By condensation of the protocatechu ester with o. methylcyclohexanone, two isomers were found as well.

Delft, September 1934.

Chemistry. — *Researches on fat metabolism V. Some experiments on the degradation of unsaturated fatty acids in the living organism.* By P. E. VERKADE and J. VAN DER LEE. (From the chemical laboratory of the Dutch Commercial University at Rotterdam.) (Communicated by Prof. J. BÖESEKEN.)

(Communicated at the meeting of September 29, 1934).

§ 1. In connection with the discussion of the investigations of LEATHES and MEYER—WEDELL [1]) and those of HARTLEY [2]) on desaturation of fatty acids in the liver, LEATHES and RAPER, in their wellknown monograph [3]), make the following remark:

> "The exact significance of the process of desaturation is not known. It is true that the unsaturated fatty acids are more liable to oxidative attack because of their double linkages than the saturated acids, but there is no proof that this accepted fact of the chemical laboratory applies equally to that of the living organism where oxidations are brought about by very different methods. It may be accepted provisionally, however, that the unsaturated acids are probably liable to attack in the body at the positions of the double linkages and that a rupture of the fatty acid chain at these points precedes the oxidation of the fragments so produced."

The probability of the mode of degradation of unsaturated and also of saturated fatty acids, briefly indicated in the above quotation, was already put forward as early as 1913 by LEATHES in the first edition of the above-mentioned monograph. The ideas expressed there have been amply discussed and partially contradicted by BLOOR [4]). We need not discuss this paper here. BLOOR's assertion that this mode of degradation was already suggested by LEATHES in a lecture delivered before the Harvey Society in 1909 [5]) is incorrect.

Recent researches, such as those of SINCLAIR [6]) and those on the nature of the fatty acids present in sheep or pig liver [7]), have shown that the

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[1]) J. Physiol. **38** (1909), Proc. Physiol. Soc., p. 38.

[2]) Ibid. **38**, 353 (1909).

[3]) The Fats (LONGMANS, GREEN and Co., London, 1925), p. 194.

[4]) J. metabolic Research **4**, 549 (1923).

[5]) Lancet, 1909, I, 593.

[6]) J. Biol. Chem. **95**, 393; **96**, 103 (1932); **100**, LXXXVII (1932).

[7]) TURNER, Biochem. J. **24**, 1327 (1930); CHANNON, IRVING and SMITH, ibid. **28**, 840 (1934).

basis on which the desaturation hypothesis of LEATHES rests is much weaker than was fairly generally accepted before. At present there is no proof whatever for the correctness of this hypothesis and it seems indeed doubtful to us that desaturation of fatty acids *in the sense meant by* LEATHES should take place in the living organism. However, in this paper we shall not discuss this problem any further.

It is, however, in our opinion, quite worth while to go carefully into the question whether perhaps the double bonds of unsaturated fatty acids may indeed be points of attack for the degradation of these acids in the living organism. It seems to us to be not at all improbable *a priori* that the degradation of a certain part of the unsaturated component acids of our food fats begins with a rupture of the carbon chain at the positions of the double bonds which are already present in these acids. The work discussed below is to be considered as a very first contribution in answer to this question. Encouraged by the success of our simple *in vivo* experiments with triglycerides derived from normal saturated fatty acids [8]), we have now made some similar experiments with triglycerides, containing unsaturated fatty acids as component acids, in the hope that we might perhaps succeed in isolating from the urine of the subjects partial degradation products of these fatty acids, pointing in this direction.

LEATHES, and LEATHES and RAPER respectively, assume the rupture of the unsaturated fatty acid chains to occur in such a manner that carboxyl groups are formed at the positions of the carbon atoms, forming the double bonds; oleic acid, for example, would thus break up into azelaic acid and pelargonic acid. In our opinion, this is *a priori* by no means certain. It is possible, for example, — work of QUAGLIARIELLO and his collaborators [9]) points in this direction — that the first phase of the one-sided β-oxidation is a desaturation of the fatty acid at the $\alpha\beta$-position, while it is often assumed that in this mode of degradation the two carbon atoms are split off in the form of acetic acid; thus, if the one-sided β-oxidation does indeed proceed in this way, the degradation products expected by the above-mentioned investigators are not formed on the rupture of the carbon chain of an $\alpha\beta$-unsaturated fatty acid. It does not seem impossible to us that the positions of the double bonds may in certain cases influence the nature of the eventual degradation products. Theoretical speculations on the possible course of the mode of degradation of unsaturated fatty acids, under discussion here, are meanwhile of very little use. OPPENHEIMER [10]), who as well as WIELAND [11]), accepts the possibility of such a rupture of the

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[8]) VERKADE, ELZAS, VAN DER LEE, Miss DE WOLFF, Mrs. VERKADE-SANDBERGEN and VAN DER SANDE, Z. Physiol. Chem. **215**, 225 (1933); VERKADE and VAN DER LEE, Biochem. J. **28**, 31 (1934); Z. Physiol. Chem. **225**, 230 (1934); **227**, 213 (1934).

[9]) MAZZA and ZUMMO, Rend. R. Accad. Naz. Lincei (6) **18**, 461 (1933); QUAGLIARIELLO, Progr. scient. 9e Congr. intern. de Chimie, Madrid, 1934, p. 98.

[10]) Chem. Grundlagen der Lebensvorgänge (GEORG THIEME, Leipzig), 1933, p. 143.

[11]) Über den Verlauf der Oxydationsvorgänge (FERD. ENKE, Stuttgart), 1933, p. 44.

carbon chain, correctly confines himself to the remark that two substances would then be formed from, for example, oleic acid, each with a chain of nine carbon atoms. Experimental work will have to show whether such a rupture of the chain of unsaturated fatty acids takes place in the living organism and which fission products may then be formed.

The problem of the catabolism of unsaturated fatty acids in the human organism is for various reasons of the greatest importance. Such fatty acids generally form a very considerable percentage of the component acids of our food fats. A study of the constitution of the unsaturated fatty acids occurring as such — oleic acid, linolic acid, linolenic acid, vaccenic acid, palmitoleic acid, erucic acid, etc. — shows at once that for these acids the degradation principles, known from the saturated fatty acids, *viz.* the β- and the ω-ovidation, by no means work out. If the β-oxidation should indeed take place via an $\alpha\beta$-unsaturated acid as intermediate product, nevertheless the double bonds, already present in the unsaturated component acids of our food fats, lie mostly in such positions that they render the unhindered continuation of the process of β-oxidation impossible. It is certainly a remarkable fact that until now, at least as far as we know, attention has never been drawn to this point.

§ 2. *Triundecylenin.*

The very pure sample of triundecylenin used by us (saponification value 284.1, calculated 285.1 ; iodine value 128.5, calculated 129.0) was obtained by esterification of carefully purified undecylenic acid (setting-point 23.6°; equivalent weight 185.0, calculated 184.16 ; iodine value 137.5, calculated 137.8) with glycerol in the presence of some zinc as a catalyst [12]) and refined according to the method used commercially.

OZAKI [13]) had already prepared this triglyceride before by esterification of the components by means of Twitchell acid and used it for feeding experiments on rats. It then appeared that within 2—3 hours after administration of a diet, consisting of a fat free basal diet with 5 or 2.7 % triundecylenin, the experimental animals died showing symptoms of paralysis. Upon examination in the same way the triundecylin, obtained by catalytic reduction of the triundecylenin used, appeared on the other hand, to be not only not poisonous but even nutritious. From this fact OZAKI draws the conclusion that "die giftigen Eigenschaften des Triundecylenins deshalb auf die ungesättigte Bindung im Molekül zurück zu führen sind".

Previous researches [8]) had shown us that in the series of simple triglycerides of the normal saturated fatty acids the terms with term-number 9 (tricaprin) and 10 (triundecylin) are by far the most strongly diacidogenic. Consequently it was very attractive to us also to include in

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

[13]) Biochem. Zeitschr. **177**, 156 (1926).

our researches a corresponding *un*saturated glyceride, *viz.* triundecylenin. For our researches on fat metabolism, published as yet, we constantly made use of human subjects; we have only very recently started experiments on animals in various directions. We therefore thought it advisable to make use of *human subjects* also in the planned experiments with triundecylenin. However, as a consequence of the observations by OZAKI on the deleterious action of this triglyceride, we had to take great care with such experiments and, at any rate in the beginning, only we ourselves might act as subjects. One of us partook of 15 g of triundecylenin, together with a liberal amount of carbohydrate, without externally perceptible injurious consequences; with the other one, on the contrary, the administration of 25 g of this triglyceride occasioned a slight nephritis (acute and marked proteinuria; in the sediment numerous hyaline and some granular cylinders, as well as fairly many red corpuscles). We therefore thought it advisable to give up further similar experiments.

It may be incidentally observed that this remarkable injurious action of triundecylenin on the kidneys should be investigated more closely in various directions. We hope soon to find an opportunity to do this.

To both subjects the triundecylenin was administered together with a liberal amount of carbohydrate. The addition of carbohydrate to the fat took place only because in our previous work on fat metabolism we had observed its stimulating action on the production of dioic acid acidosis and diaciduria; we now hoped to be able in this way to stimulate also in the case of triundecylenin the accumulation of partial degradation products of undecylenic acid in the blood and their excretion in the urine. The urine was collected during about 24 hours after the administration of the fat. *In both cases we succeeded with perfect certainty in isolating a very small amount of sebacic acid.* The method of working up the urine need not be described here in detail, as it agreed in principle with that, already communicated in previous papers.

From the urine of the subject V., to whom 25 g of triundecylenin was administered, about *10 mg* of crude sebacic acid with a melting-point of 122—127° was obtained. The mixed melting-point with a sebacic acid specimen, obtained in the usual manner from castor oil and melting at 132.5—133.5°, amounted to 125—131°. This substance was recrystallised from hot water and analysed:

2.500 mg → 5.38 mg CO_2 and 1.90 mg H_2O; C **58.7** % H **8.5** %
Calculated for $C_{10}H_{18}O_4$: C 59.36 % H 8.98 %.

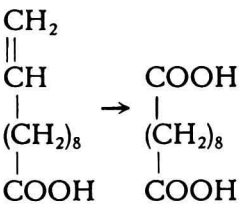
For the subject V. D. L., to whom *15 g* of triundecylenin had been administered, the amount of crude sebacic acid was about *20 mg*. After crystallisation from benzene and then from water, the product melted at 128—131°; mixed melting-point 129—133°. This substance was analysed:

3.991 mg → 8.74 mg CO_2 and 3.13 mg H_2O; C **59.7** % H **8.8** %
3.235 mg → 7.08 mg CO_2 and 2.54 mg H_2O; C **59.7** % H **8.8** %

It is to be regretted that because of the deleterious action of triundecylenin on the kidneys experiments with the administration of larger doses of this fat were inadmissible; without doubt larger amounts of sebacic acid would then have been excreted in the urine.

In judging this result, the following should be taken into account. Various investigators (BAER and BLUM; MORI; FLASCHENTRÄGER; SMITH) have proved that dogs and rabbits are able to burn higher normal saturated dicarboxylic acids. Sebacic acid was examined in this respect by BAER and BLUM [14]) with phlorhizin diabetic dogs and by FLASCHENTRÄGER [15]). By the latter, for example, 10 g of sebacic acid in the form of its sodium salt were subcutaneously injected into a healthy dog, weighing 21.2 kg, in amounts of 0.5 g twice a day; from the urine, collected throughout a fortnight, 61 % of the injected sebacic acid could be recovered, so that 39 % had been burnt, unless, of course, retention of the acid then still took place, which is indeed highly improbable. It should be remarked here that such injection experiments may easily give a too unfavourable impression of the combustibility of a substance, since at each injection the organism is suddenly flooded with this substance. Experiments by ANDERSEN [16]) further showed that adipic acid, administered *per os*, can be burnt also in the human body. The obvious conclusion that this will thus be the case with sebacic acid as well finds an indirect support in the results of researches recently published by us [17]). After administration *per os* of tricaprin, the urine of the subjects appeared to contain some suberic and adipic acid in addition to large amounts of sebacic acid; it is highly probable, not to say certain, that the first-mentioned acids were at any rate partly formed by degradation of sebacic acid. From all this it may certainly be concluded that the small amounts of sebacic acid, isolated by us from the urine after administration of triundecylenin, are in any case only part of those originally formed.

There is, therefore, according to us, not the slightest possible doubt that *in the degradation of triundecylenin in the human organism sebacic acid is formed as an intermediate product. This is only possible if the double bond of undecylenic acid is a point of attack in the degradation of this acid*:



[14]) HOFMEISTERs Beitr. **11**, 101 (1908).
[15]) Z. Physiol. Chem. **159**, 297 (1927).
[16]) Cf. FLASCHENTRÄGER, loc. cit., p. 299.
[17]) VERKADE and VAN DER LEE, Z. Physiol. Chem. **225**, 230 (1934).

§ 3. *Olive oil and rape oil.*

We included the first mentioned fat in our researches in view of its very high content of *oleic acid* as component acid. This acid is partly present as triolein, partly in the form of mixed triglycerides; in the cold pressed olive oil used by us ("extra vierge" of Etabl. C. Castel et Fils in Nice) triolein is decidedly the principal component. However, as follows from the results of one of our previous papers [18]), combined with not yet published results of experiments with synthetic mixed triglycerides, it is indifferent whether we have to deal with either simple or mixed triglycerides.

Considerable amounts of olive oil were administered fairly rapidly to the healthy subjects V. D. L. and V. — *e.g.* 150 g in three equal portions at about equal time intervals in the course of 10 hours — and together with a liberal amount of carbohydrate. While working up the urines, collected during 24 hours after administration of the first portion of fat, we noticed nothing of the presence of any substance, which might be considered as a partial degradation product of oleic acid; it is unnecessary to describe the method of working up the urine.

The fatty oils from the family Cruciferae are characterized by a high percentage (generally about 40—50 %) of *erucic acid* as a component acid; this is probably mainly present in the form of mixed triglycerides [19]).

To the healthy, sober subject V. D. L. we administered 110 g of oil of Brassica campestris L. in three practically equal portions at about equal time intervals in the course of 8 hours, together with a liberal amount of carbohydrate. While working up the urine, collected again during 24 hours, we did not find any indication in this case either of the presence of any substance, which might be regarded as a partial degradation product of erucic acid.

It stands to reason that from the negative results of our experiments with olive oil and rape oil the conclusion may not be drawn that the double bond of oleic acid or erucic acid is not a point of attack for the degradation of these acids. It is indeed quite well conceivable that under the conditions of our experiments the degradation products, formed by fission of the molecule at the position of the double bond, are not accumulated in the blood to a sufficient extent to lead to their excretion in the urine. Why this is then not the case after administration of large quantities of these fatty oils at a great rate, whereas it occurs after administration of only small amounts of triundecylenin (comp. § 2), is a question which lends itself splendidly to theoretical speculations but which, at the present stage of our work, is unsuitable for an exact discussion.

§ 4. For the sake of completeness the investigations of SMITH on the catabolism of oleic acid have yet to be discussed here. Indeed, in his

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[18]) VERKADE and VAN DER LEE, Z. Physiol. Chem. **225**, 230 (1934).

[19]) Cf. TÄUFEL and BAUSCHINGER, Z. Untersuch. Lebensm. **56**, 265 (1928).

second paper on this subject [20]) this author arrives at the following general conclusion:

> "The accumulating evidence thus seems to indicate that the metabolic oxidation of fatty acids proceeds in a manner independent of the presence or position of a double bond".

Apart from the fact that our experiments with triundecylenin, described in § 2, positively lead to the exactly opposite conclusion, much can be said against the argumentation employed by SMITH. In the first place all experiments made by him are based on the already mentioned hypothesis that azelaic and pelargonic acid should be produced on the rupture of the carbon chain of oleic acid at the position of the double bond. At most SMITH might consequently have concluded that *such* a rupture does not take place in the organism. The far more general conclusion cited above may not be drawn from the results of his experiments; for, as we incidentally pointed out in § 1, we need not by any means *a priori* and of necessity accept that the mode of fission of oleic acid, assumed by SMITH, is the only possible one.

SMITH's evidence consists of the results of feeding experiments on dogs. In the course of 6 days these dogs had administered to them in their food 20—45 g of azelaic acid in the form of its sodium salt, *i.e.* "amounts smaller than would be produced normally in the body if oxidation took place at the unsaturated linkages". Of course, what is meant here by SMITH is: smaller than the amount, which would be formed intermediarily if all unsaturated food fatty acids were to be *exclusively* attacked in the way suggested by LEATHES and RAPER. This, however, is an exceedingly rash suggestion. It is indeed very likely that the unsaturated fatty acids are catabolised in various ways; for the saturated fatty acids this has already been pointed out with certainty, merely as a result of our work. On an average about 60 % of the administered azelaic acid appeared to be excreted in the urine. During the various periods of 6 days the excreted amount varied greatly; it amounted to at most 91 % of 31.0 g and at the least 29 % of 35.3 g. It is this on the whole rather bad utilization of azelaic acid administered *per os*, which made SMITH doubt the correctness of the hypothesis in question and even made him draw the general conclusion cited above. This doubt is a result of SMITH's highly curious conception [21]) that "if the short chain mono- and dibasic acids, formed in the laboratory oxidation of the unsaturated acids, are fed to animals and are utilized, the theory stands; if they are not burned, the theory lacks direct support and falls". (!) It is unnecessary to point out here that SMITH made use of the results of his feeding experiments with azelaic acid for unpermitted interpretations.

§ 5. The researches discussed above have, to express it cautiously,

[20]) J. Biol. Chem. **103**, 531 (1933).

[21]) Ibid. **67**, Proc. Am. Soc. Biol. Chem. p. XXVII (1926).

produced a clear indication *that the double bonds of unsaturated fatty acids may indeed be points of attack for the degradation of these acids in the living organism, and that also in this mode of degradation normal saturated dicarboxylic acids are formed as intermediate products, or at any rate may be formed in certain cases.* It becomes constantly more apparent that the latter acids play a very important part in fat metabolism.

A further investigation into the catabolism of the unsaturated fatty acids is no doubt highly necessary.

We wish to make grateful acknowledgment to the HOOGEWERFF-Fonds of the Hague and the ERASMUS-Stichting of Rotterdam for a grant-in-aid of this work.

Astronomy. — *On the foundations of the theory of relativity, with special reference to the theory of the expanding universe.* By W. DE SITTER.

(Communicated at the meeting of October 27, 1934).

The restricted theory of relativity was based on the postulate of invariance of the laws of nature for LORENTZ-transformations, and the line-element of space-time accordingly was taken to be

$$ds^2 = -dx_1^2 - dx_2^2 - dx_3^2 + c^2\,dt^2 . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

In the general theory of relativity the invariance is postulated for *all* transformations of coordinates, and the line-element (1) is replaced by the more general riemannian form

$$ds^2 = g_{\alpha\beta}\,dx_\alpha\,dx_\beta , \qquad (\alpha, \beta = 1 \ldots 4) \quad (2)$$

with the restriction, however, that it shall be of index + 1, and therefore *locally* reducible to the galilean form (1). In other words the fundamental hypothesis is:

I. The laws of nature are expressible as equations between tensors in a continuous four-dimensional manyfold, the line-element (2) of which is reducible to the form

$$ds^2 = -a^2\,d\sigma^2 + v^2\,dt^2, \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

a and v being real functions of the four variables x_1, x_2, x_3, x_4, and

$$d\sigma^2 = \gamma_{pq}\,dx_p\,dx_q \qquad (p, q = 1, 2, 3)$$

being a positive definite quadratic form of the differentials of the three variables x_1, x_2, x_3.

In the popular expositions of the theory too much stress has as a rule