Chemistry. — *Researches on Fat metabolism* **XI.** *Injection experiments on dogs with sodium salts of normal saturated dicarboxylic acids.* By P. E. VERKADE, J. VAN DER LEE, A. J. S. VAN ALPHEN and M. ELZAS.

(Communicated at the meeting of March 26, 1938.)

§ 1. It was shown in communication VIII of this series [1]) that sodium salts of normal saturated dicarboxylic acids supplied *per os* to one and the same dog under as nearly as possible the same conditions are excreted in the urine to an extent which decreases very typically on ascent of the homologous series. We have now repeated these experiments — the intention of doing so had already been announced — with the understanding that the sodium salts were supplied by injection. In the first mentioned work there is the possibility that the results were influenced by possible differences in the resorbability of the dicarboxylic acids or of their sodium salts by the intestinal wall; this is obviously excluded in the injection experiments described below.

§ 2. The healthy adult dogs Polly (about 7 kg, ♂) and Terry (about 9.5 kg, ♂) served for the experiments in question. Twice a day always at the same time (about 9 h. 30 and 14 h. 30) for two successive days the dogs received a subcutaneous injection of 20 cm^3 of a sterile solution of the disodium salt of the dicarboxylic acid to be investigated. The injection fluids were obtained by adding together the pure dicarboxylic acid and an aqueous solution of the theoretical amount of pure sodium carbonate and boiling until the carbon dioxide set free was completely removed. They always contained 0.0103 grammol. of dicarboxylic acid per 100 cm^3 and will thus always have been practically isotonic with the blood. They were feebly alkaline with respect to phenolphthalein; the p_H of the injection fluids prepared from the dicarboxylic acids included in this investigation (C_6, C_8, C_{10}, C_{13}) was not appreciably different, as both the first and the second dissociation constants of these acids differ fairly little from each other [2]).

Thus in these experiments the effect of supplying equimolecular amounts of the various dicarboxylic acids was compared, whereas this was done with equal amounts by weight of the disodium salts in our previous work, hence with a number of dicarboxylic acid molecules which decreases rapidly on ascent of the homologous series; for example, the ratio of the number of molecules then amounts to 1 : 0.66 for adipic acid (C_6) and brassylic acid (C_{13}). The procedure now adopted seems to us to be definitely more correct.

The injections took place in the inner surface of the thigh or in the chest and with the C_6-, C_8- and C_{10}-acid without any phenomena worthy of mention. With the C_{13}-

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[1]) P. E. VERKADE, J. VAN DER LEE, A. J. S. VAN ALPHEN and M. ELZAS, Proc. Royal Acad. Amsterdam, **40**, 746 (1937); Z. physiol. Chem. **250**, 47 (1937).

[2]) See, for example, G. L. VOERMAN, Rec. trav. chim. **23**, 265 (1904); R. GANE and C. K. INGOLD, Journ. chem. Soc. **1928**, 1598; **1931**, 2153.

acid a considerable oedematous swelling and red coloration of the skin rapidly appeared at the injection places. These symptoms usually disappeared in the course of a few days; once an abscess was formed, which on opening furnished a considerable amount of a haemorrhagic mucopurulent exudate in which no dicarboxylic acid could be detected. Precisely with a view to the occurrence of such symptoms it seemed to us advisible to extend the injections over not more than two days. On continuing them longer the danger would indeed become continually greater that the series of comparative experiments with different sodium salts would have to be broken off untimely.

It may be mentioned here in passing that much more violent symptoms occurred after injections of the disodium salt of the C_{16}-dicarboxylic acid, carried out in quite the same way. It seems that the toxic action of the higher dicarboxylic acids administered subcutaneously increases on ascent of the homologous series. It would be of interest to institute a careful investigation into this matter. Such work is indeed on our program.

During the different experiments with the same dog we attempted to get the latter to consume always twice a day at fixed times a meal which was the same in composition and weight (during the injection days 50 g dog biscuit + 50 g lean horse meat, afterwards only 50 g dog biscuit). Thanks also to the fairly small extent of the meals we generally succeeded in this. Such a regulation of the food intake appeared to us to be desirable for safety in these comparative experiments, since in man we have observed an influence of carbohydrate intake on the extent of the diaciduria occurring after the administration of triundecylin. This fact has already been mentioned several times; we hope to discuss it extensively in later publications. Water was always provided *ad lib.* for the test animals. A reasonable interval always occurred between successive experiments with the same dog, during which the animal resumed its normal life.

The urine was collected on the two injection days and several (at least 6) succeeding days. We have indicated elsewhere [3]) to a sufficient extent the way in which the urine was worked up and the isolation of the dicarboxylic acid excreted therein unchanged. We have not troubled ourselves this time with the lower dicarboxylic acids produced therefrom by β-oxidation.

§ 3. The table below gives a review of the results obtained. As a consequence of the small amount of adipic acid (1.20 g) injected on the one hand and the fairly large solubility of this acid in water on the other hand, we have unfortunately not succeeded in making an exact determination of the amount of adipic acid excreted in the urine. This was all the less possible as certainly only a fairly small (dog Polly) or small (dog Terry) percentage of the amount of acid injected proved to be excreted.

	Amount injected	Excreted unchanged	
		dog Polly	dog Terry
C_6 adipic acid	1.20 g	several %	a few %
C_8 suberic acid	1.43 g	about 66 %	about 57 %
C_{10} sebacic acid	1.66 g	„ 16 %	„ 16 %
C_{13} brassylic acid	2.01 g	none	none

[3]) See P. E. VERKADE and J. VAN DER LEE, Z. physiol. Chem. **227**, 215 (1934); P. E. VERKADE, J. VAN DER LEE, A. J. S. VAN ALPHEN and M. ELZAS, Proc. Royal Acad. Amsterdam, **38**, 945 (1935).

On comparing these results with those of the experiments with sodium salts supplied orally [1]) the difference in the fate of adipic acid strikes one. In the first case this acid is catabolised much better than suberic acid and in the second case somewhat worse. Unfortunately we have not yet succeeded in finding a really satisfactory explanation for this contrast. We presume that an investigation of what happens to the sodium salts or the dicarboxylic acids first in the stomach and later in the intestine will be able to clear up this point.

For the rest the results of the two researches show a striking agreement. We are now again led to the conclusion that *the "combustibility" of these acids in the organism increases rapidly on ascent of the homologous series* and this time this conclusion is, from the nature of the experiments, really undeniable.

It may be mentioned here that the investigation of the sodium salts occurred in the order C_{10}, C_{13}, C_8, C_6 with the dog Polly and in the order C_{13}, C_{10}, C_8, C_6 with the dog Terry, so that there can be no question of the results being explained by the animals gradually "learning better" how to degrade the dicarboxylic acid.

At the time of the publication of communication VIII we were not aware of the results of the latest feeding experiments with sodium salts of dicarboxylic acids by K. BERNHARD and Miss M. ANDREAE [4]), nor of those of the injection experiments with monoethyl esters of dicarboxylic acids by K. BERNHARD [5]). Hence we now make the remark that it is incomprehensible to us how Miss ANDREAE [6]) can still express a doubt of the correctness of the conclusion from our feeding experiments then already published in a preliminary form [7]). Indeed, the results of the investigations from FLASCHENTRÄGER's laboratory just quoted, although dealing with different test subjects or dogs and not always obtained under comparable conditions, do nevertheless as a whole point in the same direction. We shall esteem it a favour if FLASCHENTRÄGER and his coworkers will repeat carefully both our feeding experiments and our injection experiments.

§ 4. We now come to the question of the cause of the increase of the "combustibility" of the dicarboxylic acids with ascent in the homologous series. It was already mentioned in communication VIII that by the term "combustibility" we merely mean to indicate in a very general way the possibility of being catabolised and that the "velocity of oxidation" of a dicarboxylic acid — already in itself a very complex concept — need in no wise be *a priori* regarded as the only factor which determines its "combustibility".

F. P. MAZZA [8]), using the manometric technique of Barcroft-Warburg, has investigated the velocity of oxidation of some normal saturated

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[4]) Miss M. ANDREAE, Inaug.-Diss. Univ. Zürich, 1937; K. BERNHARD and Miss M. ANDREAE, Z. physiol. Chem. **245**, 103 (1937).

[5]) Z. physiol. Chem. **246**, 133 (1937)

[6]) Loc. cit., p. 14.

[7]) P. E. VERKADE and J. VAN DER LEE, Rec. trav. chim. **54**, 893 (1935).

[8]) Arch. sci. biol. (Ital.) **22**, no. 3 (1936).

dicarboxylic acids (C_4, C_5, C_6, C_8, C_9, C_{10}, C_{18}) by surviving liver and kidney tissue from the rabbit and the guinea pig. We shall not discuss this work in detail here. MAZZA formulates a number of conclusions which fit well with our own results and with our ideas — one may refer here merely to the somewhat vague conclusion that "gli acidi a massa molecolare maggiore sono più facilmente ossidati" — and which would for that matter be thankfully accepted by us were it not that we are very doubtful whether these conclusions are really all completely justified by the experimental data which when all said and done are rather meagre. Caution is still to be advised all the more as some analogous experiments bij EDSON [9]) with the liver tissue of the rat have furnished a different picture on the whole of the oxidisability of the dicarboxylic acids (C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{16}). A new and careful comparative investigation of the oxidisability of these dicarboxylic acids by surviving tissue is definitely desirable.

In our opinion the desired explanation is not or at least not mainly to be sought in an increase of the "velocity of oxidation" of the dicarboxylic acids on ascent of the series. We sometimes tend rather to the supposition *that the dicarboxylic acids can be stored temporarily in the body with increasing ease on ascent of the series.* They will then be excreted unchanged in the urine to a decreasing extent and it will be possible for them to be gradually burned to an increasing extent. It may be regarded as a point in favour of this supposition that the amount of lower dicarboxylic acids excreted in the urine and formed by β-oxidation of the dicarboxylic acid administered also appears to become smaller if the latter is situated higher in the series. At any rate the amount of "difficultly combustible" dicarboxylic acids present in the urine in our feeding experiments with hexadecanedioic acid [1]) on dogs was found to be strikingly small. It may be remarked that if an increase of the "velocity of oxidation" of the dicarboxylic acids were of predominant importance, one would rather expect the opposite.

We believe that we can reject out of hand the possibility of a considerable increase in the threshold concentration of the dicarboxylic acids in the blood on ascent of the homologous series, by means of which the increase of the "combustibility" of these acids could also be explained.

§ 5. There is a parallelism between the diacidogenic action of the lower, simple, normal saturated triglycerides with the dog [10]) and the "combustibility" of their ω-oxidation products when supplied to it by injection. It is indeed understandable that there is a tendency to reduce the explanation of the first mentioned phenomenon to that of the latter one. In our opinion it is however a question whether that would be permissible,

[9]) Biochem. J. **30**, 1855 (1936).

[10]) P. E. VERKADE, J. VAN DER LEE and A. J. S. VAN ALPHEN, Z. physiol. Chem. **247**, 111 (1937).

that is to say, whether the "combustibility" of the dicarboxylic acids is the only necessary element for the explanation of the diacidogenic action of the triglycerides. There is namely fairly certainly no question of any such parallelism with man. The variation of the diacidogenic action of the triglycerides on ascent of the homologous series is different in this case from that with the dog[11]), while the "combustibility" of the dicarboxylic acids, in so far as one can form an opinion at present[4]), shows qualitatively the same picture with both. The explanation of the first mentioned phenomenon would thus have to be different here, must be sought more deeply than in the case of the dog. This does not seem to us acceptable for the time being.

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11) P. E. VERKADE and J. VAN DER LEE, Biochem. J. **28**, 31 (1934).