Chemistry. — *Researches on fat metabolism.* VI. *Experiments with α. lauro-βγ. diundecylin.* By P. E. VERKADE, J. VAN DER LEE and K. HOLWERDA. (Communicated by Prof. J. BÖESEKEN.)

(Communicated at the meeting of March 30, 1935.)

§ 1. We have felt justified in restricting the experiments, referred to in a previous paper [1]), with synthetically prepared mixed triglycerides containing diacidogenic and non-diacidogenic fatty acids as component acids, at any rate for the present, to those described below with *α. lauro-βγ. diundecylin.* This substance was obtained by the method of FISCHER, BERGMANN and BÄRWIND [2]); starting from acetone glycerol and lauryl chloride, α. monolaurin was prepared, which was then caused to react with undecoyl chloride in chloroform solution and in the presence of quinoline. After crystallisation from 96 % alcohol, the product obtained set at 28°.3; the saponification number was 276.4 (calc. 275.7).

36 g of this glyceride, melted in coffee, together with a liberal amount of carbohydrate, were administered to the sober healthy subject V. From this time onward the urine was collected with intervals of 1—2 hours, and tests were made for the presence of dicarboxylic acid, formed by ω. oxidation of fatty acid. The urine was collected until two successive portions no longer gave a positive reaction. Not until then did the subject again take food. The method of working up the urine need not be described here, as it agreed in principle with those, already communicated in previous papers. A quantity of 0.47 g of *undecanedioic acid* could be isolated from the urine. M.p. 107—109°; the mixed m.p. with a sample, obtained by the method of WALKER and LUMSDEN [3]) and melting at 110—111°, was 108—111°.

0.1914 g → 18.30 cm³ 0.0954 n. NaOH; equiv. wt. 109.4, calc. 108.1.

A similar experiment was made on the subject V. D. L. After administration of 35 g of the mixed triglyceride, in two equal portions with an interval of 4 hours and both times together with a liberal amount of carbohydrate, 0.27 g of *undecanedioic acid* could be isolated from the urine.

By these experiments it has been demonstrated, — and this more

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

[2]) Ber. **53**, 1589 (1920).

[3]) J. Chem. Soc. **79**, 1191 (1901); dissertation HARTMAN, Delft 1925, p. 27.

distinctly than was already previously [1]) done —, that not only simple but also mixed triglycerides may give rise to diaciduria. The *qualitative* results of these experiments conform to those of our previous work with triundecylin and trilaurin [2]): abundant excretion of undecanedioic acid and no excretion of dodecanedioic acid in the urine; indeed, no indication has been found of the presence of the latter acid.

§ 2. A direct *quantitative* comparison of the diaciduria caused by α. lauro-$\beta\gamma$. diundecylin on the one hand and an equal weight of a mixture of triundecylin and trilaurin in the molecular proportion 2 : 1 on the other hand is out of the question. For, as we pointed out briefly in a previous paper [2]), the amount of dicarboxylic acid excreted after administration of always the same amount of a diacidogenic triglyceride to one and the same subject under apparently similar conditions, can vary fairly considerably, owing to many, partly obvious circumstances.

On many grounds it is nowadays fairly generally accepted that fat as such is either not or hardly resorbed. Premising that the resorption by the cells of the intestinal mucosa is preceded by a complete hydrolysis of the fat to glycerol and fatty acids, we have succeeded in making a comparison of the diacidogenic properties, as indicated above, in a simple *indirect* way. A possible difference in the capacity to cause diaciduria can then only have its origin in a difference in the velocity, with which in the alimentary tract (mainly in the intestine) fatty acid, and particularly undecoic acid — for lauric acid is hardly diacidogenic — is liberated from α. lauro-$\beta\gamma$. diundecylin or from the corresponding mixture of triundecylin and trilaurin. We took advantage of the fact that in our laboratory extensive investigations are being made on pancreas lipase, to compare also the behaviour *in vitro* of the above mentioned mixed triglyceride and of the mixture of the two simple triglycerides towards this enzyme under various conditions, hoping thus to obtain useful indications regarding the course of their saponification in the alimentary tract.

For the purpose of these saponification experiments pure samples of trilaurin and triundecylin were prepared by esterification of the pure fatty acids with glycerol in the way described by VERKADE, VAN DER LEE and Miss MEERBURG [3]); they were finally crystallised from acetone. The sample of α. lauro-$\beta\gamma$. diundecylin, described in § 1, was also still further purified by crystallisation from this solvent.

A mixture of trilaurin and triundecylin in the molecular proportion 1 : 2 becomes just completely liquid at body-temperature (about 37°); it may be incidentally remarked that, on administration of such a completely melted mixture to a subject, partial setting in the body is decidedly out

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[1]) VERKADE and VAN DER LEE, Z. physiol. Chem. **225**, 230 (1934).
[2]) VERKADE and VAN DER LEE, Biochem. J. **28**, 31 (1934).
[3]) Rec. trav. chim. **51**, 850 (1932).

of the question. However, by way of precaution, we made our saponification experiments at 40°. In all experiments 0.4 millimols of triglyceride (= 1.2 milliequivalents of fatty acid) and 25 cm³ of an aqueous solution (see the tables) were brought into a stoppered bottle of 50 cm³ capacity and kept for some time in the thermostat. Then the pancreas extract — an aqueous dilution 1 : 10 of an extract of pancreas powder with 90 % glycerol prepared in principle by WILLSTÄTTER's method [1]) — was added and the bottle placed in the shaking-apparatus, built in the thermostat. After shaking for a certain time, the saponification was practically put to a stop by addition of 75 cm³ alcohol, and the quantity of fatty acid formed was determined by titration with sodium hydroxide. It stands to reason that all experiments of a series were carried out simultaneously and consequently under equal conditions; *e.g.*, all the bottles, belonging to the same series of measurements, were shaken in absolutely the same way. The technique of such saponification experiments will be discussed in detail elsewhere in another connection.

The following three series of measurements were made. The results are expressed in % of the quantity of fatty acid present in the glyceride; the accuracy amounts to about 1 % fatty acid.

Series I.

Aqueous solution: 9 cm³ (0.2 mol. NaH_2PO_4 + 0.2 n. NaCl) + 6 cm³ 0.2 mol. Na_2HPO_4, diluted to 25 cm³.
Initial p_H: about 6.5.
Pancreas extract: 1 cm³.
Indicator in the titration of the fatty acid: thymolphthalein.

	fatty acid, formed in		
	20′	45′	90′
α. lauro-βγ. diundecylin	17 %	27 %	40 %
trilaurin + triundecylin 1 : 2	14 %	26 %	39 %

Series II.

Aqueous solution: 15 cm³ (0.2 mol. H_3BO_3 + 0.05 n. NaCl) + 6 cm³ 0.05 mol. $Na_2B_4O_7$ (Palitzsch), diluted to 25 cm³.
Initial p_H: about 8.0.
Pancreas extract: 0.7 cm³.
Titration of the fatty acid after addition of glycerol with phenolphthalein as indicator.

	fatty acid, formed in		
	15′	45′	90′
α. lauro-βγ. diundecylin	14 %	24 %	32 %
trilaurin + triundecylin 1 : 2	13 %	25 %	34 %

[1]) Z. physiol. Chem. **125**, 150 (1923).

Series III.

Aqueous solution: 10 cm³ (0.2 mol. H_3BO_3 + 0.05 n. NaCl) + 6 cm³ 0.05 mol. $Na_2B_4O_7$ (Palitzsch) + 1 millimol. sodium glycocholate, diluted to 25 cm³.

Initial p_H: about 8.2.

Pancreas extract: 1 cm³.

Titration as in series II.

	fatty acid, formed in		
	10′	40′	80′
α. lauro-βγ. diundecylin	6 %	21 %	38 %
trilaurin + triundecylin 1 : 2	4 %	22 %	37 %

From the results of these experiments it is evident that *in weak acid as well as in weak alkaline media, and in the latter case with and without addition of sodium glycocholate, no difference is perceptible in the rate with which under the influence of pancreas extract fatty acid is liberated from α. lauro-βγ. diundecylin and from the mixture of trilaurin and triundecylin.* The objection can, of course, be made that perhaps in both cases the product of hydrolysis is not the same mixture of lauric acid and undecoic acid (and their sodium salts), and consequently the perfect agreement of the results of our saponification experiments is only apparent. For various reasons, which cannot be discussed here in detail, we consider this very unlikely; in the course of the work on pancreas lipase carried out in our laboratory it appeared for example that under the conditions of our experiments undecoic acid and lauric acid and also sodium undecoate and sodium laurate have a different effect on the velocity of saponification of a triglyceride with pancreas lipase.

It is not our intention to try to explain here this interesting result from a consideration of the kinetics of the reaction. From the work on the hydrolysis of triacetin in aqueous solution with sodium hydroxide [1]) or hydrochloric acid [2]) it appears that here the ester groups are all — at any rate to a close approximation — hydrolyzed with the same speed, and that the rate of hydrolysis of a certain ester group is independent of the fact whether a neighbouring group is hydrolyzed or not. The same result was obtained by TREUB [3]) in his work on the hydrolysis of trilaurin in sulphuric acid solution. It would be the simplest explanation to assume that this applies also to the saponification in heterogeneous media under the influence of pancreas extract; in our experiments the undecoyl groups as well as the lauryl groups would then always be split off at the same rate, independent of their manner of linkage in a simple or in a mixed trigly-

[1]) JUL. MEYER, Z. physik. Chem. **67**, 257 (1909).

[2]) JUL. MEYER, Z. Electrochem. **13**, 485 (1907); GEITEL, J. prakt. Chem. **55**, 417 (1897); **57**, 113 (1898); YAMASAKI, J. Amer. Chem. Soc. **42**, 1455 (1920).

[3]) J. chim. phys. **16**, 107 (1918).

ceride. But with these emulsions the kinetics of the saponification is certainly more complicated [1]).

In our opinion we are now certainly justified in making the supposition *that also in the alimentary tract the two fatty acids will be liberated at the same rate from α.lauro-βγ.diundecylin and from the corresponding mixture of trilaurin and triundecylin.* On the basis of the above premise regarding the resorption of fat by the cells of the intestinal mucosa this implies, however, *that in these two cases the capacity to cause diaciduria is equally large.*

We wish to make grateful acknowledgment to the Hoogewerff-Fonds for a grant in aid of this work.

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[1]) Comp. TREUB, Proc. Roy. Acad. Sci. Amsterdam **20**, 345 (1917); J. chim. phys. **16**, 137 (1918).

Histology. — *A new procedure for the detection of gold in animal tissues: physical development.* By W. J. ROBERTS. (From the laboratory for Embryology and Histology, Utrecht, Holland. Director: Prof. J. BOEKE). (Communicated by Prof. J. BOEKE).

(Communicated at the meeting of April 27, 1935).

As recent researches have shown, gold-compounds, injected therapeutically or experimentally, can to a certain degree be traced in the Reticulo-Endothelial system. We therefore wished to include these substances in our experiments on the behaviour of the R.E.S. towards vital dyes, etc., and started studying the several possibilities for the detection of gold in animal tissues. It is, however, superfluous to describe the methods in question in detail, since in 1933 Mr. P. ANSTETT (Comparaison entre les méthodes de détection de l'or dans les tissus au cours de la chrysothérapie, Thése de Lyon) has published a critical study of the several ways for the detection of gold; a simple enumeration of these processes will therefore be sufficient here. The principle techniques then are the following:

1. chemical analysis of tissue-ashes;
2. spectral analysis of the tissue in bulk;
3. reduction of gold bij means of $SnCl_2$ (CHRISTELLER);
4. reduction of gold by means of metallic silver, derived from $AgNO_3$ by photochemical decomposition in the presence of the tissue (BORCHARDT);
5. spectral analysis of very limited tissue-areas.

Whereas the first and second techniques naturally do not aim at histolo-