidation of dicarboxylic acids in the organism; VERKADE and VAN DER LEE had already pointed this out in their above mentioned paper and had in connection herewith drawn attention to the markedly increased excretion of oxalic acid in the urine after administration of adipic acid, observed by MORI [4]) on rabbits and by ANDERSEN [5]) on men and dogs. We have now been able to furnish this required proof in a convincing manner.

§ 2. Two adult healthy dogs of 12 and 13 kg weight respectively served us for the experiments in question. These received twice a day at always the same times (11 h. and 17 h.) for three successive days 2,5 g of the disodium salt of sebacic acid or undecanedioic acid as an addition to their meal which usually consisted of as nearly as possible equal amounts of dog biscuit (20—30 g) and lean horseflesh (10—15 g). As much water as they desired was supplied. A part of the dicarboxylic acid administered was excreted in the urine. We leave this phenomenon without further discussion here; we shall deal with it separately in our next paper in

[4]) J. Biol. Chem. **35**, 341 (1918).

[5]) Comp. FLASCHENTRÄGER, Z. physiol. Chem. **159**, 299 (1927).

which we wish to prove that the way of degradation of the normal saturated fatty acids found by us is by no means restricted to the lower members of this series, as has been suggested from different sides in recent literature *). The urine was either passed freely by the experimental animals or if necessary obtained by catheterization. Tests were made in our usual way — namely by acidifying the urine with concentrated hydrochloric acid or phosphoric acid — for the presence of sebacic acid or undecanedioic acid in the successive portions. The collection of the urine was suspended after this test had come out negative for a considerable time; a stay of 6 days in all in the test-cages was always found sufficient.

Two such experiments were made with each dog with each of the two acids. The dogs thus received in total 30 g of each disodium salt, that is, 24.6 g sebacic acid and 24.9 g undecanedioic acid, administered per os. The urines of both dogs were worked up separately and furnished qualitatively — and we are only concerned with that aspect here — the same results.

After administration of sebacic acid (C_{10}) the urine was found to contain suberic acid (C_8) and adipic acid (C_6); after administration of undecanedioic acid (C_{11}), azelaic acid (C_9) and pimelic acid (C_7) were found to be present. A convincing proof is thus furnished that β-oxidation of dicarboxylic acids occurs in the dog. Therefore in our opinion it cannot be in doubt that this process also occurs in the human body — it seems unnecessary to us to prove this by special experiments — and the suberic acid, for example, found in the urine of the test subjects after administration of tricaprin (see § 1) is certainly, at any rate partially, produced by β-oxidation of sebacic acid, the ω-oxidation product of capric acid.

The ratio in which the dicarboxylic acids C_{10}, C_8 and C_6 or C_{11}, C_9 and C_7 are present in the urine, is not the same with different test animals or even in different experiments with the same test animal; however, the dicarboxylic acid C_{10} or C_{11} always predominates to a marked extent. In working up the urine one must consequently deal with it according to the circumstances and there is no object in describing in detail the methods we used. It will be enough to add the following remarks on it.

The urine was heated to boiling and then treated with an excess of concentrated hydrochloric acid. On cooling an appreciable amount of sebacic acid or undecanedioic acid separated out in an easily filtrable form. The filtrate was evaporated as far as possible on a water-bath. The residue was treated with concentrated hydrochloric acid and again evaporated as far as possible; this treatment was repeated several times. Then water was added and the mass made strongly alkaline with caustic potash; it was then again evaporated to dryness as far as possible on a water-bath. Finally the above mentioned treatment with concentrated hydrochloric acid was repeated several times. This preliminary treatment of the urine was

*) A recent paper by FLASCHENTRÄGER and BERNHARD [Helv. chim. acta **18**, 962 (1935)] has forced us to discuss this question in a preliminary paper to be published in the November issue of the Rec. trav. chim.

in the first place with the object of hydrolysing possibly present condensation products of the partial degradation products of sebacic acid or undecanedioic acid sought by us with glycine, glucuronic acid, etc.; furthermore hippuric acid is removed hereby, this acid being sometimes very troublesome in the further operations and less darkly coloured etherial extracts are thus obtained. The residue was now rinsed with water into an extractor and extracted continuously for several days with ether. The mass, remaining after distilling off the ether, was dried and subsequently extracted several times with 125 cm^3 of boiling benzene. On distilling off the benzene a mass of crystals soaked with syrup (*A*) remained.

From this product (*A*) the above mentioned partial degradation products of sebacic acid, or undecanedioic acid could be isolated, mainly by fractional crystallization from water and from a mixture of acetone and petroleum ether and further, among other things, by fractional precipitation of the copper salts. Some data regarding the products isolated are given below.

Adipic acid.

M.P. of the acid isolated 151—152°; the mixed M.P. with a preparation of adipic acid obtained by oxidation of cyclohexanol with nitric acid and melting at 153°, was 151—152°.

120.7 mg → 17.13 cm^3 0.0965 n NaOH. Equiv. wt. **73.0**; calc. 73.04
3.713 mg → 6.73 mg CO_2 and 2.21 mg H_2O; C **49.4** %, H **6.7** %
Calc. for $C_6H_{10}O_4$: C 49.29 %, H 6.90 %

Suberic acid.

M.P. 140—141°; mixed M.P. with a preparation made from castor oil and also melting at 140—141° unaltered.

162.9 mg → 19.24 cm^3 0.0965 n NaOH. Equiv. wt. **87.7**; calc. 87.06
3.892 mg → 7.91 mg CO_2 and 2.74 mg H_2O; C **55.4** %, H **7.9** %
4.295 mg → 8.72 mg CO_2 and 2.99 mg H_2O; C **55.4** %, H **7.8** %
Calc. for $C_8H_{14}O_4$: C 55.14 %, H 8.11 %

Pimelic acid.

M.P. of the product isolated 102—104°; the mixed M.P. with a pimelic acid preparation made by VON BRAUN's method and melting at 104.5—105° was 103—104°.5.

138.2 mg → 17.76 cm^3 0.0965 n NaOH. Equiv. wt. **80.6**; calc. 80.05
3.604 mg → 7.00 mg CO_2 and 2.45 mg H_2O; C **53.0** %, H **7.6** %
3.635 mg → 7.04 mg CO_2 and 2.47 mg H_2O; C **52.8** %, H **7.6** %
Calc. for $C_7H_{12}O_4$: C 52.47 %, H 7.56 %

Azelaic acid.

M.P. 105—107°. The mixed M.P. with an azelaic acid preparation made from castor oil and melting at 106°.5—107°.5, was 106—107°; with an undecanedioic acid preparation synthesized by WALKER and LUMSDEN's method and melting at 110° (ratio of the weights about 1 : 1), it was about 90—96°.

81.6 mg → 8.08 cm³ 0.1054 n NaOH. Equiv. wt. **95.8**; calc. 94.06
4.210 mg → 8.89 mg CO_2 and 3.20 mg H_2O; C **57.6** %, H **8.5** %
3.867 mg → 8.18 mg CO_2 and 2.96 mg H_2O; C **57.7** %, H **8.6** %
Calc. for $C_9H_{16}O_4$: C 57.41 %, H 8.56 %

It is perhaps useful to refer here to the following. HALLE [6]) in his investigations on the high molecular acids of Japan wax pretends to have observed that mixed melting point determinations are completely untrustworthy with high molecular homologous dicarboxylic acids; no depressions of the melting points were found in mixtures of certainly not identical dicarboxylic acids (with only slightly differing melting points). Very definite depressions were however found with the lower members of the series of normal saturated dicarboxylic acids in which we are at present interested — such is seen above to be the case already for azelaic acid + undecanedioic acid — and identification of these acids is thus very well possible in this way.

We have not attempted to attain a more or less quantitative determination of the amounts of adipic acid and suberic acid, or pimelic acid and azelaic acid present in the urine. These amounts were small in comparison with the amounts of sebacic acid or undecanedioic acid catabolised by the test animals; in general some per cent by weight of the acid administered were excreted in the form of lower dicarboxylic acids.

We have looked also for the presence of *succinic acid* in the urine passed after administration of sebacic acid, however without positive results. We refer to communication IX of this series in which we shall make some observations on the role of succinic acid in fat catabolism.

§ 3. Some years ago FLASCHENTRÄGER [7]) incidentally raised the question whether the normal saturated dicarboxylic acids are or are not attacked in the organism by β-oxidation and even made the experiments on animals which might have enabled him to answer this question. For example this investigator injected subcutaneously 10 g of sebacic acid as sodium salt in doses of 0.5 g twice a day into a normal dog of 21.2 kg weight. It is very probable — there is no single basis for an *essential* distinction in this respect between dicarboxylic acid administered per os and subcutaneously — that the urine collected in this experiment will have contained lower dicarboxylic acids besides unchanged sebacic acid by the animal. Curiously enough it does not appear from FLASCHENTRÄGER's paper that this question has further occupied his attention. It is only mentioned very summarily that 61 % of the sebacic acid administered is excreted in the urine.

Feeding experiments on normal dogs have been carried out by SMITH [8])

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[6]) Diss. Leipzig, 1928, p. 56.

[7]) Z. physiol. Chem. **159**, 297 (1927).

[8]) J. Biol. Chem. **103**, 531 (1933); Comp. VERKADE and VAN DER LEE, Z. physiol. Chem. **230**, 214 (1934).

with large quantities of sodium azelate. It should certainly have been possible to detect the presence of pimelic acid in the urine. However this investigator has also satisfied himself with a study of the excretion of unchanged azelaic acid.

§ 4. It should not remain unnoticed that PONSFORD and SMEDLEY—MAC LEAN [9]), in their investigations on the oxidation of several normal saturated dicarboxylic acids by hydrogen peroxide at 60° in the presence of a copper salt, have been able to isolate some products which must be considered as β-oxidation products of these acids. Thus acetone was obtained from glutaric acid, acetonylacetone from suberic acid. In the latter case β-oxidation has thus taken place at both ends of the dicarboxylic acid molecule. Correctly or incorrectly — we leave this at present quite undecided — hydrogen peroxide is frequently considered as the oxidizing agent whose action closely agrees with the oxidation processes taking place *in vivo*.

§ 5. As a consequence of the simple *in vivo* experiments here described the scheme for the degradation of the normal saturated fatty acids given at the beginning of § 1 may now be considered as completely established. As has already been pointed out in § 2, we shall discuss the general validity of this scheme in communication VIII of this series. In a brief discussion of our work ARTOM [10]) has, without any explanation, put forward a small extension to this scheme; he reproduced it as follows:

$$\begin{array}{l} CH_3{-}(CH_2)_n\ {-}COOH \xrightarrow{\omega} HOOC{-}(CH_2)_n\ {-}COOH \\ \quad\downarrow\beta \qquad\qquad\qquad\qquad\quad \downarrow\beta \\ CH_3{-}(CH_2)_{n-2}{-}COOH \xrightarrow{\omega} HOOC{-}(CH_2)_{n-2}{-}COOH \\ \quad\downarrow\beta \qquad\qquad\qquad\qquad\quad \downarrow\beta \\ CH_3{-}(CH_2)_{n-4}{-}COOH \xrightarrow{\omega} HOOC{-}(CH_2)_{n-4}{-}COOH \end{array} \quad \beta\,\beta$$

The intention of this small change of the scheme is not clear to us. Perhaps ARTOM desires to put forward the possibility that a dicarboxylic acid with $(x{-}4)$ carbon atoms is produced from a dicarboxylic acid with x carbon atoms by perfectly simultaneous β-oxidation at both ends of the molecule; thus without the dicarboxylic acid with $(x{-}2)$ carbon atoms — or an active form of it — occurring as intermediate product. Such a perfectly simultaneous course of both β-oxidations requires a mutual dependence of the two oxidation processes, that is to say, a very complicated reaction mechanism which is certainly extremely improbable. We should then be inclined to think rather of possibility of a δ-oxidation.

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[9]) Biochem. J. **28**, 892 (1934).

[10]) Annual Review of Biochemistry **4**, 216 (1935).