RESEARCHES ON FAT METABOLISM

XII. THE INFLUENCE OF CARBOHYDRATE ON DIACIDURIA

by

P. E. VERKADE, J. VAN DER LEE, AND M. ELZAS
Laboratory for Organic Chemistry, Technische Hoogeschool, Delft (Netherlands)

§ 1. In previous papers[1] arguments have been presented which are in our opinion strongly in favour of the concept that all normal saturated fatty acids are broken down not only by *unilateral β-oxidation*, but also by *ω-oxidation* and subsequent *bilateral β-oxidation* of the dicarboxylic acids formed. For a number of lower fatty acids the last-mentioned type of oxidative degradation has been proved with certainty by means of experiments on human beings and animals, and for the higher fatty acids it has been shown to be very probable.

As concerns the breakdown of unsaturated fatty acids *in vivo* actually very little is known yet with certainty. It has been suggested elsewhere by one of us[2], upon grounds which we consider acceptable, that there also the two types of oxidative degradation in question may play a part.

It should be mentioned here that a somewhat divergent view is defended by FLASCHENTRÄGER and especially by his former collaborator BERNHARD, and most recently in a paper by the latter[3]. According to these workers, only those normal saturated fatty acids containing 8–11 carbon atoms undergo ω-oxidation. This view seems to us insufficiently founded and highly illogical; to contest it at length would, however, be out of place in the present paper. War conditions have unfortunately so far prevented us from presenting arguments against the work of BERNHARD, but we hope soon to be able to do so.

For the rest it is obvious that the possibility of still other oxidation pathways of the fatty acids than the two mentioned above must be kept in mind.

§ 2. It has long been known that there exists a very typical relation between the oxidative degradation of the fatty acids and carbohydrate metabolism. We are thinking here of the phenomena of *ketosis* and *ketonuria*, and particularly of those cases where — SNAPPER[4] has shown that this is not always so — a greatly increased production of ketone bodies in the liver is the dominating cause of the accumulation of these substances in the blood and thus of their excretion with the urine. Briefly, but also very superficially, it may be said that the phenomena mentioned occur when exogenic carbohydrate is either not available in sufficient quantity (for instance, hunger ketosis) or cannot be metabolised in the proper way (for instance, diabetic ketosis or ketosis in glycogen disease = hepatomegalia glycogenica).

For the sake of completeness attention may be called to the following. Some years ago, particularly by the school of SZENT-GYÖRGYI[5], the curious conception has been

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brought forward that pyruvic acid and hence carbohydrate is the mother substance of the ketone bodies. It will already be plain from the above that we cannot agree with this view[6]. It is not to be denied that under certain conditions the conversion of pyruvic acid into ketone bodies has been realized *in vivo* (see, for instance, the work of ANNAU[7] and of KREBS and JOHNSON[8] with surviving tissues), but on the other hand it is quite impossible to disregard the extensive body of facts which point distinctly to fatty acids (fat) as the ketogenic substances *par excellence*. We consider it unnecessary to cite literature in support of this statement, except to quote the beautiful work of WEINHOUSE *et al.*[9], who demonstrated the formation of ketone bodies from carboxyl-labelled caprylic and butyric acid.

For the present it is sufficient for our purpose to state that *the phenomena of ketosis and ketonuria are related to carbohydrate metabolism*. We need not enter here into considerations on the nature of this relation. In our opinion the results of the work to be discussed in the present paper may be able to throw some light on this complicated problem.

§ 3. Shortly after starting our work on fat metabolism it was found[10] that after administration of certain monoacid saturated triglycerides to healthy subjects, accumulation of the dicarboxylic acid formed by ω-oxidation of the component acid in the blood and consequently excretion of this substance with the urine occurs: this as a result of the striking fact[11], which actually has little or nothing to do with fat metabolism, that a number of normal saturated dicarboxylic acids, namely those with 6–11(12) carbon atoms, are for some reason difficultly combustible *in vivo*. We then called the two phenomena in question *dioic acid acidosis* (the name *diacidosis* would be shorter, but in our opinion less clear) and *diaciduria*, respectively.

As a matter of course, directly after the discovery of these two phenomena, the question arose as to *whether they might not also be related to carbohydrate metabolism*. This was indeed found to be the case; in a further study of the diaciduria produced by triundecylin, addition of a liberal amount of carbohydrate to the diet proved to lead in general to a pronounced rise of the undecanedioic acid excretion. This fact was published long ago[12], and it has been repeatedly made use of in our further researches on fat metabolism. A description of the work carried out before the war by which we demonstrated the existence of the relation in question is, however, still lacking, mainly because we wished to supplement our data in a certain direction. Circumstances being still unfavourable, however, we are presenting our investigations now in their present form. It may be remarked that even so the results are in our opinion quite certain and trustworthy.

The monoacid saturated triglycerides are *diacidogenic* to very different degrees, *i.e.*, the administration of these substances under otherwise as far as possible similar conditions to the same subject or the same animal leads to very different degrees of dioic acid acidosis and diaciduria. In man these phenomena are by far the most striking after consumption of tricaprin or triundecylin[10]; we are not at present in a position to say which of these triglycerides is the more affective. Because of this these two substances were the most suitable for the experiments on human beings which we had in mind. For a purely incidental reason we have until now used triundecylin exclusively: the quantitative determination of undecanedioic acid (the ω-oxidation product of undecanoic acid, the component acid of triundecylin) in the urine was found to be easier in various respects, which we shall presently mention, than that of decanedioic acid = sebacic acid (the ω-oxidation product corresponding to tricaprin).

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So far we have concerned ourselves only with diaciduria. No direct experiments with regard to dioic acid acidosis, *e.g.*, determinations of the dioic acid content of the blood, have been carried out.

§ 4. The following method was worked out for the *quantitative determination of undecanedioic acid in urine.*

An aliquot part of the urine was treated at room temperature with 5 % of its volume of 85 % phosphoric acid and then extracted with vigorous shaking with an equal volume of ether. This extraction was repeated twice. The filtered extracts were combined and the ether distilled off. The residue was dried at 80–90° for not too long a time.

On shaking urine which has been treated with phosphoric acid with ether very troublesome emulsions are often formed. This can be prevented by shaking the urine in advance for a short time with a little active charcoal. For that purpose we used carbo animalis Ph. Ned. Ed. IV, 5–10 mg of which per 100 ml of urine was always found to be sufficient.

The residue was boiled under reflux first with 100 ml and then three times with 50 ml portions of benzene. The hot benzenic extracts were poured through a small filter and combined in a 750 ml flask. After the addition of 100 ml of water the benzene was very carefully distilled off.

In this extraction with benzene the hippuric acid present in very variable, but sometimes quite large quantities remains almost entirely undissolved; this acid is very little soluble even in boiling benzene[13]. Reference is made to § 5, where the results are given of some control determinations of undecanedioic acid in urine to which a considerable amount of hippuric acid had been added.

The solubility of the normal saturated dicarboxylic acids in benzene shows a pronounced alternation, *i.e.*, the acids with an odd number of carbon atoms are more soluble than their nearest neighbours with an even number of carbon atoms; the latter are very little soluble even in boiling benzene. This is the first reason why we preferred the quantitative determination of an odd dicarboxylic acid, namely undecanedioic acid, to that of an even dicarboxylic acid, and why triundecylin and not tricaprin was used in the experiments to be described below.

The remaining hot aqueous solution was filtered with suction through a GOOCH crucible, the bottom of which was covered with two hardened filters, in order to remove a little very sticky oil. The flask and the GOOCH crucible were rinsed with boiling water. The filtrate was transferred gradually and quantitatively to a small beaker and concentrated on a small flame to about 40 ml.

If during the concentration of the aqueous solution any appreciable amount of sticky oil separated out, which might happen especially in dealing with larger quantities of urine (for instance, 500 ml), then after partial evaporation the solution was again filtered through a small wad of cotton.

The beaker with the aqueous concentrate was placed in the ice-chest. The following day the undecanedioic acid which had crystallised out — after inoculation if necessary — was transferred quantitatively into a weighed glass filter-crucible, using only the mother liquor which was kept ice-cold. The crucible with contents was then washed a few times with a few ml of ice-cold water and finally dried at about 90°, and weighed.

The solubility of undecanedioic acid in cold water is very slight; according to WALKER and LUMSDEN[14] 100 ml of solution saturated at 20° contain only 14 mg of this acid.

The solubility of the normal saturated dicarboxylic acids in water alternates in the same way as that in benzene, *i.e.*, acids with an odd number of carbon atoms are more soluble in water than their nearest neighbours with an even number of carbon atoms. The higher even dicarboxylic acids present however the disadvantage that they crystallise slowly and have a tendency to form supersaturated solutions. This is the second reason why we preferred the quantitative determination of undecanedioic acid over that of sebacic acid and thus used triundecylin and not tricaprin in our investigations.

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§ 5. In Table I the results are given of a number of control determinations. The amounts of pure undecanedioic acid shown were dissolved by warming in 500 ml of urine. After cooling — undecanedioic acid is naturally considerably more soluble in urine than in water — the determination of the acid was carried out as described in § 4. The results leave nothing to be desired.

TABLE I

UNDECANEDIOIC ACID IN 500 ml OF URINE

Added	Found	Added	Found
60 mg	63 mg	162 mg	162 mg
70 ,,	73 ,,	180 ,,	175 ,,
89 ,,	86 ,,	200 ,,	199 ,,
119 ,,	121 ,,	207 ,,	200 ,,
158 ,,	157 ,,	208 ,,	207 ,,

Equally satisfactory were the results of the two following control determinations (Table II), where a considerable amount of hippuric acid had also been added to the urine. In order to be able to judge the degree of purity of the undecanedioic acid isolated, the melting point and the equivalent weight as found by titration (indicator phenolphthalein) are also given; pure undecanedioic acid melts at 110.5–111.5°, its equivalent weight is 108.1.

TABLE II

HIPPURIC ACID	UNDECANEDIOIC ACID			
Added	*Added*	*Found*	*m.p.*	*equiv. wt*
200 mg in 500 ml of urine	100 mg	97 mg	108–110°	109.0
200 mg in 500 ml of urine	200 ,,	198 ,,	109–111°	109.8

The determination of undecanedioic acid in the urine of the subjects was always performed at least in duplicate. In Table III the results are given of a number of randomly chosen duplicate determinations; the agreement is always sufficient and in fact is generally excellent. The amount of urine used in these determinations was usually appreciably smaller than in the control determinations discussed above; further particulars with regard to this point are in our opinion unnecessary.

TABLE III

	UNDECANEDIOIC ACID			
Test person V. D. L.	59–62	106–106	215–217	376–378
,, ,, D.	55–57	140–141	239–240	
,, ,, V.	40–45	130–131	219–223	
,, ,, V. D. B.		139–144	263–264	

The possibility has of course also been considered that the urine of subjects who had taken triundecylin might contain some or even all of the undecanedioic acid in a conjugated form (for example, combined with glycocoll or glucuronic acid). We believe

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we are justified in stating that this is not the case. We have repeatedly determined the content of undecanedioic acid in urines which had been pretreated by boiling for a considerable time with hydrochloric acid or with caustic soda solution; the results obtained in these determinations always agreed very well with those obtained from urines which had not been pretreated. For the sake of brevity we have omitted these data.

§ 6. As regards the arrangement and course of our experiments with triundecylin the following general details may suffice:

As far as was known or could be determined by an external examination by one of us (ELZAS), our subjects were all in good health. They took in the morning on an empty stomach 25 g of triundecylin, melted in broth with as little fat as possible or preferably in black coffee, with or without a liberal amount of carbohydrate. During the whole experiment they kept as quiet as possible and were only supplied, if desired, with a limited amount of water. At intervals of 1 to 1½ hours the urine was carefully collected and each time some ml of it tested for the presence of undecanedioic acid by the addition of 85 % phosphoric acid; the undecanedioic acid separated out directly or after some time, depending on the concentration, in a characteristic way in crystalline form. This sensitive reaction was if necessary performed by the subjects themselves. As a rule, at a certain moment a positive reaction was obtained. The time between the consumption of the test breakfast and the occurrence of a positive reaction naturally varied; the same was true of the length of time during which undecanedioic acid was excreted. These facts require further discussion which will be found in § 11. The urine was still collected for several hours after the reaction for undecanedioic acid had become negative again. These experiments seldom took more than fifteen hours, often not more than twelve. In those cases where the reaction for undecanedioic acid failed to give a positive result the collection of the urine was continued for 24 hours, sometimes even longer. The different portions of urine were combined and any undecanedioic acid which was then still undissolved because of the use of phosphoric acid as a reagent was brought into solution by the addition of caustic soda solution, whereupon the whole was brought to a known volume. Finally the content of undecanedioic acid was determined at least in duplicate in a suitable aliquot part of this diluted urine.

After some days, during which the subjects lived a normal life, a similar experiment was carried out, this time omitting or adding carbohydrate as the case might be.

§ 7. With respect to § 6 the following remarks must be made.

a. As subjects, in addition to two of the present authors (V. and V. D. L.), there were a teacher (V. D.), a number of students of the Nederlandsche Handels-Hoogeschool in Rotterdam (BE., BEN., BR., V. BU., DE., KR. and V. D. M.) and three nurses (V. D. B., BL. and V. S.); the ages are shown in the tables in § 9 and varied from 19 to 47 years.

Obviously it would have been desirable to have prescribed bedrest for our subjects during the whole test period (from the first to the last day on which triundecylin was administered) and also a few days before it began, and to have given them on the free days a fixed diet. For obvious practical reasons, however, this was unfortunately impossible; we were compelled to give our subjects absolute freedom before and between the test days, and we could only try to impress upon them the necessity of keeping quiet and eating as normally and regularly as possible. Consequently it was inevitable that the condition of our subjects, for example the state of their glycogen depots and

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the intensity of their metabolism, will by no means have been constant during the successive experiments of a single test period. This fact must certainly be regarded as a serious objection to the arrangement of this part of our work. We could indeed have kept the subjects in bed during the test days, those on which triundecylin was administered, but it seemed to us that the improvement thereby achieved in the total, necessarily somewhat unsatisfactory, situation would not have been of decisive importance.

It is nevertheless out of the question to suppose that on account of the non-constant condition of our subjects the results obtained are misleading. From the survey in § 9 appears that the administration of carbohydrate in addition to triundecylin has a distinct and generally even pronounced effect on the degree of diaciduria. This cannot be accidental: the number of experiments is too large and the differences between the results of the experiments with and without carbohydrate carried out with one and the same subject are too striking and too regular. Moreover, we sometimes began the test periods with a test with triundecylin and carbohydrate and sometimes with only triundecylin; these two procedures were found to lead to the same general picture of the results.

b. As source of carbohydrate we used chiefly white bread and further, cane sugar and orange. Our intention of carrying out further experiments using only cane sugar as the carbohydrate unfortunately could not be realized because of external circumstances. The question of course now arises as to whether the protein of the white bread may have played some part. Must the increased excretion of undecanedioic acid on the test days when white bread was one of the components of the diet be ascribed wholly or partly to this protein, in connection for instance, with its strong specific dynamic action? In our opinion this question may be answered with a decided negative. In the first place the amount of protein administered to the subjects — the protein content of white bread being at the time about 7.5 %, this amount was about 4–12 g — is certainly too small to cause the often pronounced effect in question. In the second place the experiments to be discussed in § 10 and § 12 provided clear arguments in the same direction.

c. Taking the carbohydrate content of white bread to be 45 % and that of orange 10 %, the total amount of carbohydrate in the experiments discussed here amounted to 36–102 g. Thus per gram of triundecylin 1½ to 4 g of carbohydrate was administered. This statement naturally brings to mind the well-known investigations of SHAFFER, WOODYATT, and others on the anti-ketogenic effect of glucose and the ketogenic: anti-ketogenic balance in man (the so-called "ketogenic: anti-ketogenic ratio"), but of course without desiring to suggest here any definite analogy or even placing a high value on that work; we simply mention that according to clinical investigations by WOODYATT[15] for the undisturbed progress of fat catabolism, thus in order to avoid ketosis and ketonuria, the combustion of 1 g of glucose per 1.5 g of "fatty acid" (the common component acids of foodstuffs) is necessary.

d. VERKADE and VAN DER LEE[16] have shown that after administration of triundecylin, in addition to undecanedioic acid nonanedioic acid (azelaic acid) and heptanedioic acid (pimelic acid) were also excreted with the urine of the subjects. It would therefore have been more correct in the experiments described in this paper to have determined not the content of undecanedioic acid in the urine, but the total amount of the dicarboxylic acids with odd number of carbon atoms. This, however, would have made our work very much more difficult; azelaic acid and pimelic acid, especially the latter, are considerably more soluble than undecanedioic acid. We felt justified in this omission

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because, according to our experience as recorded in the paper just cited, which is in agreement with that obtained in experiments by us and other investigators on dogs, the amount of the lower dicarboxylic acids compared with that of undecanedioic acid is always very slight.

§ 8. By way of example the records of several characteristic experiments are given below. No further explanation is necessary.

EXPERIMENT 5, 18 July 1932; subject V., ♂, 41 years.
8 h 25 g of triundecylin in fat-free broth, 70 g of white bread, 15 g of cane sugar, 20 g of orange (48 g of carbohydrate, 5.5 g of protein)

	VOLUME OF URINE	UNDECANEDIOIC ACID
10 h	200 ml	—
11 h	45 ,,	+
12 h	35 ,,	+++
13 h 30	80 ,,	+++
15 h	70 ,,	++
16 h 30	50 ,,	+
18 h	25 ,,	—

Urine collected until 20 h, the combined portions then diluted to 1000 ml; 2 × 250 ml of this liquid analysed: 219 and 223 mg, respectively, of undecanedioic acid. Thus total excreted *0.88 g of undecanedioic acid.*

EXPERIMENT 6, 25 July 1932; subject V., ♂, 41 years.
8 h 25 g of triundecylin in fat-free broth

	VOLUME OF URINE	UNDECANEDIOIC ACID
10 h	100 ml	—
11 h	30 ,,	+
12 h	25 ,,	+
13 h 30	25 ,,	++
15 h	35 ,,	+
16 h	15 ,,	—

Urine collected until 18 h, the combined portions then diluted to 1000 ml; 2 × 250 ml of this liquid analysed: 40 and 45 mg, respectively, of undecanedioic acid. Total excreted *0.17 g of undecanedioic acid.*

In both these experiments the test breakfast was well tolerated; no digestive disturbances occurred, which would of course have rendered the experiment worthless. This remark applies as well to all other experiments dealt with in this paper.

EXPERIMENT 20, 28 June 1932; subject Ben., ♂, 22 years.
8 h 25 g of triundecylin in fat-free broth, 160 g of white bread, 20 g of cane sugar, 100 g of orange (102 g of carbohydrate, 12 g of protein)

	VOLUME OF URINE	UNDECANEDIOIC ACID
10 h 30	135 ml	—
12 h 30	140 ,,	—
14 h	120 ,,	+
15 h	85 ,,	++
16 h	60 ,,	+++
17 h 30	290 ,,	++
19 h	80 ,,	++
20 h 30	105 ,,	+
22 h	30 ,,	—

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Urine collected until 8 h the following morning, the combined portions then diluted to 1500 ml; 2 × 250 ml of this liquid analysed: 175 and 173 mg of undecanedioic acid. Total excreted *1.05 g of undecanedioic acid.*

EXPERIMENT 21, 3 July 1932; subject Ben., ♂, 22 years.
8 h 25 g of triundecylin in fat-free broth

Undecanedioic acid reaction negative in all portions of urine. Urine collected until the following morning 8 h, the combined portions then made up to 1500 ml; 2 × 500 ml of this analysed: only *a very small amount of undecanedioic acid* was found to have been excreted.

EXPERIMENT 45, 8 February 1937; subject v. d. B., ♀, 25 years.
8 h 25 g of triundecylin in black coffee, 100 g of white bread (45 g of carbohydrate, 7.5 g of protein).

	VOLUME OF URINE	UNDECANEDIOIC ACID
9 h 15	100 ml	+
10 h 15	125 ,,	+
11 h 15	145 ,,	+
12 h 15	145 ,,	+
13 h 15	65 ,,	+
14 h 15	55 ,,	++
15 h 15	60 ,,	++
16 h 15	70 ,,	+
17 h 15	40 ,,	+ (very weak)
18 h 15	35 ,,	—

Urine collected until 20 h 15, combined portions then diluted to 1000 ml; 2 × 300 ml of this liquid analysed: 263 and 264 mg of undecanedioic acid. Thus total excreted *0.88 g of undecanedioic acid.*

EXPERIMENT 46, 11 February 1937, subject v. d. B., ♀, 25 years.
8 h 25 g of triundecylin in black coffee

	VOLUME OF URINE	UNDECANEDIOIC ACID
9 h 15	110 ml	+ (very weak)
10 h 15	105 ,,	+ (weak)
11 h 15	170 ,,	+ (very weak)
12 h 15	150 ,,	+ (very weak)
13 h 15	70 ,,	+ (very weak)
14 h 15	45 ,,	+ (weak)
15 h 15	30 ,,	+ (weak)
16 h 15	35 ,,	+
17 h 15	30 ,,	+ (weak)
18 h 15	15 ,,	—

Urine collected until 20 h 15, combined portions diluted to 1000 ml; 2 × 400 ml of this liquid analysed: 144 and 139 mg of undecanedioic acid. Total excreted *0.35 g of undecanedioic acid.*

It should be noted here that the indications of the intensity of the undecanedioic acid reaction used in the above tables have of course only a relative value.

§ 9. Table IV contains the results of all our experiments. The symbols + or — in the column with the heading test breakfast indicate whether or not carbohydrate was given in addition to 25 g of triundecylin. The figures in this column indicate the amounts in grams of carbohydrate and protein, respectively, in the diet.

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TABLE IV

EXPT.	DATE	SUBJECT	TEST BREAKFAST	UNDECANEDIOIC ACID IN URINE
1	31. 5.'32	V., ♂, 41 years	—	0.32 g
2	9. 6.'32	ditto	+ 77, 8.5	0.52
3	14. 6.'32	ditto	—	0.11
4	18. 6.'32	ditto	+ 66, 6	0.54
5	18. 7.'32	ditto	+ 48, 5.5	0.88 g
6	25. 7.'32	ditto	—	0.17
7	2. 8.'32	ditto	+ 48, 4	0.29
8	9. 8.'32	ditto	—	0.16
9	19. 8.'32	ditto	+ 57, 4.5	0.66
10	13. 5.'32	V. D. L., ♂, 31 years	—	0.42 g
11	16. 5.'32	ditto	+ 83, 10	1.59
12	20. 5.'32	ditto	—	0.19
13	23. 5.'32	ditto	+ 83, 10	1.51
14	14. 7.'32	ditto	+ 88, 10.5	0.60
15	23. 7.'32	ditto	—	0.28
16	31. 7.'32	ditto	+ 78, 9	0.90
17	5. 8.'32	ditto	—	0.31
18	27. 6.'32	BE., ♂, 19 years	—	0.57 g
19	30. 6.'32	ditto	+ 88, 8.5	1.49
20	28. 6.'32	BEN., ♂, 22 years	+ 102, 12	1.05 g
21	3. 7.'32	ditto	—	trace
22	2.11.'32	ditto	—	trace
23	9.11.'32	ditto	+ 72, 12	0.44 g
24	7. 6.'32	DE., ♂, 21 years	+ 81, 10	1.11 g
25	10. 6.'32	ditto	—	0.27
26	20. 6.'32	ditto	+ 68, 6	0.72
27	23. 6.'32	ditto	—	0.61
28	2. 2.'33	ditto	+ 36, 5.5	0.75 g
29	9. 2.'33	ditto	—	0.35
30	9. 6.'32	V. BU., ♂, 20 years	+ 54, 6	0.08 g
31	13. 6.'32	ditto	—	none
32	16. 6.'32	ditto	+ 53, 7	none
33	31. 1.'33	ditto	+ 67, 8.5	none
34	1.10.'32	V. D., ♂, 47 years	—	none
35	8.10.'32	ditto	+ 58, 4.5	0.31 g
36	15.10.'32	ditto	—	trace ?
37	29.10.'32	ditto	+ 38, 4	0.23 g
38	5.11.'32	ditto	—	trace

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EXPT.	DATE	SUBJECT	TEST BREAKFAST	UNDECANEDIOIC ACID IN URINE
39	23.11.'35	KR., ♂, 19 years	+ 74, 9	0.03 g
40	30.11.'35	ditto	—	none
41	24. 2.'37	BR., ♂, 19 years	+ 45, 7.5	0.69 g
42	1. 3.'37	ditto	—	0.29
43	6. 3.'37	V. D. M., ♂, 34 years	—	0.76 g
44	13. 3.'37	ditto	+ 45, 7.5	1.09
45	8. 2.'37	V. D. B., ♀, 25 years	+ 45, 7.5	0.88 g
46	11. 2.'37	ditto	—	0.35
47	16. 2.'37	V. S., ♀, 24 years	+ 45, 7.5	0.95 g
48	22. 2.'37	ditto	—	0.47

It is clear from Table IV that different subjects have very different tendencies to diaciduria. In some cases (for example subject V. BU., experiments 30–33) there is practically no such tendency; other subjects (for example subject V. D. L., experiments 10–13), on the contrary, are able under suitable conditions to excrete very considerable amounts of undecanedioic acid. The undecanedioic acid found in the urine is certainly only a part and probably even only a small part of that which is formed by ω-oxidation of undecanoic acid, the component acid of triundecylin. This is evident from the results of catabolism experiments carried out on men by BERNHARD and Miss ANDREAE[17] and by EMMRICH and EMMRICH-GLASER[18] with normal saturated dicarboxylic acids; it is here indeed a question only of dicarboxylic acids with an even number of carbon atoms, but there is no reason whatever to assume in this respect a contrast between the even and odd members of this homologous series, as appears for instance from the results of similar work carried out on dogs[19]. In this connection the well-known fact must not be forgotten, and we have already demonstrated this on earlier occasions, that, because of an enhanced reactivity of newly-formed molecules, reactions often proceed differently or go further than corresponds to the properties of the ordinary (non-active) forms of these molecules[20]. It is just the normal saturated dicarboxylic acids which offer excellent examples of this phenomenon; in a paper by one of us (VERKADE[21]) cases are enumerated in which dicarboxylic acids formed by oxidation processes *in vitro* proved to possess a much enhanced susceptibility for oxidative degradation. By blindly applying the results of experiments with dicarboxylic acids administered *per os*, where the organism is suddenly more or less overloaded with these substances, to cases where these acids are formed *in vivo* more or less gradually as intermediate products, a too unfavourable impression would certainly be obtained of their assimilability *in vivo*. Taking all this into consideration, the experiments described in this paper again lead to the conclusion that, of course under appropriate conditions, a considerable percentage of the undecanoic acid administered in the form of triundecylin may undergo ω-oxidation.

DEUEL and Miss GULICK[22] found that women have a much greater tendency to

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hunger ketosis and the resulting ketonuria than men; during the first day of fasting, for example, their female subjects excreted on the average 10 times as much ketone bodies as the males. To explain this observation the investigators mentioned suggest the possibility that in the male the glycogen reserve is larger or is more economically used during fasting. With regard to the development of diaciduria we have until now found no evidence of any influence of the sex of the subject, but the number of persons examined in that respect by us (only ten male and two female) is still very small. If there is any truth in the suggestion of DEUEL and Miss GULICK, the probability of an influence of sex on the development of dioic acid acidosis and diaciduria may in our opinion be regarded as considerable. Such a phenomenon might be brought to light only by an examination of a large number of male and female subjects.

As a rule considerably more undecanedioic acid was excreted when the test breakfast included also white bread, cane sugar and orange; we feel justified in concluding (cf. § 7) that this was because in addition to triundecylin a liberal amount of carbohydrate was administered. As good examples illustrating this fact experiments 10–13 and 34–38 may serve. There can be no question of mere coincidence here; the number of experiments is too large and the difference between the results of the experiments with and without carbohydrate is too striking and too regular; only the results of experiments 7 and 27 do not fit perfectly into the picture. As far as any excretion of undecanedioic acid took place in experiments with the subjects V. BU. and KR., who obviously had practically no tendency toward diaciduria, this occurred during experiments with test breakfasts containing carbohydrate. *In our opinion it is undeniable that there is a clear relation between the degree of diaciduria and the administration or omission of carbohydrate.* For further considerations on this point we refer to § 13.

§ 10. In a few cases we combined experiments such as described in § 6–9 with others in which 10 g of cane sugar was administered every hour to the subject after a test breakfast containing carbohydrate plus triundecylin; this was continued until the excretion of undecanedioic acid had stopped, that is for many hours. In Table V the results are given of these series of experiments, whose number we should like to have been larger. The new type of experiment is indicated by ++ in the column with the heading test breakfast. The test breakfast consisted of 25 g of triundecylin (—) or of the same amount of this triglyceride and 100 g of white bread (+ and ++). The figures

TABLE V

EXPT.	DATE	SUBJECT	TEST BREAKFAST	UNDECANEDIOIC ACID IN URINE
49	10.3.'37	V. D. L., ♂, 36 years	+ 45, 7.5	0.32 g
50	17.3.'37	ditto	++ 45, 7.5	0.72
51	24.3.'37	ditto	—	0.20
41	24.2.'37	BR., ♂, 19 years	+ 45, 7.5	0.69 g
42	1.3.'37	ditto	—	0.29
52	8.3.'37	ditto	++ 45, 7.5	1.02
43	6.3.'37	V. D. M., ♂, 34 years	—	0.76 g
44	13.3.'37	ditto	+ 45, 7.5	1.09
53	19.3.'37	ditto.	++ 45, 7.5	1.33

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in the column with the heading test breakfast have the same significance as those in the corresponding column of Table IV. The order of succession of the three types of experiments was varied expressly and for obvious reasons.

In all three cases, and therefore independently of the order of the experiments of a series, the amount of undecanedioic acid excreted was clearly largest when cane sugar had been administered regularly during the test day. This fact provides experimental support for the conviction already expressed in § 7, that the phenomenon is one connected with carbohydrate and not with protein. These series of experiments thus corroborate the conclusion we reached in § 9; *there is undoubtedly a relation between the degree of diaciduria and the degree of administration of carbohydrate.*

§ 11. The progress of the excretion of undecanedioic acid should now be dealt with. How much time elapses between the consumption of the test breakfast and the beginning of the excretion of the acid in question? How long does the latter continue and when does it reach a maximum? All these questions relate, of course, to each of the three types of experiments discussed above. While the data at our disposal are only qualitative in nature, — it would obviously have been much better to determine the amount of undecanedioic acid excreted in the urine quantitatively at regular intervals, for instance every hour —, they are in our opinion nevertheless sufficient for a very tentative answer to the questions just proposed.

It would be reasonable in every way to expect that in the experiments, performed on the same subject, with and without administration of carbohydrate in addition to triundecylin, there would exist a clear systematic difference in the progress of the excretion of undecanedioic acid, in the sense that as a rule after the consumption of a test breakfast containing carbohydrate the diaciduria would begin earlier or later and would last longer or shorter than after the consumption of a test breakfast free of carbohydrate. Curiously enough, this is not, or at least not clearly, the case, as is evident from Figs. 1–2 where the duration of the diaciduria is represented graphically for a number of experiments; the moments of beginning and ending of the excretion of undecanedioic acid estimated by aid of our qualitative test for this acid (cf. the experimental records given by way of examples in § 8) will be subject to an error of not more than ½ hour. In the cases of V. and BE., where the excretion of undecanedioic acid lasted for a relatively short time, the moment of maximum excretion could be estimated with not too great uncertainty (Fig. 1); it appeared to vary rather little in the different experiments and no systematic effect of the administration or omission of carbohydrate on its position could be detected. The facts under consideration are certainly interesting and will be discussed somewhat further in § 13.

The time between the consumption of the test breakfast and the beginning of the excretion of undecanedioic acid varied considerably among the different subjects. A few examples: in the case of V. D. it was about 6 hours, in that of V. about 3 hours, and in that of V. D. B. only about 1 hour; these values are of course only approximate. The time of only about 1 hour between intake of the triundecylin *per os* and excretion in the urine of a degradation product thereof, as observed several times by us and with different subjects, is strikingly short. There is no reason at all to assume here any exceptional position for the combined or free undecanoic acid: the successive even and odd members of the series of normal saturated fatty acids, or those of the corresponding series of monoacid triglycerides certainly show a gradual change in behaviour as far as the

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different phases of fat metabolism are concerned; it is not necessary to cite here literature on this wellknown fact. We therefore feel justified in the general conclusion *that the assimilation of fat (fatty acids) in the human organism*[23] *can take place much more rapidly than has, so far as we know, been hitherto ascertained or assumed.*

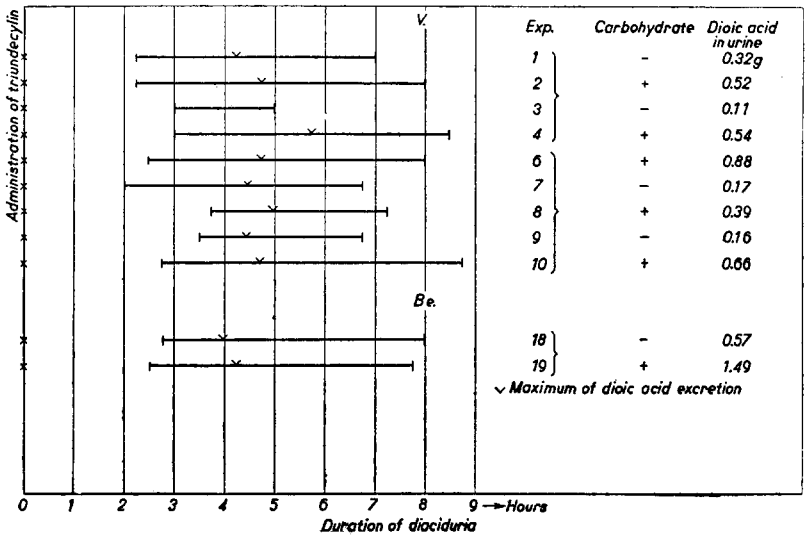

Fig. 1

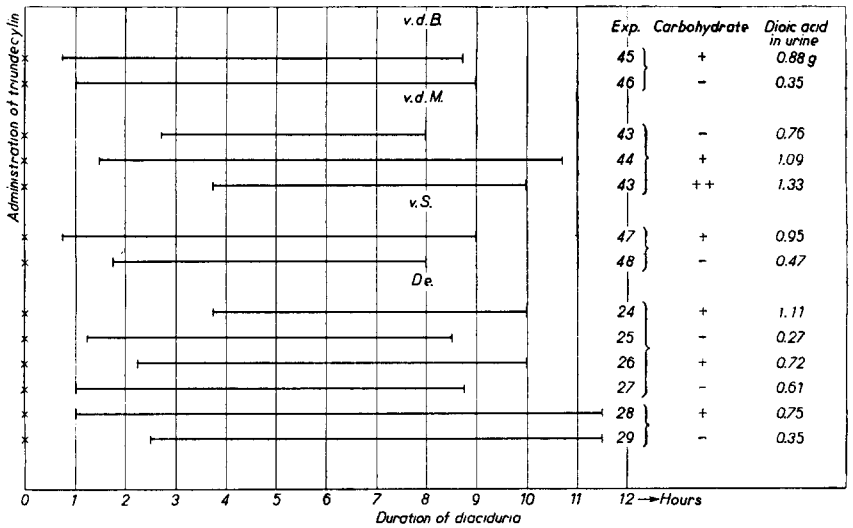

Fig 2

Appreciable differences were also encountered in the duration of the diaciduria in the different subjects. We refer here to the graphical representations (Figs. 1–2) of the experiments on V., Be., v. d. B., and De. In the case of the first two subjects mentioned the excretion of undecanedioic acid lasted appreciably shorter than in the case of the

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last two; especially DE. Does this mean that there are individual differences in the tendency towards development of diaciduria? We do not at all consider this to be out of the question, but we do not feel justified in reaching a decision on the basis of the data available at present. Further experimental investigation of this problem is needed.

To form an opinion on the problem just proposed is made difficult by the fact that the momentary condition of the subject will certainly and indeed obviously exert an influence on the development of the diaciduria. The experiments on DE., and especially those on V. D. L. and BR., which for incidental reasons are not represented in graphical form, show this plainly. In this connection it should be mentioned that in some cases, for instance in experiments 29, 49 and 54, the excretion of undecanedioic acid was irregular; in experiment 54 it was even interrupted for not less than about 2 hours.

§ 12. In order to obtain some insight into the nature of the relation demonstrated above between the degree of diaciduria and the supply of carbohydrate, the result of the experiment now to be described is in our opinion very important. The arrangement of this experiment is similar to that of those described in communication I of this series[7], to which reference may be made for particulars.

After a day of fasting in order partially to exhaust the glycogen depots, a quantity of 100 g of triundecylin, as a component of a diet very low in carbohydrate, was given for six successive days to the completely healthy subject V. D. L., who in previous experiments had shown a strong tendency to diaciduria. It is unnecessary to give details of the diet; it was as far as possible the same for all the test days and contained in addition to the triundecylin, estimated on the basis of the Netherlands foodstuffs tables of COHEN TERVAERT, an average of about 20 g of carbohydrate (almost entirely in the form of orange pulp), 90 g of protein and 85 g of fat, thus making a total of about 2200 calories. This diet was of course distributed over the day, obviously in as far as possible the same way for the successive test days; thus the triundecylin was taken in five portions of 20 g, melted in fat-free broth, at about 11, 14, 17, 20 and 23 h. During the test days and also during the fast day the subject was allowed water, tea (without milk or sugar), and fat-free broth; care was taken, however, that the intake of these beverages was regular and normal.

At 8 h blood was taken of the subject before breakfast for a determination of the carbon dioxide combining capacity of the bloodplasma[24]. The first meal was then eaten, which like all the following meals was followed by a short rest. Between meals until about 18 h some light laboratory work was done. The performance of much work would obviously have been undesirable for several reasons, for instance because of the lightness of the diet, which contained only about 2200 cal or only about 30 cal per kg body weight, while the basal metabolism of the subject (♂, 40 years old, height 170 cm, weight 72 kg), calculated according to HARRIS and BENEDICT, already amounted to 1704 cal. During the experiment the weight of the subject decreased only from 73.2 to 71.1 kg.

The urine was carefully collected by the subject over periods of 24 hours; each period was terminated by the collection of the urine voided directly upon rising. For each day's urine we determined the acidity using FOLIN's method[25], the ammonia content by means of FOLIN's method as modified by BARRENSCHEEN and WILHELM[26] (removal of the ammonia from the urine which had been made alkaline with soda by means of a powerful stream of air) — the formol titration, as would be expected, gave concordant results — and the content of undecanedioic acid.

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At the beginning of the first and at the end of the last test day the subject swallowed some carmine in order to mark the faeces formed during the test days. These were collected as carefully as possible. The dry matter of the different portions of faeces was found always to contain an entirely normal amount of "neutral fat + free fatty acid". The faeces contained a total of 17.2 g of this, while about 1100 g of fat, including 600 g of triundecylin, was consumed by the subject. There is thus no question of appreciable amounts of triundecylin having escaped absorption in the intestine. As has also been shown already in communication I of this series[7], the resorption of triundecylin leaves nothing to be desired. Establishing this fact is of course also of great importance in connection with the experiments described in §§ 6–11.

Table VI gives the results obtained. It is very interesting that the amount of undecanedioic acid excreted during the successive test days decreased regularly and rapidly. In our opinion it is certainly not rash to infer from this *that the dioic acid acidosis caused by the administration of triundecylin decreased regularly and rapidly during the experiment.* In agreement with this conclusion the changes in the acidity of the urine, and also — although the differences are here very small — those in the carbon dioxide combining capacity of the bloodplasma, indicate a decreasing acidosis. As to the amount of ammonia excreted, the inertia of the organism in its production is manifested here as always.

TABLE VI

	Plasma carbon dioxide combining capacity vol. %	Acidity of urine in ml 0.1 *n* alkali per day	Undecanedioic acid in urine g per day	Ammonia in urine g per day
fast day . . .	46	106	—	0.46
1st diet day .	42	258	4.29	0.70
2nd ,, ,, . .	—	451	2.85	1.24
3rd ,, ,, . .	43	476	1.38	1.89
4th ,, ,, . .	—	422	1.16	2.29
5th ,, ,, . .	44	376	0.74	2.48
6th ,, ,, . .	48	328	0.64	2.27

In a few other and less complete experiments of shorter duration with the subjects V. D. L. and V. this decrease in the dioic acid acidosis was found to be confirmed.

§ 13. The question now remains as to how the results of the experiments described in §§ 6–11, and that of § 12, are to be explained. For the present we shall confine ourselves to a few remarks only. We hope to go more deeply into the problem in a subsequent paper.

Since in the experiment described in § 12 the diet given to the subject and the experimental conditions were, so to say, always the same during the successive test days, it is in our opinion obvious *that the observed decrease in diaciduria, and therefore in dioic acid acidosis, had an endogenic cause.*

One might be inclined to try to arrive at the primary cause for the general result of the experiments dealt with in §§ 6–11 by supposing that the absorption of triundecylin in the intestine is accelerated by the simultaneous presence of carbohydrate, and the fat in question is consequently catabolised more rapidly. The relation observed in these ex-

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periments between the degree of diaciduria and the presence or absence of carbohydrate in the diet (§§ 6–9) or the amount of carbohydrate administered (§ 10) would then have a more or less exogenic cause. We think such an explanation to be unacceptable, however, especially in view of the fact that the course of the experiment described in § 12, which we are convinced is inextricably bound up with that of the experiments of §§ 6–10, would then still remain an open question. It should be noted here that as far as we are aware nothing is known about any difference in rate of absorption in the above sense[27]. We do not think such a difference probable. In this connection the fact mentioned in § 11 may be recalled — without attaching much significance to it — that in the experiments with and without carbohydrate in addition to triundecylin, performed on one and the same subject, no systematic difference was observed in the course of the excretion of undecanedioic acid.

Furthermore, one might be inclined to explain to some extent the course of the experiment of § 12 on the assumption that the organism gradually "learns" to catabolise completely the administered "körperfremde" fat (fatty acid); in other words, the question of habituation might arise. Such an assumption is however unsatisfactory, particularly for a reason analogous to the one already brought forward above: it is obvious that the course of the experiments discussed in §§ 6–11, the relation therein manifested between the degree of diaciduria and the amount of carbohydrate administered in addition to triundecylin would then remain completely unexplained; with regard to the experimental data in question nothing, of course, can be done with such vague concepts as "learning" of "habituation". In this connection the significance of the fact that triundecylin (undecanoic acid) is "körperfremd" — that is to say, does not occur normally in foodstuffs or body fat — might also be discussed. We shall not, however, go into this point here; we feel sure that the fact in question is of no consequence.

An explanation must be sought which is equally applicable to both types of experiments discussed.

In our view it is self-evident that in this connection special attention should be devoted to the liver. Indeed, in carbohydrate as well as in fat metabolism the liver plays an extremely important part. In the experiment discussed in § 12, after a fast day triundecylin was given to the healthy subject for six successive days as part of a diet very poor in carbohydrate, so that the glycogen reserve in the liver certainly underwent a gradual and considerable decrease; the amount of undecanedioic acid excreted daily also decreased gradually and very considerably. In the work discussed in §§ 6–11 administration of carbohydrate in addition to triundecylin on an empty stomach to the healthy subjects leads quickly to an increase in the glycogen store in the liver; in experiments of this type the amount of undecanedioic acid excreted was found as a rule to be definitely larger than in those experiments where only triundecylin was given to the same subject. *There is therefore most probably a relation between the degree of diaciduria, a consequence of the occurrence of a dioic acid acidosis, and the glycogen store in the liver, in the sense that they decrease or increase conjointly.*

Decrease in the store of glycogen in the liver leads to a pathological state. We must therefore consider a decrease in the diaciduria, and thus a decrease in the dioic acid acidosis, actually as a pathological fact. This seems to be a contradiction in terms. It must not be forgotten, however, that our experiments were always — and of course expressly — performed with a triglyceride whose component acid gives on ω-oxidation

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one of the "difficultly combustible" dicarboxylic acids[11]. This difficult oxidative degradation of the dicarboxylic acids with 8–11 (12) carbon atoms *in vivo* is, as already pointed out in § 3, a fact which actually has nothing to do with fat metabolism.

In view of what follows it seems useful to say again that with regard to dioic acid acidosis and diaciduria there is no contrast between fatty acids with even and odd number of carbon atoms. In principle, we might as well have carried out our experiments with tricaprin, *i.e.*, with a triglyceride whose component acid, the normal saturated fatty acid with ten carbon atoms, occurs as such in certain edible fats (for instance, in coconut fat). It was only for the experimental reasons mentioned in § 3 that we preferred to use triundecylin.

The oxidative degradation *in vivo* of fatty acids with an even number of carbon atoms (body fat, ordinary food fats) may under suitable conditions give rise to ketosis and ketonuria. In healthy persons — and we confine ourselves to such cases here because the work described in this paper was performed on healthy subjects — these phenomena become evident on fasting or after administration of a diet rich in fat and poor in carbohydrate, that is on decreasing the store of glycogen in the liver. Ketosis and ketonuria are typical pathological phenomena. These are generally familiar facts which thus need no illustration here[28].

Ketosis and dioic acid acidosis, ketonuria and diaciduria must therefore be considered as antagonistic processes with reference to the significance of the glycogen store in the liver.

We realize that the conclusion just arrived at is by no means a final one. For a further discussion of the problems involved we must refer, however, to another paper which we hope to publish in the not too distant future.

We wish to express our thanks to Mr J. H. VAN DER NEUT for his assistance in carrying out the undecanedioic acid determinations.

SUMMARY

1. The influence of carbohydrate on the excretion of undecanedioic acid in the urine after administration of triundecylin to healthy subjects has been examined.

2. In order to make this research possible, a method for the quantitative determination of undecanedioic acid in urine has been worked out.

3. After simultaneous administration of triundecylin and carbohydrate the amount of undecanedioic acid excreted in the urine is generally considerably larger than after administration of only triundecylin.

4. Administration of carbohydrate at regular intervals after the simultaneous supply of triundecylin and carbohydrate causes a further increase of the undecanedioic acid excretion.

5. There is thus undoubtedly a relation between the degree of diaciduria and the supply of carbohydrate in the diet.

6. Some remarks are made with regard to the onset, the maximum, and the duration of undecanedioic acid excretion. It was found that the assimilation of fat (fatty acids) in the human body can take place much more rapidly than has hitherto been ascertained or assumed.

7. On administering a daily quantity of 100 g of triundecylin, as part of a constant diet very low in carbohydrate, to a healthy person for six successive days under as nearly as possible constant conditions, the amount of undecanedioic acid excreted in the urine showed a regular and rapid decrease.

8. This observed decrease in the diaciduria, and therefore in the dioic acid acidosis, has an endogenic cause. In our opinion it is evident that special attention in this regard should be devoted to the liver.

9. There is probably a relation between the degree of diaciduria, as a consequence of dioic acid acidosis, and the glycogen store in the liver, in the sense that they decrease or increase together.

10. Ketosis and dioic acid acidosis, and ketonuria and diaciduria must than be considered as antagonistic processes with regard to the significance of the glycogen store in the liver.

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RÉSUMÉ

1. L'influence des hydrates de carbone sur l'excrétion de l'acide undécanedioïque par l'urine, après ingestion de triundécyline par des individus normaux, a été étudiée.

2. Cette étude a été rendue possible grâce à la mise au point préalable d'une méthode de dosage de l'acide undécanedioïque dans l'urine.

3. Après ingestion simultanée de triundécyline et d'hydrate de carbone, la quantité d'acide undécanedioïque excrétée dans l'urine est généralement beaucoup plus forte qu'après ingestion de triundécyline seule.

4. L'ingestion d'hydrate de carbone à intervalles réguliers après l'ingestion simultanée de triundécyline et d'hydrate de carbone, provoque un nouvel accroissement de l'excrétion de l'acide undécanedioïque.

5. Il existe ainsi certainement une relation entre le degré de diacidurie et la quantité d'hydrate de carbone fournie par le régime.

6. Quelques remarques sont faites en ce qui concerne le moment auquel commence l'excrétion de l'acide undécanedioïque, le moment auquel cette excrétion est maximum, et la durée de cette excrétion. Il a été constaté que l'assimilation des graisses (acides gras) chez l'homme peut se faire beaucoup plus rapidement qu'on ne l'avait soupçonné jusqu'ici.

7. Lorsque l'on fait ingérer à un individu normal une quantité journalière de 100 g de triundécyline, comme constituant d'un régime de composition constante et de teneur très faible en hydrate de carbone, cette ingestion étant faite pendant 6 jours successifs et dans des conditions aussi constantes que possible, la quantité d'acide undécanedioïque excrétée dans l'urine décroît rapidement et régulièrement.

8. Cette diminution de la diacidurie et, par conséquent, celle de l'acidose dioïque est d'origine endogène. Il apparaît que le foie doit jouer ici un rôle important.

9. Il existe probablement une relation entre le degré de la diacidurie qui résulte de l'acidose dioïque et la réserve en glycogène dans le foie, de telle manière que l'un et l'autre augmentent ou diminuent parallèlement.

10. La cétonémie et l'acidose dioïque, la cétonurie et la diacidurie, peuvent être considérées respectivement comme des phénomènes antagonistes en ce qui concerne la signification de la réserve en glycogène du foie.

ZUSAMMENFASSUNG

1. Der Einfluss von Kohlenhydrat auf die Ausscheidung von Undecandisäure im Harn nach Verabreichung von Triundecylin an gesunde Personen wurde untersucht.

2. Um diese Untersuchung möglich zu machen, wurde eine Methode für die quantitative Undecandisäurebestimmung im Harn ausgearbeitet.

3. Nach gleichzeitiger Verabreichung von Triundecylin und Kohlenhydrat ist die im Harn ausgeschiedene Undecandisäuremenge im allgemeinen beträchtlich grösser als nach ausschliesslicher Verabreichung von Triundecylin.

4. Kohlenhydratverabreichung in regelmässigen Zeitabständen nach gleichzeitiger Gabe von Triundecylin und Kohlenhydrat verursacht eine weitere Zunahme der Undecandisäureausscheidung.

5. Es besteht also zweifellos eine Beziehung zwischen dem Grade der Diacidurie und dem Kohlenhydratgehalt der Kost.

6. Einige Bemerkungen werden gemacht über den Beginnzeitpunkt, das Maximum und die Dauer der Undecandisäureausscheidung. Es wurde festgestellt, dass die Assimilation von Fett (Fettsäure) im menschlichen Körper viel schneller stattfinden kann als bisher bekannt war oder angenommen wurde.

7. Wenn eine tägliche Menge von 100 g Triundecylin, als Bestandteil einer konstanten, sehr kohlenhydratarmen Kost, einer gesunden Person an sechs aufeinanderfolgenden Tagen unter so weitgehend möglich konstanten Bedingungen verabreicht wurde, zeigte die im Harn ausgeschiedene Undecandisäuremenge eine regelmässige und schnelle Abnahme.

8. Diese Abnahme in der Diacidurie, also auch die in der Disäureacidose, hat eine endogene Ursache. Unserer Meinung nach ist es deutlich, dass in diesem Zusammenhang die Leber besondere Aufmerksamkeit verdient.

9. Es besteht wahrscheinlich eine Beziehung zwischen dem Grade der Diacidurie, eine Folge einer bestehenden Disäureacidose, und dem Glycogenvorrat der Leber, und zwar so, dass beide zugleich ab- und zunehmen.

10. Ketose und Disäureacidose, Ketonurie und Diacidurie müssen dann, was die Bedeutung der Glycogenreserve in der Leber betrifft, als antagonistische Prozesse betrachtet werden.

References p. 56.

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[26] *Die Laboratoriumsmethoden der Wiener Kliniken*, 1928, p. 614.

[27] The investigations carried out by I. BANG [*Biochem. Zeitschr.*, 91 (1918) 104, 111], J. J. VAN LEEUWEN [*Thesis*, Leiden 1930; *Ned. Tijdschr. Geneeskunde*, 79 (1935) 3983] etc. on the variation of the content of neutral fat in the blood after the administration of fat alone or of fat together with carbohydrate can of course give us no help here; the development or non-development of an alimentary hyperlipaemia is certainly partly controlled by endogenic factors. Moreover, if we did use the data in question in order to draw a conclusion about the rate of absorption of fat in the intestine, the conclusion would be just the reverse of the supposition discussed above.

[28] Cf. for example H. S. RAPER AND E. C. S. SMITH, *J. Physiol.*, 62 (1926) 17; J. R. MURLIN, E. S. NASSET, WM. R. MURLIN, AND R. S. MANLY, *J. Nutrition*, 12 (1936) 645; N. BLIXENKRONE-MÖLLER, *Z. physiol. Chem.*, 252 (1938) 117; etc.

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