CXXXV. THE FATE IN THE ANIMAL BODY OF PHENYLSUCCINIC ACID AND β -PHENYLHEXOIC ACID.

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IN a previous paper, Clutterbuck and Raper [1925] have shown that when the ammonium salts of fatty acids are oxidised with hydrogen peroxide, oxidation, besides taking place at the β -carbon atom, as described by Dakin [1908, 1, 2, 3], also takes place at the γ - and δ -carbon atoms. The γ - and δ -keto-acids formed in this way, on further oxidation, yield succinic acid. Since succinic acid occurs in the body in various tissues, and an enzyme, succinoxydon, capable of oxidising it, is widely distributed in animal tissues, it seemed possible that the process described above, which takes place *in vitro*, might be of biological importance. Little is known about the mode of production of succinic acid in the body and the experiments mentioned above suggested that oxidation of fatty acids at the γ - or δ -carbon atom might be one means by which succinic acid arises in metabolism. The experiments described in this paper are among several which have suggested themselves as likely to throw light on this hypothesis.

It was thought that if a fatty acid with a normal chain of four or more carbon atoms were substituted in the β -position by a phenyl group, so as to render the β -carbon atom less accessible to oxidation, then phenylsuccinic acid should be produced if γ oxidation, with removal of four carbon atoms, took place.

If phenylsuccinic acid is not easily oxidised in the body then it should be excreted in the urine if it were produced as pictured above. Administration of phenylsuccinic acid to a dog (dose 0.22 g. per kg.) showed in fact that it was not easily oxidised, since 61 % of the amount given was recovered from the urine. The main experiment was therefore undertaken. The acid chosen was β -phenylhexoic acid, which, if it were oxidised at the γ -carbon atom, would produce acetic acid and phenylsuccinic acid.

On administering this acid subcutaneously in the form of its sodium salt, 45 % of it was recovered from the urine chiefly as a compound with glycuronic acid, but not a trace of phenylsuccinic acid could be detected. The amount of unoxidised acid was probably greater than this since the method of isolation of the acid from the urine is not strictly quantitative. It appears therefore that β -phenylhexoic acid is oxidised with difficulty in the body and that phenylsuccinic acid is not one of its oxidation products. It seems probable therefore that in this instance γ -oxidation does not take place. Other experiments which have been designed to answer the question are being taken in hand.

EXPERIMENTAL.

Phenylsuccinic Acid.

The specimen used¹ was prepared by the method of Lapworth and McRae [1922]. It was converted into the sodium salt and an amount of this containing 1.4 g. of the acid was injected subcutaneously, on each of two successive days, in 10 % solution into a dog weighing 6.5 kg. The urine was collected from the time of the first dose to 48 hours after the last dose had been given. The urine, concentrated on the water-bath, was acidified with phosphoric acid and shaken out with ether and then extracted with the same solvent for 18 hours in a continuous extractor. On removal of the ether a crystalline mass was obtained. This was extracted several times with warm light petroleum, to remove traces of higher fatty acids, dissolved in hot water, clarified with charcoal, evaporated to small bulk and left to crystallise. In this way 1.71 g. of practically pure phenylsuccinic acid was recovered, M.P. 167°, equivalent 96.9 (calc. 97). The ether extract of the urine obtained by continuous extraction contained a small amount of an acid much less soluble in cold ether than phenylsuccinic acid. It was separated from the latter acid by means of a little warm ether and melted at 140–2°. It was not phenaceturic acid since a mixed M.P. determination caused the M.P. to drop to 110–17°. The amount obtained was too small for further investigation. In this experiment, therefore, 61 % of the phenylsuccinic acid given was recovered from the urine. The recovered acid, examined in 4.3 % solution in alcohol, was optically inactive.

β -Phenylhexoic Acid.

This acid was prepared by condensing butyrophenone with bromoacetic ester in dry benzene solution using a modification of the Reformatsky [1887] and Lindenbaum [1917] methods.

The crude β -phenyl- β -*n*-propylhydracrylic ester thus obtained was gradually dissolved in sulphuric acid and poured on to ice [Schroeter, 1907, 1908]; β -*n*-propylcinnamic acid separated and was recrystallised from light petroleum. The acid was neutralised with sodium hydroxide and reduced in

¹ This was kindly presented to us by Professor Lapworth.

the usual way with sodium amalgam. The crude β -phenylhexoic acid was recovered by acidification with hydrochloric acid and extraction with ether. It was dried and fractionated *in vacuo*. The acid was collected at 187–9° (20 mm.). Equivalent 188.5 (calc. 192). The glycine compound was prepared in case it might be required for purposes of identification. The acid chloride was prepared from a small portion of the acid by the action of phosphorus trichloride and converted into the glycine compound by the Schotten-Baumann method. A little unchanged acid was removed from the product of this reaction by extraction with light petroleum and the β -phenylhexoylglycine crystallised from aqueous alcohol. M.P. 131°. (N = 5.7 %, $C_{14}H_{19}O_3N$ requires N = 5.6 %.)

The same dog which had received the phenylsuccinic acid was given, subcutaneously, 1.5 g. of the β -phenylhexoic acid as the sodium salt in 10 % solution on each of two successive days. It produced no ill effects. The urine, collected for the succeeding 72 hours after the first dose, was worked up as in the experiment with phenylsuccinic acid. The ether extract of the acidified urine on evaporation left a straw-coloured syrup. It was extracted several times with warm light petroleum and this removed some unchanged acid together with traces of higher fatty acids. The latter were removed as insoluble barium salts and the unchanged acid recovered by acidification and extraction with light petroleum. That portion of the original ether extract which was insoluble in light petroleum failed to crystallise and inoculation with a trace of phenylsuccinic acid failed to bring about crystallisation. Since it reduced Fehling's solution slightly on boiling, the presence of a glycuronic acid compound was suspected. The syrup was therefore decomposed by boiling for 6 hours with *N*/50 sulphuric acid and again extracted with ether. The greater part of the ether extract was soluble in light petroleum and consisted of unchanged β -phenylhexoic acid. That part which was insoluble in light petroleum was boiled with 20 % hydrochloric acid and yielded a little more acid soluble in light petroleum. In all 1.36 g. of unchanged acid was recovered. Equivalent 196 (calc. 192). The aqueous solution obtained by hydrolysis with *N*/50 sulphuric acid after ether extraction was neutralised with sodium hydroxide and concentrated to about a tenth its volume on the water-bath. The solution thus obtained reduced Fehling's solution strongly on heating, gave the orcinol reaction and a crystalline osazone which could not be recrystallised owing to decomposition. All these properties being characteristic of glycuronic acid there is no doubt that the unchanged acid was present in the urine largely as an ether-soluble glycuronic acid compound. The unchanged acid recovered, as described above, was optically inactive. It amounted to 45 % of that administered.

SUMMARY.

1. Phenylsuccinic acid administered subcutaneously to a dog was excreted unchanged in the urine to the extent of 61 %.

2. β -Phenylhexoic acid administered similarly was excreted partly unchanged and partly as a glucuronate; 45 % of the acid was recovered in this way. Phenylsuccinic acid was not detected as one of its oxidation products.

3. These observations indicate that β -phenylhexoic acid does not undergo γ -oxidation in the body since this should lead to the formation and excretion of phenylsuccinic acid.

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