CCCXXI. AN INVESTIGATION OF THE RATES OF DIGESTION OF STARCHES AND GLYCOGEN AND THE BEARING ON THE CHEMICAL CONSTITUTION.

II. LIVER AMYLASE.

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GREAT diversity of opinion exists as to the nature of the end-products and the course of hydrolysis of glycogen by liver amylase.

Macleod [1926] stated that the final product was maltose which was, however, liable to be hydrolysed further to glucose. Osborne & Zobel [1903], working on muscle juice, found that maltose was the chief end-product together with a trace of glucose. Hollander [1934], using rat liver preparations and starch as substrate, found maltose as the sole end-product. He arrived at the conclusion that the liver preparations contain two amylases comparable with α - and β -plant amylases. Tebb [1894; 1897], using glycogen and a dried pig liver preparation, found glucose as the sole product. Preliminary saline perfusion of the liver through the portal vein, to remove all the blood, and subsequent drying provided a liver preparation which still had marked maltase activity. Hodgson [1936], using acetone-extracted and dried rabbit liver preparations, claimed, it appears without satisfactory experimental evidence, that glucose was the sole end-product and was unable to detect the presence of maltose at any stage of hydrolysis. These results are referred to again later. Barbour [1929], who used glycerol extracts of rabbit muscle, claimed to have obtained a trisaccharide as the sole product of hydrolysis. This trisaccharide is stated to have a reducing power 30 % of that of glucose (by Shaffer-Hartman method), a specific rotation $[\alpha]_D^{181}$, to be readily transformed into an anhydride and to form an osazone crystallizing in small star-shaped aggregates of needles. This trisaccharide is not identifiable with Pringsheim's [1924] amylotriose, obtained by the breakdown of glycogen and amylopectin with concentrated HCl. Lohmann [1926], using KCl extracts of frog muscle, similar to those used by Meyerhof [1926] studying simultaneously the production of lactic acid by glycogen, describes, in addition to glucose, the formation of a trisaccharide which he considers to be probably identical with Pringsheim's amylotriose. Case [1931], without sufficient experimental data, claims to have repeated and confirmed Barbour's results. Carruthers & Wei Yung-Lee [1935] also repeated Barbour's work, but contrary to this worker found that maltose, and not a trisaccharide, was the chief product of hydrolysis. They also obtained evidence of the formation of some glucose. Carruthers [1935] explained Barbour's results as due to inhibition of the maltase activity of his preparations by the glycerol employed in the extraction.

In the present investigation liver preparations from the cat, rat, rabbit, pig, ox, guinea-pig and frog were employed. The preparations were made according to the method suggested to me by Prof. J. Mellanby and as used and described

by Hodgson [1936]. Eadie [1927] pointed out the necessity for perfusing the liver to remove all blood, which contains some amylase, before testing the amylolytic activity of the liver preparation. Consequently a similar preparation was made from the liver of a rat which had been perfused until blood-free. In all cases the maltase activity of the dried liver preparations was tested by incubating 0.5 g. of the liver preparation with 25 ml. 2% maltose solution, after addition of a few drops of toluene, for 24 hours at 37°. The reducing power of the digest was determined by Hanes's method after suitable preliminary dilution and precipitation of proteins with "colloidal iron" and acetic acid.

An osazone was also made. In all the cases tested (the perfused liver preparation which was all employed in the glycogen hydrolysis was not tested) a considerable amount of glucose was formed, showing that all these preparations contain maltase. It must be emphasized, however, that the amount of liver preparation employed in this test was relatively very much greater than that used in the subsequent hydrolyses. The capacity of these preparations to form glucose from maltose might be due to the maltase activity of the liver tissue itself or to the maltase activity of the blood in the preparation. (A comparison of the hydrolysis by the unperfused and perfused rat liver preparations is of significance in this connexion.) The maltase activity of the serum of the rat, rabbit, cat and ox was tested in a similar manner to that described above, in this case employing 5 ml. serum. In the case of the rat serum, there was a quantitative conversion of maltose into glucose, an almost quantitative conversion with the ox serum, whereas both cat and rabbit serum showed no maltase activity. These results are in general accord with those of Barnes & Mackay [1936]. Hynd & Macfarlane [1927], determining the maltase activity of whole blood, found that pig's blood alone gave positive results and obtained negative results with the blood of the mouse, rat, guinea-pig, rabbit, kitten, ox and sheep. Tebb [1894] also found that pig serum had marked maltase activity.

Course of digestion of glycogen by liver amylase.

Digestion mixtures were set up containing 1.0 g. glycogen (Kahlbaum) in 160 ml. 0.1% NaCl, 40 ml. phosphate buffer pH 6.4, 1.0 g. liver preparation and 1 ml. toluene.

A control mixture was set up omitting the glycogen. The hydrolysis of potato starch was also followed in some cases. The digestion mixtures were placed in an incubator at 37° and samples removed, boiled, filtered and analysed at intervals up to 3 days. To test Carruthers's criticism of Barbour's work, 0.25 g. glycogen dissolved in 30 ml. 0.1% NaCl, 10 ml. glycerol, 10 ml. phosphate buffer pH 6.4 and 0.25 g. pig liver preparation were incubated for 17 hours.

The following estimations were performed:

- (1) Unchanged polysaccharide.
- (2) Total reducing power.
- (3) Reducing power due to sugar alone.
- (4) Reducing power after acid hydrolysis.
- (5) Lactic acid.

The unchanged polysaccharide was estimated as described for glycogen in a previous paper [Glock, 1936]. The sugar determinations in each case were performed according to the ferricyanide method of Hanes [1929]. The total reducing power was determined on 5 ml. samples of a 1 in 10 dilution of the digest.

A further 10 ml. of the digest was diluted to 50 ml. with absolute alcohol, to precipitate any unchanged glycogen and dextrins, left for 2-3 hours or longer and

filtered. Two 20 ml. portions of the filtrate were taken and the alcohol removed by evaporation. The reducing power of one portion was determined directly after suitable dilution and of the other after acid hydrolysis followed by subsequent neutralization and dilution. 20 ml. of the digest were employed for the estimation of lactic acid, by the method of Friedemann & Graesser [1933]. Lactic acid determinations were not made in the early stages of hydrolysis. In each case an osazone was made after alcohol precipitation and subsequent evaporation of the alcohol. The results are represented diagrammatically in Fig. 1. The reducing sugar for convenience is calculated and expressed as "maltose" although in some cases the sugar was known to consist partly or entirely of glucose.

Fig. 1.

DISCUSSION OF RESULTS.

From Fig. 1 it appears that in the breakdown of glycogen to sugar there is no appreciable formation of reducing dextrin, since the reducing power of the digest after alcohol precipitation of dextrans and unchanged glycogen is only very slightly different from that before precipitation. It would seem, therefore, that in the hydrolysis by liver amylase, sugar is chiefly split off successively from the glycogen molecule and is only formed to a much smaller degree from simple dextrans produced intermediately. In this respect the action of liver amylase is different from those of salivary, malt and pancreatic amylases.

From the results of the hydrolysis of potato starch by liver amylase it is seen that starch is broken down rather more easily than glycogen, but this difference between the rates of breakdown of the two polysaccharides by liver amylase is not nearly as marked as with salivary, pancreatic and malt amylases.

In all cases the formation of lactic acid was insignificant, the amount produced varying from 0.87 to 4.76 %.

Three different types of osazone were observed. The first was the "*iso-maltose*" type consisting of relatively large rosettes of very fine needles, sometimes tufted at their free extremities. This type of osazone was observed in the early stages of hydrolysis with all the liver preparations and throughout the hydrolysis with the cat, rabbit and perfused rat liver preparations, with the pig liver preparation in the presence of glycerol and generally in addition to glucosazone in the later stages of hydrolysis with the rat liver preparation. The second type of osazone (or hydrazone?) was one not previously observed. It consisted of large pale yellow fern-like crystals. Owing to their pale yellow colour and to the fact that they were relatively insoluble in hot water, it was considered possible that the crystals might be those of a hydrazone and not of an osazone. This osazone was generally found after 42 hours' hydrolysis with the cat and rat liver preparations. The third type of osazone, glucosazone, was the only product formed by the pig liver preparation after 17 hours and longer and was also formed by the rat liver preparation, but in the latter case an additional osazone was always obtained.

The results of acid hydrolysis corroborate the view that glucose is the sole product of hydrolysis with the pig liver preparation. The first type of osazone is probably produced by maltose, perhaps modified in crystalline form by admixture with unprecipitated dextrin, since the amount of glucose formed on acid hydrolysis in the later stages of hydrolysis was 100–110 % of the theoretical yield.

If graphs are plotted of the percentage formation of sugar calculated as maltose against time it is seen that with the pig and rabbit liver preparations the formation of reducing sugar follows a normal hyperbola, whereas this is not true with the rat (perfused and unperfused) and cat liver preparations. In the latter cases there is a sudden fall in the reducing power which finally rises slowly after 42 hours. This type of hydrolysis curve would appear to be due to the reversible action of the liver amylase, the breakdown phase being predominant in the early stages of digestion and the synthetic phase in the later stages, probably initiated by accumulation of the hydrolysis products of the breakdown phase. The possible criticism that the decrease in the reducing power in the later stages might be due to the formation of hexosephosphates is disproved by the fact that a similar type of curve was obtained using the rat liver preparation but replacing the phosphate buffer by a citrate buffer of the same pH. This decreased reducing power also cannot be accounted for by increased lactic acid formation, for in no case was the percentage formation greater than 5.

It is interesting to note, in this connexion, that the third type of osazone described above was only observed in the 42 hours' digest with the rat and cat preparations and is, therefore, probably correlated with the decreased reducing power. The breakdown of potato starch by rat liver preparations, however, follows a normal course.

The conversion of glycogen into glucose by liver preparations depends on the concentration of amylase and maltase. If both enzymes are very active, it is possible to get 100 % conversion into glucose: this is the case with the pig liver preparation. If the concentration of either amylase or maltase is reduced, the yield of glucose will be less: this seems to be the case with the rat preparation. If the concentration of maltase is very small, the breakdown will stop at the maltose stage and will not proceed as far as glucose. It was shown, as already described, that all the preparations possessed maltase activity. In the case of the cat and rabbit preparations this activity must have been much less than that of the pig and rat preparations, so that in the amount of preparation which

was used in the glycogen hydrolysis, the maltase concentration was negligible. If the maltase activity of the preparation were due to the blood it contained, then one would suppose that after perfusion of the liver to render it blood-free, the breakdown of glycogen would not proceed further than the maltose stage. This was found to be true with the dried preparation from the perfused rat liver.

That the end-product in this case was not glucose was further substantiated by the fact that after alcohol precipitation and subsequent evaporation of the alcohol, the liquid was not fermented by a yeast known to be incapable of fermenting maltose. In the case of the rat, the maltase activity of the serum was found to be so great that the ability of the unperfused liver preparation to form glucose might easily be attributed to the maltase activity of the blood. It is doubtful, however, whether this is true of the pig liver preparation since in this case there was normally 100 % conversion into glucose. (That perfused pig liver preparations still possess marked maltase activity has been shown by Tebb [1894].) The breakdown of glycogen would also stop at the maltose stage if the maltase activity of the preparation were inhibited, for example, by the method of preparation. The pig liver preparation, in the presence of glycerol, failed to produce any glucose after 17 hours. This also supports Carruthers's opinion of Barbour's work.

From the values of the reducing power before and after acid hydrolysis, the relative amounts of glucose and maltose at each stage of digestion can be calculated. At the end of 17 hours the sugar formed consisted in the case of the pig preparation of 16 % maltose and 84 % glucose and in the cases of the rat, guinea-pig and ox liver preparations of 93 % maltose and 7 % glucose, 91 % maltose and 9 % glucose and 79 % maltose and 21 % glucose, respectively. After 42 hours the values were for the pig preparation 100 % glucose, for the rabbit 100 % maltose and for the rat 88 % maltose and 12 % glucose. The necessity for estimating the reducing power both before and after acid hydrolysis in order to form an opinion as to the nature of the sugar produced is obvious when the results obtained with the pig preparation are considered: after 7½ hours only 15 % of the sugar was glucose, after 17 hours 84 %, whereas at 42 hours there was a 100 % yield.

A final 100 % yield of glucose does not necessarily mean that no maltose is produced intermediately. Hodgson [1936], without having performed any acid hydrolyses, claims that glucose is the sole product of hydrolysis of glycogen by rabbit liver preparations. It is obvious from the above results that such an assumption is unfounded.

SUMMARY.

1. The hydrolysis of glycogen, and in a few cases of potato starch, by acetone-extracted and dried liver preparations of the rat, cat, rabbit and pig in the presence of phosphate buffer of pH 6.4, was followed. The course of hydrolysis of glycogen was also followed using a perfused rat liver preparation and in addition the behaviour of the pig liver preparation in the presence of glycerol was studied.

2. The maltase activity of each liver preparation was tested, in all cases with a positive result. The maltase activity of the serum was also tested. That of the rat serum was found to be very great, that of the ox quite large, whereas cat and rabbit sera possessed no maltase activity.

3. In the case of the pig liver preparation only was there quantitative conversion into glucose. This formation of glucose is inhibited by the presence of glycerol.

4. Maltose was the sole end-product with the cat, rabbit and perfused rat preparations.

5. The unperfused rat liver preparation produced maltose alone in the early stages of hydrolysis, but this was gradually converted into glucose as digestion proceeded.

6. In the cases of the rat (perfused and unperfused) and cat liver preparations the reducing power showed a steady decrease from 17 to 42 hours. This can only be explained by a reversal of the enzymic activity.

8. That the production of glucose by the rat liver preparation could be due to the maltase contained in the blood has been proved since no glucose was produced by the perfused rat liver preparation.

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