CLXXXIV. THE BEHAVIOUR OF δ -KETO-*n*-HEXOIC ACID IN THE PERFUSED LIVER.

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IN an investigation of the oxidation products produced from the ammonium salts of the saturated fatty acids by hydrogen peroxide it was shown by Clutterbuck and Raper [1925, 1] that γ - and δ -oxidation occurred, in addition to the β -oxidation which had been described previously by Dakin [1908, 1, 2, 3]. The γ - and δ -keto-acids produced in this way on further oxidation yield succinic acid, this change being more pronounced with the δ - than with the γ -keto-acids. This mode of oxidation of fatty acids may be of biological interest. Attempts have been made therefore to find out whether it occurs in the body. The results of the administration of β -phenyl-*n*-hexoic acid have already been described [Clutterbuck and Raper, 1925, 2]. If this acid underwent γ - or δ -oxidation and were further split between the γ - and δ -carbon atoms it should yield phenylsuccinic acid; the latter was however not found to occur. Since a large part of the β -phenylhexoic acid administered was recovered in the urine and was therefore apparently difficult to oxidise, the result was inconclusive. The experiments described in the present paper aimed at getting a more definite answer to the question as to whether succinic acid may be obtained by the oxidation of fatty acids in the body following δ -oxidation of the carbon chain. The sodium salt of δ -keto-*n*-hexoic acid has been subjected to liver perfusion with a view to determining whether it gives rise to acetoacetic acid or not. The following two modes of oxidation may be considered to represent the possible methods of breakdown.

If scheme (a) is correct then perfusion of δ -keto-*n*-hexoic acid through the isolated liver should not give rise to the formation of acetoacetic acid, whereas under scheme (b) a considerable production of acetoacetic acid would be expected. It has also to be borne in mind that acetic acid under certain conditions

gives rise to acetoacetic acid in the liver, but, as will be shown presently, this even if produced under scheme (a) could not account for the results obtained.

The experiments described consist of a comparison of the yields of acetone substances produced by *n*-hexoic acid and δ -keto-*n*-hexoic acid under conditions as nearly alike as possible. Three experiments were carried out with each acid and it was found that the δ -keto-acid, although yielding somewhat less acetone and β -hydroxybutyric acid than *n*-hexoic acid, gave amounts which were so large that there seems to be no doubt that it undergoes oxidation according to scheme (b). These experiments therefore furnish no support for the view that δ -oxidation of fatty acids followed by scission between the γ - and δ -carbon atoms occurs in the animal body.

After the perfusion both the blood and the liver were examined for the presence of succinic acid. None was found in the blood but small amounts were obtained from the liver. The presence of succinic acid in the liver did not depend however on the nature of the acid undergoing oxidation, since it was found on all occasions whether *n*-hexoic or δ -keto-*n*-hexoic acid had been added to the perfusing blood or when no addition had been made. It may be pointed out that Magnus-Levy [1902] found succinic acid in the liver of dogs and other animals after autolysis. The amount was independent of the period of autolysis and it seems reasonable to believe that it may have been present in the fresh organ.

The possibility that the acetone substances obtained from δ -keto-*n*-hexoic acid might have been produced from acetic acid has been referred to. It has been shown by Loeb [1912] that when the liver is perfused with blood containing sodium acetate a definite but small formation of acetoacetic acid occurs. This we have confirmed. But using for the perfusion the same conditions as were used for δ -keto-*n*-hexoic acid, the amount of acetone substances produced was very much smaller than with the latter acid. We conclude therefore that the formation of acetone substances when δ -keto-*n*-hexoic acid is oxidised in the liver must be due mainly to β -oxidation.

EXPERIMENTAL.

The δ -keto-*n*-hexoic acid was prepared as described by Clutterbuck and Raper [1925, 1]. When required 2 g. of the crystalline acid hydrate (equivalent to 1.76 g. acid) were dissolved in a small amount of water, neutralised with sodium hydroxide and added to the blood.

Perfusion.

The apparatus used for the perfusion was that described by Burn and Dale [1926] with the exception of the oxygenator. As originally described we found this to be inadequate when a dog's liver is used, although in our experience with a cat's liver, because of its smaller size, oxygenation of the blood has been sufficient. The modification we have introduced is illustrated by Fig. 1. In Burn and Dale's apparatus the blood after leaving the liver is led on to a

silver disc spinning rapidly at the top of a wide glass cylinder. This throws it on to the wall of the cylinder and it runs slowly to the bottom where it forms a reservoir. From this the blood is now drawn up to the pump which circulates it through a heating coil to the liver. Instead of depending for oxygenation on the contact of the film of blood on the wall of the cylinder with oxygen led into its interior as originally described by Burn and Dale, we have bubbled oxygen directly through the reservoir of blood at the bottom of the cylinder. For this purpose a Jena glass-filter (593/L7) in the shape of a thistle funnel was used. This produces a stream of extremely fine bubbles which form a compact froth on the surface of the blood. When a layer of froth about 10 cm. deep

Fig. 1

has been formed the oxygen may be shut off until absorption or bursting of the bubbles has reduced the layer to about 5 cm. It should then be increased again to 10 cm. by further admission of oxygen. The blood percolating through this fine froth becomes completely oxygenated.

The dogs were anaesthetised with ether and bled from the abdominal aorta. Warmed Ringer's solution was introduced into the distal end of the divided aorta between the intervals of bleeding. In all about 400–500 cc. of blood diluted with Ringer's solution were usually obtained. The bile duct, hepatic artery, and tributaries of the portal vein were then ligatured and a cannula was inserted into the vein itself. The thorax was immediately opened, an incision was made into the right auricle and 1–2 litres of ice-cold normal saline were passed through the liver. The object of this was to cool the liver so that it did not suffer from oxygen lack during the subsequent part of the operation for its removal when it had no blood supply. Having cooled the liver in this way a cannula was inserted into the inferior vena cava above the diaphragm and the vessel was then ligatured in the abdomen just above the suprarenal glands. The liver was then removed and placed in the perfusion apparatus.

The organ was perfused for 75 min. after the temperature of the entering blood had reached 38°.

Analyses.

Shaffer's method [1908] was used for estimating acetone, acetoacetic acid and β -hydroxybutyric acid in the blood after perfusion. Schenk's reagent was used for precipitating the blood proteins. As a rule 50 cc. of blood were taken for the estimation. The presence of substances other than acetone which appear in the distillate when either acetone and acetoacetic acid or β -hydroxybutyric acid are being determined necessitated the treatment of these distillates by Masuda's method [1912]. The figures in Table I give the results obtained. The acetone and acetoacetic acid are reckoned as acetone in the whole of the blood which was used in the perfusion. The figures for β -hydroxybutyric acid represent the acid itself and not acetone in the total blood.

Table I.

Experi- ment	Addition to blood	Acetone +aceto- acetic acid mg.	β -Hydroxy- butyric acid mg.	Wt. of liver g.	Volume of blood used cc.	Glycogen content of liver
1	Nil.	Trace	Trace	225	490	Poor
2	Nil.	7.7	2.8	200	400	Poor
3	Nil.	1.5	9.5	200	400	Rich
4	2 g. <i>n</i> -hexoic acid as Na salt	4.1	166	225	400	Poor
5	2 g. <i>n</i> -hexoic acid as Na salt	4.6	215	180	400	Poor
6	2 g. <i>n</i> -hexoic acid as Na salt	22.5	242	165	485	Moderate
7	2 g. δ -ketohehoic acid hydrate as Na salt	22.6	65	450	465	Poor
8	2 g. δ -ketohehoic acid hydrate as Na salt	21.4	124	270	425	Poor
9	2 g. δ -ketohehoic acid hydrate as Na salt	59	143	225	565	Rich
10	2 g. sodium acetate	28.4	14.5	285	460	Rich

Further examination of the liver and blood. This was undertaken to estimate the amount of unchanged δ -keto-acid in the blood and liver after the perfusions with δ -keto-acid and also to find out whether succinic acid was present. An approximate method of estimating the δ -keto-acid by conversion into the semi-carbazone was worked out and the percentage recovery by this method was determined when varying amounts of the acid were added either to whole blood or to a mixture of 2 parts of blood with 1 part of normal saline. The aqueous extract of liver or an aliquot part of the blood filtrate (after removing proteins by Schenk's reagent and mercury as sulphide) was neutralised, evaporated to small bulk, saturated with ammonium sulphate and filtered. The precipitate was washed with small amounts of saturated ammonium sulphate, and the united filtrate and washings were strongly acidified with syrupy phosphoric acid. This solution was extracted for 40 hours with ether in a continuous extractor. The ether was removed by distillation. The residue

was extracted with warm benzene, the benzene-insoluble residue being reserved for the detection of succinic acid as described later. After distilling off the benzene a few drops of water were added to the residue followed by solid semicarbazide hydrochloride and powdered sodium acetate. The semicarbazone of the keto-acid rapidly separated out. It was filtered off, washed with water, dried in a vacuum desiccator and weighed. The results shown in Table II were obtained when the amounts of δ -keto-acid stated were added to 100 cc. blood or the blood-saline mixture.

Table II.

Keto-acid added g.	Keto-acid recovered g.	% recovery
0.2195	0.1935	88
0.395	0.334	84
0.615	0.543	88
	Mean	86

In the three perfusion experiments (Nos. 7, 8 and 9) in which 2 g. of the keto-acid hydrate (equivalent to 1.76 g. keto-acid) were used the amounts of keto-acid in the whole blood at the end of the perfusion and assuming on the basis of the control experiments an 86 % recovery were, 0.41, 0.24 and 0.20 g. respectively. The amount recovered from the liver was smaller, namely 0.10, 0.035 and 0.023 g. so that a considerable amount of the acid had disappeared during the perfusion. The yield of acetone substances shows that some of the acid had undergone β -oxidation, but it is also probable that a portion had been reduced to δ -hydroxy-*n*-hexoic acid. This may account for the smaller yield of acetone substances compared with that obtained from *n*-hexoic acid under the same conditions.

That portion of the ether extract which was insoluble in benzene was neutralised with *N* NaOH and precipitated with 5 % silver nitrate. The silver precipitate was washed with a little water and rubbed up with 10 % sulphuric acid and the solution was then extracted five times with ether. The ether was removed by distillation and the crystalline residue washed with a small amount of ether and dried. The m.p. and the pine splinter reaction showed that it was succinic acid. In a control perfusion without any addition to the blood, and in those perfusions in which *n*-hexoic or δ -keto-hexoic acid had been added, succinic acid was always found in the liver in small amount. On the other hand, none was found in the blood in the perfusions with δ -keto-hexoic acid. The blood was not examined in the other experiments.

SUMMARY.

1. The sodium salt of δ -keto-hexoic acid on perfusion through the liver yields a considerable amount of acetone substances. This indicates that it undergoes β -oxidation and not further γ - or δ -oxidation.
2. Succinic acid was normally found in the liver in small amount. Its presence is not apparently related to any addition made to the perfusing blood.

3. An improved method of oxygenating the blood in perfusion experiments is described.

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