CLVI. STUDIES ON KETOSIS: II. THE OXIDATION OF KETONE BODIES BY THE ISOLATED LIVER OF THE RAT.

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It has been generally assumed that the liver is primarily concerned in the metabolism of the ketone bodies. It was thought that an investigation of the oxidation of these substances by liver preparations—using the oxygen uptake as a measure of oxidation—might throw further light on the changes occurring in the body during the adaptation to a fat diet and possibly on the part played by antiketogenic factors in the metabolism of these substances.

Methods.

The oxygen uptake has been measured upon 0·5 g. quantities of fresh liver preparations from the rat by means of the modified Barcroft differential blood-gas analysis apparatus as employed in this laboratory. The reaction bottles were calibrated to take 4 cc. of fluid; and four apparatus were used at the same time in one bath at 37°.

Various methods of preparing the liver so as to give good agreement between the several samples have been tried. The method finally adopted was the following. The rat was anaesthetised with chloroform and bled as thoroughly as possible. About 3 g. of liver were removed under conditions as aseptic as possible, placed upon a glass plate and covered with a small metal capsule sealed round the edges with plasticine. The whole was immersed in a freezing mixture of ice and salt until the tissue was just frozen through (15 minutes). The capsule was then removed and the liver cut by hand into slices as uniform as possible of rather less than 0·5 mm. thickness. Consecutive slices were transferred to four watch glasses so as to give the most uniform distribution possible until these contained 0·5 g. of tissue. With these precautions excellent agreement could be obtained between four samples from a given liver.

The tissue was then transferred to the reaction bottles and 4 cc. of buffer solution added to each. The buffer employed was either phosphate mixture

of p_H 7·4 or Locke-Ringer solution to which a knife-point of solid calcium carbonate was added. When the usual amounts of tissue were present the filtrate from the latter mixture had a p_H of about 7·4. It is of interest to note in passing that no difference was to be observed between the rate of oxygen uptake by the tissue in phosphate buffer and the tissue in Ringer. Hopkins has recently found that the oxidations catalysed by glutathione are markedly inhibited in phosphate buffer.

All the substances whose oxidation has been studied have been added so as to give a concentration of 1/25th molecular in the presence of the tissue, the reaction of the solutions being adjusted to p_H 7·4 by addition of sodium hydroxide or hydrochloric acid.

Acetoacetic acid has been prepared by the method of Hurtley as employed by Shaffer [1921]. Inactive β-hydroxybutyric acid neutralised with sodium hydroxide has been employed. These two substances have been adjusted to the same molecular concentration by means of estimations by the method described in the preceding paper.

Mention may here be made of a possible source of error. If the sodium hydroxide used in neutralisation contains much carbonate and if sufficient time be not allowed for equilibration in the water-bath, the subsequent absorption of the carbon dioxide liberated on warming will give a false appearance of increased oxygen uptake.

Results.

Under these conditions the liver of the rat shows an increased uptake of oxygen in the presence of β-hydroxybutyric acid and of acetoacetic acid. The intensity of this oxidation was rather variable and seemed to be particularly marked in large old rats. Fig. 1 shows a typical experiment. It will be seen that the acetoacetic acid is more readily oxidised than the β-hydroxybutyric acid; though even in the case of the former the amount of oxygen taken up does not exceed 1/5th of an atom of oxygen per molecule of acid in five hours.

Fig. 2 shows an experiment upon the liver of a similar rat after 36 hours' fast, *i.e.* a liver which must have been almost entirely free from glycogen. There is no apparent change in the oxidation of the ketone bodies.

Fig. 3 represents a similar experiment after the rat had been for three days on a diet of butter fat alone; *i.e.* at a time when the ketosis was probably most intense. There is again no definite change in the oxidation.

Fig. 4 is from a similar rat after keeping for ten days on a diet of butter fat; *i.e.* after the rat had become adapted to the fat diet. The liver at this stage was very small and laden with fat, so that the results are not strictly comparable with the preceding. The total oxygen uptake is very much reduced, but the oxidation of the acetoacetic and β-hydroxybutyric acids still occurs.

According to the theory of antiketogenesis supported by Shaffer [1921] the oxidation of the ketone bodies by the tissues can only proceed after molecular union with some carbohydrate derivative has taken place.

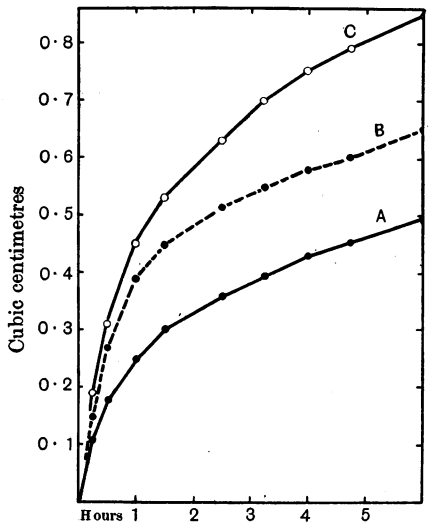

Fig. 1. Uptake of oxygen by 0·5 g. quantities of liver preparation from normal rat. *A*, liver alone. *B*, liver plus β-hydroxybutyric acid. *C*, liver plus acetoacetic acid.

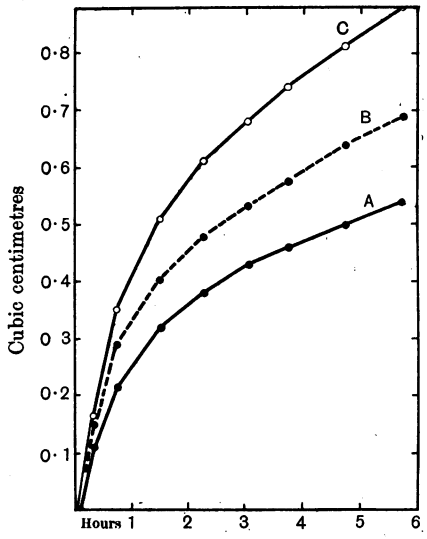

Fig. 2. Same as Fig. 1. Rat, fasted for 36 hours.

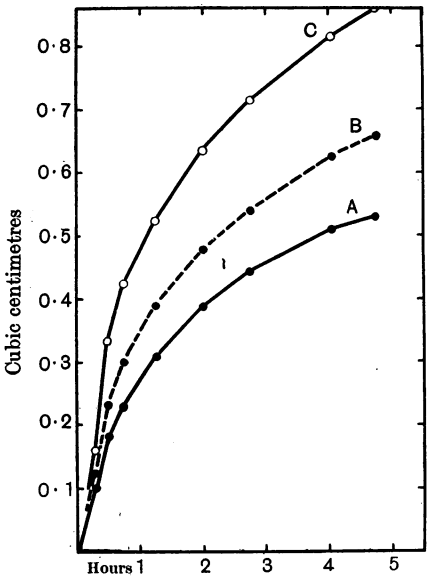

Fig. 3. Same as Fig. 1. Rat, after feeding for 3 days on butter fat alone.

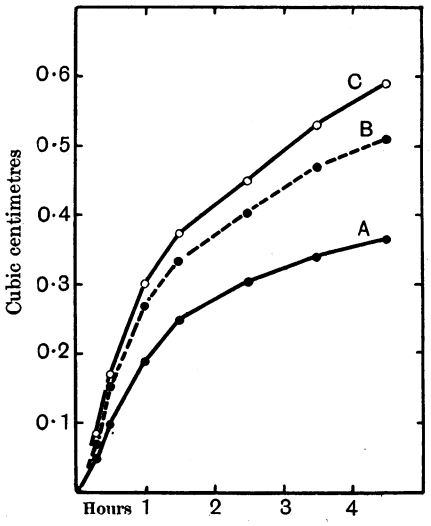

Fig. 4. Same as Fig. 1. Rat, after feeding for 10 days on butter fat alone.

An attempt was made to test this theory by experiments carried out on the lines described. Since these experiments have yielded uniformly negative results, they can be referred to very briefly.

Glucose added to the fresh liver preparation in a concentration of 1/25th molecular does not lead to an increased oxygen uptake; nor is the oxidation of the ketone bodies increased by the presence of glucose under these conditions.

Shaffer considers that the "catalysis" of the oxidation of acetoacetic acid by hydrogen peroxide in alkaline solution *in vitro* is due to its union with some oxidation product of the glucose. Since the main product of the action of hydrogen peroxide on glucose is glucosone, the above experiments were repeated with this substance. Glucosone was prepared by the method of Fischer [1889], and added in about the same concentration as was the glucose.

Glucosone caused no increase in the oxygen uptake, and it did not increase the oxidation of acetoacetic acid or β-hydroxybutyric acid.

A similar experiment was carried out with a solution of glucose which had been partially oxidised by the addition of one molecular equivalent of hydrogen peroxide in the presence of 1: 10,000 Fe and allowing to stand for twelve hours. Such a solution of mixed oxidation products of glucose caused a very marked increase in oxygen uptake. The uptake during four hours by the controls was 500 c.mm. of oxygen and in the presence of the above mixture 1079 c.mm. This increase was not due to the iron present, which alone in the same concentration caused a doubtful increase of about 10 %. The oxidation of the ketone bodies was not increased in the presence of these mixed oxidation products of glucose.

It has been suggested that before glucose can take part in metabolic changes it must first be "activated," and that such activation may be equivalent to the "dissociation" of glucose by alkalies [Woodyatt, 1915]. An 8 % solution of glucose was therefore treated with 10 % sodium hydroxide at room temperature for fifteen hours, neutralised to p_H 7·4 with hydrochloric acid and the same experiment repeated. Such a solution of partially broken down glucose caused a very slight increase in oxygen uptake (10 %) but did not augment the oxidation of the ketone bodies.

Woodyatt has further suggested that in the body one of the functions of the internal secretion of the pancreas is to activate glucose so that it can enter into combination with the ketone substances. The same technique has been used to investigate the question. Sections of the fresh pancreas of the rat were cut under the same conditions as those of the liver and added to the liver preparation. The oxidation of acetoacetic acid and β-hydroxybutyric acid was not increased in the presence of pancreas, either alone or with the addition of glucose.

The experiments were repeated using insulin in place of fresh pancreas and with the same results.

Thus under no experimental conditions to which the living rat or its tissues

after removal from the animal have been subjected could the oxidation of the ketone bodies by the liver be definitely influenced. If the liver is indeed the main locus of the breakdown of the ketone bodies and if this oxidation which has been studied is really the main oxidative process which they undergo, then in so far as the experiments throw any light on the phenomenon of ketosis they tend to support the view that ketosis is due to an increased production of ketone bodies rather than to a diminished oxidation; or that possibly their normal fate is synthetic rather than oxidative and that this change is interfered with.

It is of interest that acetoacetic acid appears to be more readily oxidised by the liver of the rat than does β-hydroxybutyric acid in the same molecular concentration. If, as is generally held, the normal conversion of these substances is from acetoacetic acid to β-hydroxybutyric acid it was to be expected that the latter would be the more readily oxidised even when the inactive acid was used. This difference in the oxidisability of the two acids may possibly have some bearing on the relative concentrations in which they appear in the urine during alkalosis in the rat.

Summary.

A technique is described by which accurate sampling of fresh tissue is obtained for comparative measurements of oxygen uptake.

The fresh liver of the rat shows an increased uptake of oxygen in the presence of acetoacetic acid and inactive β-hydroxybutyric acid. When present in the same molecular concentrations the former causes the more marked increase.

The oxidation of the ketone bodies by the liver does not appear to be affected by fasting the animal for thirty-six hours, nor at any stage in the adaptation to a diet of fat.

The oxidation of the ketone bodies is not affected by the presence of glucose or its breakdown products, nor by the presence of pancreatic extracts or insulin.

It is suggested that ketosis is not due to a diminished direct oxidation of ketone bodies.

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