Chemistry. — *Researches on fat metabolism.* IV. *Two-sided β-oxidation of the dicarboxylic acids formed by ω-oxidation of saturated fatty acids.* By P. E. VERKADE and J. VAN DER LEE. (Communicated by Prof. G. VAN ITERSON Jr.).

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§ 1. In our second paper [1]) on fat metabolism we pointed out by means of experiments on two healthy subjects that in the series of simple triglycerides of the normal saturated fatty acids the terms with term-numbers [2]) 9 (tricaprin) and 10 (triundecylin) are the most strongly *diacidogenic*; the accumulation in the blood (*dioic acid acidosis*) and the excretion in the urine (*diaciduria*) of dicarboxylic acid, formed by *ω-oxidation* of the component acid, is by far the most pronounced with these two triglycerides. Since the first moment of our discovery of dioic acid acidosis and diaciduria [3]) we have been convinced that the formation of dicarboxylic acids by means of ω-oxidation is the first phase of an as yet unknown way of degradation of the saturated fatty acids — these only are dealt with in this paper — in the organism. The frequently very abundant excretion of undecanedioic acid after administration of triundecylin and particularly, since a fatty acid with an even number of carbon atoms is concerned here and such fatty acids are the component acids of our food fats, of sebacic acid after partaking of tricaprin or elmseed oil [4]), in our opinion points already in this direction; it seems to us to be highly improbable that in the catabolism of these substances by healthy persons such large quantities of these acids should be formed as useless by-products.

Does indeed the degradation of the fatty acids, besides in the way discovered by KNOOP and henceforth indicated by us as *one-sided β-oxidation*, take place also in another way, in which dicarboxylic acids, formed by ω-oxidation, play a part? It would seem to us that we have been successful in being able to give an affirmative answer to this question, owing to the fact that from the urine of persons who had taken large amounts of tricaprin or triundecylin we could isolate characteristic substances, which certainly — at any rate partly — are partial degradation products of the above-mentioned acids. *After administration of tricaprin we found in addition to a large quantity of sebacic acid* (C_{10}) *in the urine*

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[1]) VERKADE and VAN DER LEE, Biochem. J. **28**, 31 (1934).

[2]) VERKADE and COOPS, Rec. trav. chim. **47**, 568 (1930).

[3]) VERKADE, ELZAS, VAN DER LEE, Miss DE WOLFF, Mrs. VERKADE—SANDBERGEN, and VAN DER SANDE, Z. physiol. Chem. **215**, 225 (1933).

[4]) VERKADE and VAN DER LEE, ibid. **225**, 230 (1934).

also small quantities of suberic acid (C_8) *and adipic acid* (C_6), *while after administration of triundecylin the urine appeared to contain besides a large quantity of undecanedioic acid* (C_{11}) *also azelaic acid* (C_9) *and pimelic acid* (C_7) *in small quantities.* Further on these interesting facts will be discussed in detail.

§ 2. In the work now to be discussed we started from the, in our opinion, rational thought that an accumulation of such partial degradation products in the blood and consequently their excretion in the urine may be expected the sooner if the subject has a strong dioic acid acidosis. Therefore we took for this work only subjects who, as was known to us, usually had a strong tendency to dioic acid acidosis. Secondly we had to administer fats to them, which exclusively contained strongly diacidogenic fatty acids as the component acids. Obviously it was then desirable to make use of one of the two strongly diacidogenic *simple* triglycerides (tricaprin and triundecylin); the isolation of partial degradation products, which might eventually be present in the urine, would thus be greatly facilitated, if these products, as might be expected and indeed appeared to be the case, would be specific for the ω-oxidation product. Thirdly, besides the triglyceride the subjects were always given a liberal amount of carbohydrate; as will be proved in a subsequent paper, such an addition to the diet leads with most people, among them also the subjects used in this investigation, to a pronounced rise of the dioic acid acidosis and the diaciduria.

§ 3. *Presence of pimelic acid and azelaic acid in addition to undecanedioic acid in the urine after administration of triundecylin.*

In total a quantity of 480 g of pure triundecylin (setting-point 29°.95; saponification number 281.2, calculated 282.2) was taken by three healthy subjects (V. D. L., V., and D.). It may be mentioned here that this fat was obtained by esterification [1]) of glycerol with undecylic acid of setting-point 28°.35, which was prepared by hydrogenation of pure undecylenic acid. It seems superfluous to summarize the quantities of triundecylin and carbohydrate, administered on the various test-days, as well as their distribution over the day. The urine of the subjects was collected from the moment that the first portion of fat was taken till for some hours no excretion of undecanedioic acid took place any longer; the presence of undecanedioic acid in the urine was again tested for by addition of some concentrated phosphoric acid.

The collected urine was heated to the boiling-point and then treated with hydrochloric acid. On cooling 19.5 g of undecanedioic acid separated

[1]) Comp. VERKADE, VAN DER LEE and Miss MEERBURG, Rec. trav. chim. **51**, 850 (1932).

out. The filtrate was made strongly alkaline with sodium hydroxide and evaporated as far as possible on the water-bath. The residue was then taken up in water, strongly acidified with hydrochloric acid, and again evaporated as far as possible on the water-bath. The purpose of this preliminary treatment of the urine was to make practically sure that possibly present condensation products of the partial degradation products sought, with glycine, glucuronic acid, etc., for the occurrence of which we have, however, no indications whatever, were hydrolysed. The residue was now intimately mixed with anhydrous sodium sulphate and during several days extracted with ether in a Soxhlet apparatus. The mass, left behind after distilling off the ether, was taken up in excess caustic soda solution and again evaporated, this time particularly in order to remove, at any rate partly, still present colouring matter (in this manner a part of the latter is namely made insoluble in water and ether), then acidified with hydrochloric acid and, in order to remove benzoic acid, subjected to a steam-distillation. The solution left in the flask was again for a considerable time continuously extracted with ether, whereupon the remaining mass, the ether being distilled off, was dried and subsequently extracted several times with 50 cm^3 of boiling benzene. The substance separated from the benzene solution on standing for some days in the ice-chest — it may be recalled here that the normal saturated dicarboxylic acids are only very sparingly soluble in cold benzene [1]) — was for a considerable time kept in vacuo over phosphorus pentoxide and then formed a mass of crystals soaked with some syrup. By filtering at the pump the crystals (A) were separated as far as possible from the syrup.

The crystals (A), weighing 2.5 g, were dissolved in 100 cm^3 of hot water; on cooling some impure undecanedioic acid separated out. After evaporation of the filtrate to 40 cm^3 and cooling to about 0°, 0.37 g of crystals (B_1) and a mother-liquor (B_2) were obtained. The crystals (B_1) consisted partly of *azelaic acid*. After some crystallisations from water, from benzene, and from a mixture of acetone and petroleum ether, we succeeded in obtaining this acid in a perfectly pure state. Melting-point 104°.5—105°.5; the mixed m.p. with azelaic acid, prepared in the usual manner from castor oil and melting at 106°.5—107°.5, was 105°.5—106°.5.

69.3 mg → 8.00 cm^3 0.0913 n. NaOH. Equiv. wt. **94.9**; calculated 94.06.
4.103 mg → 8.67 mg CO_2 and 3.01 mg H_2O; C **57.6** % H **8.2** %
3.953 mg → 8.33 mg CO_2 and 2.92 mg H_2O; C **57.5** % H **8.3** %
Calculated for $C_9H_{16}O_4$: C 57.41 % H 8.56%.

After evaporation to 4 cm^3 the mother-liquor (B_2) produced a fraction (C_1) of 0.96 g in addition to a mother-liquor (C_2), which after further evaporation, even after standing for months, did not crystallize and was not examined further. The crystals (C_1) were recrystallised first from a

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[1]) Comp. VERKADE and COOPS, Rec. trav. chim. **49**, 578 (1930).

little water, then from a large quantity of a mixture of acetone and petroleum ether; thus 0.35 g of pure *pimelic acid* was obtained. M.p. 104—105°: the mixed m.p. with a specimen of pimelic acid, prepared by VON BRAUN's method [1]) and melting at 104.5—105°, was 104—104°.5.

200.7 mg → 23.66 cm³ 0.1052 n. NaOH. Equiv. wt **80.6.**; calculated 80.05.
3.835 mg → 7.41 mg CO_2 and 2.55 mg H_2O; C **52.7** % H **7.4** %
4.211 mg → 8.14 mg CO_2 and 2.78 mg H_2O; C **52.7** % H **7.4** %
Calculated for $C_7H_{12}O_4$: C 52.47 % H 7.56 %.

As appears from the above, we have by no means tried to arrive at a quantitative determination of the amounts of azelaic acid and pimelic acid present in the urine. The data given, however, are in all respects sufficient to warrant the conclusion that these quantities, in comparison to the amount of excreted undecanedioic acid, were small and *that undoubtedly the quantity of azelaic acid was smaller than the quantity of pimelic acid.*

§ 4. *Presence of adipic acid and suberic acid in addition to sebacic acid in the urine after administration of tricaprin.*

The administered tricaprin (setting-point 30°.95; saponification number 302.7, calculated 303.4) was obtained by esterification of glycerol with capric acid of setting-point 31°.35, which had been prepared by elimination of carbon dioxide from pure n. octylmalonic acid. In total an amount of 360 g of this fat was taken by three healthy subjects (V. D. L., B., and V.). The urine of the subjects was again collected from the moment of partaking of the first portion of fat till for some hours no excretion of sebacic acid took place any longer.

The working up of the urine took place in an analogous manner to that described in the preceding §. The *suberic acid* obtained melted at 140—141°; on mixing with a specimen of suberic acid, prepared in the usual way from castor oil and likewise melting at 140—141°, no depression of the melting-point occurred.

4.243 mg → 8.53 mg CO_2 and 3.03 mg H_2O; C **54.8** % H **8.0** %
3.898 mg → 7.82 mg CO_2 and 2.82 mg H_2O; C **54.7** % H **8.1** %
Calculated for $C_8H_{14}O_4$: C 55.14 % H 8.11 %.

For a titration the amount of this pure product was insufficient. For this reason we also mention here the results of a titration of the acid in a previous stage of isolation; the m.p. was then 135—138° and the mixed m.p. 137—140°.

109.8 mg → 12.55 cm³ 0.0979 n. NaOH. Equiv. wt. 89.3; calculated 87.06.

The *adipic acid* obtained melted at 149—153°. The mixed m.p. with a

[1]) Ber. **37**. 3588 (1904); comp. VERKADE, HARTMAN and COOPS, Rec. trav. chim. **45**, 380 (1926).

specimen of adipic acid, obtained by oxidation of cyclohexanol with nitric acid and melting at 153°, was 152—153°.

137.1 mg → 19.08 cm³ 0.0979 n . NaOH . Equiv . wt . **73.4**; calculated 73.04.
3.848 mg → 7.01 mg CO_2 and 2.30 mg H_2O; C **49.7** % H **6.7** %
4.096 mg → 7.44 mg CO_2 and 2.50 mg H_2O; C **49.5** % H **6.8** %
Calculated for $C_6H_{10}O_4$: C 49.29 % H 6.90 %.

Here also the quantities of suberic acid and adipic acid present in the urine were small in comparison to the amount of excreted sebacic acid. *Very likely* — unfortunately we are not so certain here as in the experiments with triundecylin — *the amount of suberic acid was smaller than the amount of adipic acid.*

§ 5. On the basis of the remarkable results discussed in the two preceding §§ it is in our opinion very obvious to conclude that the normal saturated fatty acids are catabolised in a hitherto unknown way besides by one-sided β-oxidation. It should be remarked in passing that we by no means wish to exclude the possibility of the occurrence of still further mechanisms for the degradation of these acids. *By ω-oxidation of the fatty acid the corresponding dicarboxylic acid is formed which is then further catabolised via lower dicarboxylic acids, formed by β-oxidation.* It is indeed highly interesting that than also in this way of degradation the β-oxidation discovered by KNOOP [1]) plays a predominant part. One might be inclined to regard the ω-oxidation only as a measure of efficiency of the organism, by which means the fatty acid molecule obtains a second point of attack for the β-oxidation; the possibility for what henceforth will be called *two-sided β-oxidation* is now present.

The suberic acid found in the urine after administration of tricaprin — this acid is only taken here as an example — may then have originated in the two following ways: 1. by β-oxidation of sebacic acid, formed by ω-oxidation of capric acid; 2. by ω-oxidation of caprylic acid, formed by one-sided β-oxidation of capric acid. If these different ways of the possible formation of dicarboxylic acids are taken into account, a general scheme of a degradation of the normal saturated fatty acids, taking place via the dicarboxylic acids, appears to be as follows:

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\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}
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[1]) Hab.-Schrift Freiburg i.B. 1904; HOFMEISTERs Beitr. **6**. 150 (1905).

As already follows from a remark made in § 1, we may, in our opinion, put aside the possibility that dicarboxylic acids should be exclusively formed by ω-oxidation of fatty acids and should not be intermediate products, but by-products of fat metabolism, which further play no part.

§ 6. It stands to reason that it is necessary to confirm the existence of the just indicated way of degradation of the normal saturated fatty acids by further investigations. In fact, such work is already in course of preparation. In particular, of course, we are interested in the question whether after administration of e.g. sebacic acid, either per os or by injection, under suitable conditions to suitable human subjects or experimental animals, likewise suberic acid and adipic acid will be excreted in the urine. It may be incidentally remarked here that really the same question has been raised already some years ago by FLASCHENTRÄGER [1]; of an effort to answer this question there is, however, nothing to be perceived in that paper. The investigations of BAER and BLUM [2], MORI [3], FLASCHENTRÄGER [1], ANDERSEN [4], and SMITH [5], which will be discussed in detail in a subsequent communication, have at any rate already taught us that the animal and human organism are capable of burning higher normal saturated dicarboxylic acids. In our opinion they do not advance arguments at all — as has, for example, been asserted by SMITH [5] — against the conception that such dicarboxylic acids occur as intermediate products in fat metabolism. The observations by MORI [3] on rabbits and by ANDERSEN [4] on men and dogs, that after administration of *adipic acid* (C_6) a clearly increased excretion (viz. 3—4 times and 1.5—3.5 times respectively) of *oxalic acid* (C_2) took place in the urine, are highly interesting and to us probably of great importance. The question now presents itself whether this increased excretion of oxalic acid (oxaluria) may have been caused by formation of this acid through β-oxidation of adipic acid, and at the same time whether perhaps at least part of the endogenic oxalic acid normally present in the urine may be a product of fat metabolism. At present we shall not enter into this question any further.

§ 7. In the course of time the supposition or the conviction has been pronounced from many sides that the one-sided β-oxidation should not be the only way of degradation of the fatty acids in the organism. It will not do and would besides be superfluous to give a summary here of the relevant literature; it may be sufficient to refer to the text-books of HAMMERSTEN [6],

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[1]) Z. physiol. Chem., **159**, 297 (1927).
[2]) HOFMEISTERs Beitr. **11**, 101 (1908).
[3]) J. Biol. Chem. **35**, 341 (1918).
[4]) Comp. FLASCHENTRÄGER, loc. cit., p. 299.
[5]) J. Biol. Chem. **103**, 531 (1933).
[6]) Lehrb. d. Physiol. Chemie, 11th ed. 1926, p. 611.

ABDERHALDEN [1]), HAHN [2]), OPPENHEIMER [3]), and LEATHES and RAPER [4]). Thus far, however, nobody had been able to indicate another way with any certainty. We have now succeeded in doing this with a probability in our opinion bordering on certainty, and this indeed *by means of in vivo experiments, carried out with simple triglycerides, thus as it were with the fatty acids themselves.*

The advantage of *in vivo* experiments over those *in vitro* is indeed very obvious. The value of the latter — we think here, for example, of the researches by SMEDLEV-MACLEAN and PEARCE [5]) on the oxidation of fatty acids by means of hydrogen peroxide in the presence of a cupric salt, and of those of CLUTTERBUCK and RAPER [6]) in a similar direction — for our insight into fat metabolism is in our opinion often overestimated and seems to us to be generally rather small. Transferring the results of such so-called „Modellversuche" to what happens in the living organism — e.g. a possible conclusion from the results of the just-mentioned researches that in the organism γ- or δ-oxidation of the fatty acids resulting in the formation of succinic acid takes place — always contains an element of uncertainty.

The arguments in favour of the existence of the discussed possible way of degradation of the normal saturated fatty acids are actually even more cogent than those for their degradation by one-sided β-oxidation, which nevertheless is doubted by nobody. For in the latter case the formation or non-formation of ketonic bodies from the fatty acids with even or odd numbers of carbon atoms respectively is the only somewhat direct indication; thus far there is no question at all of the isolation of partial degradation products *specific to the different acid components of our food fats.* For the rest we are referred to the results of numerous experiments with *derivatives* of these fatty acids, which, mostly in the ω-position, contain a phenyl group (KNOOP, DAKIN, QUICK, a.o.) or a methyl (benzenesulphonyl)amino group (FLASCHENTRÄGER), KNOOP [7]) has already pointed out the obvious fact „dass der Einfluss des Benzolkerns im Molekül zunächst ein ganz unübersehbarer ist, bei jeder Uebertragung der an fettaromatischen Säuren gefundenen Tatsachen auf die aliphatische Reihe also Vorsicht geboten ist." Nevertheless QUICK [8]) feels justified to adduce evidence from the results of his in vivo experiments with ω-phenylsubstituted acids in favour of the conception that the fatty acids are exclusively catabolised by one-sided β-oxidation. On the strength of our researches we may now characterize this opinion as unjustified. For it has now become

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[1]) Lehrb. d. Physiol. Chemie, 6th ed. 1931, p. 114.
[2]) Grundriss d. Biochemie, 2nd ed. 1932, p. 159.
[3]) Chem. Grundlagen der Lebensvorgänge (Georg Thieme, Leipzig), 1933, p. 142—145
[4]) The Fats (Longmans, Green & Co., London), 1925, p. 193.
[5]) Biochem. J. **25**, 1252 (1931); **28**, 486 (1934).
[6]) ibid. **19**, 385 (1925).
[7]) Hab.-Schrift, Freiburg i. Br. 1904, p. 39.
[8]) J. Biol. Chem. **77**, 581 (1928).

apparent that, by introduction of a substituent in ω-position, a group which very probably is highly important for the degradation of the fatty acids, viz. the terminal methyl group, is blocked up ; ω-oxidation with formation of a carboxyl group is then no longer possible.

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Chemical Laboratory of the Dutch Commercial University.

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