CXIII. GLUCOSE DEHYDROGENASE: A NEW OXIDISING ENZYME FROM ANIMAL TISSUES.

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THE preparation and properties of an enzyme which oxidises hexosediphosphate have already been described [Harrison, 1931]. During the course of this work, experiments were carried out to see whether the oxidation of hexosediphosphate was affected by the addition of other substances. Substances similar in structure to hexosediphosphate might, from the work of Quastel and Wooldridge [1928], be expected to be adsorbed at the same enzyme surface and so inhibit the oxidation. One of the substances tested was glucose, and it was surprising to find that this substance produced not an inhibition but a marked acceleration. Further investigation showed that there is present in the hexosediphosphate enzyme preparation another enzyme capable, in the presence of a suitable hydrogen acceptor, of bringing about the oxidation of glucose. Since an enzyme which oxidises glucose has not apparently been hitherto obtained from animal tissues, it seemed desirable to study its preparation and properties in some detail. It has been found possible to obtain perfectly clear solutions of the enzyme capable of being filtered through a porcelain filter without loss of activity.

EXPERIMENTAL.

Preparation of the enzyme.

Acetone-liver was prepared by a modification of the method described by Bernheim [1928]. About $1\frac{1}{2}$ lbs. of fresh ox liver (obtained a few hours after the animal was killed) were weighed after peeling off the surrounding membrane, washed for about a minute under the cold tap, squeezed and minced twice through an ordinary fine mincer. The pulp was well stirred for about a minute with pure acetone (B.P.), using 900 cc. to 675 g. of liver, and filtered on a large Büchner funnel. The acetone treatment was repeated twice more, using half the above quantity of acetone for each extraction. After partially drying on the funnel for a short time, the product was spread out in two large desiccators over sulphuric acid and evacuated at the water-pump for several hours until most of the acetone was removed. The preparation is fairly stable if kept evacuated in the desiccator. In order to extract the enzyme, 30 g. of the

dry acetone-liver were put into a mortar with 120 cc. of distilled water and ground at intervals for 2 to 3 hours. The mixture was squeezed through coarse muslin, the residue ground up with 60 cc. of distilled water, squeezed through muslin, and the combined extracts were centrifuged for a short time. The opaque liquid was poured off (through muslin to remove the fatty layer which sometimes forms), put into thin collodion sacs, and dialysed overnight in the ice-chest against 2 litres of distilled water. The solution was then brought to about p_H 5.7 to bromocresol purple by the addition of about one drop of N HCl and the heavy protein precipitate centrifuged off. The clear or slightly opaque pigmented solution was brought approximately to neutrality by the addition of a few drops of N NaOH and was saturated with pure ammonium sulphate. After carefully pouring off from the excess of ammonium sulphate, the precipitate was filtered in the ice-chest on a starch-free filter-paper (Whatman, No. 40), partially dried by transferring to an absorbent porous tile, and finally removed from the plate and dried *in vacuo* over sulphuric acid. The enzyme, which is fairly stable in the dry form, was dissolved in water or phosphate buffer, made up to the required volume (30 cc. or 45 cc. are convenient volumes for methylene blue experiments) and brought to p_H 7.4 with N NaOH. This solution which is pigmented, but as a rule perfectly clear, was used for most of the experiments described in this paper. If the enzyme is required free from ammonium sulphate, the dry preparation is put into a wet collodion sac and dialysed against frequent changes of distilled water in the ice-chest until practically free from ammonium sulphate, after which it is centrifuged, a considerable amount of protein being thereby removed. The solution may then be concentrated by bringing to about p_H 7.8 with NaOH and distilling *in vacuo* in a large flask, keeping the temperature of the liquid below 25° and cooling the receiver in ice. The flask is heated on a water-bath at 30–35°. Frothing is sometimes troublesome at the beginning of the distillation, and it is advisable to allow a very slow stream of air (one bubble every few seconds) to bubble through a wide capillary under the surface of the liquid. If dialysis has been complete, the solution remains alkaline at the end of the distillation, and there is practically no destruction of the enzyme. The enzyme does not keep well in solution and loses some of its activity even on standing overnight in the ice-chest.

In preparing the enzyme, the following points should be noted. The temperature during the acetone treatment should not be much above 20°, and in hot weather, the acetone and the liver should be first cooled in the ice-chest. The acetone treatment should be carried out as rapidly as possible, and prolonged evacuation of the desiccators is desirable in order to remove the acetone as completely as possible. The collodion sacs used for dialysis should be kept sterile and should be renewed after using about three times, as they tend to become impermeable. In drying the enzyme, the thick absorbent porous plate is much preferable to the ordinary unglazed dinner plate. (The "Owens College" pattern from Griffin and Tatlock, Ltd., was found satisfactory.) The use of

ammonium sulphate in fairly large crystals facilitates decantation of the precipitated enzyme from the excess of ammonium sulphate. Starch-free filter-paper should always be used (except in preparing the acetone-liver) since the liver extract may break down starch to glucose.

The activity of the enzyme prepared from different samples of liver varies considerably, and occasionally an almost inactive preparation is obtained. The samples of liver which are soft and mince to a dark almost liquid pulp usually give the most active enzyme preparations. The enzyme has been prepared from the liver of the ox, sheep, cat and dog. Other tissues have not yet been examined.

Anaerobic oxidation of glucose.

The enzyme solution by itself usually shows a slight reducing power towards methylene blue varying considerably with different preparations. On adding glucose, the rate of reduction of methylene blue is greatly increased, as is shown in the following typical experiment. The methylene blue experiments were carried out at 37° in Thunberg vacuum tubes as previously described [Harrison, 1931]. 1 cc. of enzyme solution equivalent to 1 g. of acetone-liver was used in each tube together with 0.5 cc. phosphate buffer p_H 7.4 and 0.5 cc. methylene blue (1 : 5000). The glucose used was pure dextrose (A.R.), from British Drug Houses. The solutions of glucose were stored below 0° and fresh solutions were made up at frequent intervals.

Substrate added	Time of decoloration of methylene blue
None	28 mins.
0.05 cc. 2 <i>M</i> glucose	5 mins.

That the acceleration is due to glucose itself and not to an impurity is clear from the fact that different samples of pure glucose from different sources in suboptimal concentrations gave, with the same enzyme preparation, identical reduction times.

Pure fructose is not oxidised by the enzyme, and in the same concentration as glucose in the above experiment retards the residual reducing power of the enzyme preparation itself. Galactose and arabinose showed no appreciable reduction of methylene blue except in high concentrations, and it is not unlikely that the slow reduction which then occurred was due to traces of glucose present as an impurity in the sugars.

The effect of increasing the glucose concentration is shown in Fig. 1. The experiment was carried out at 37° using 1.0 cc. ammonium sulphate-free enzyme (equivalent to 1 g. acetone-liver) and 0.5 cc. methylene blue. The p_H was 7.6, and water was added where necessary to bring the total volume to 2.0 cc.

The optimum concentration of glucose is seen to be 0.25 *M* and in concentrations greater than this the velocity falls off slowly. The substrate concentration giving half the maximum velocity is about 0.07 *M* which is an exceptionally high value for an oxidising enzyme.

Lactic, citric and hexosediphosphoric acids are oxidised, though the optimum rates of oxidation with these substances are considerably smaller than that of glucose. Since, however, lactic acid is oxidised by the preparation, it was possible that the oxidation of glucose might not be a direct one, but that the glucose might first be glycolysed to lactic acid and the lactic acid then oxidised. That such is not the case is shown by the following experiment.

Fig. 1. Rate of glucose oxidation as a function of substrate concentration. Abscissa: molar concentration of glucose. Ordinate: velocity = $100/T$, where T = methylene blue reduction time in minutes.

Four small flasks *A*, *A'*, *B*, *B'*, each contained 10 cc. of enzyme solution. To duplicates *B* and *B'* were added 3.0 cc. *M* glucose, while the duplicates *A* and *A'* received 3 cc. water. The flasks were then filled with nitrogen, tightly stoppered and incubated at 37° for 2½ hours. 5 cc. of 20% trichloroacetic acid were then added to each flask, the solutions were filtered, glucose removed by the copper-lime method and lactic acid was estimated on an aliquot portion of each solution by the method of Friedemann, Cotonio and Shaffer [1927].

Flask	Mg. lactic acid
<i>A</i>	0.94
<i>A'</i>	0.87
<i>B</i>	0.72
<i>B'</i>	0.74

= 0.90
= 0.73

It is clear that there is no formation of lactic acid from the added glucose. Methylene blue experiments carried out at the same time with the same enzyme gave the following results. Each tube contained 1 cc. enzyme + 0.25 cc. methylene blue (1 in 5000) together with sufficient water to make the volume 1.55 cc.

Substrate added	Time for decoloration of methylene blue mins.
None	50
0.3 cc. <i>M</i> /1 glucose	5
0.1 cc. <i>M</i> /10 lactate	25
0.05 cc. <i>M</i> /10 hexosediphosphate	19
0.05 cc. <i>M</i> /10 hexosediphosphate + 0.1 cc. <i>M</i> /10 lactate	14
0.05 cc. <i>M</i> /50 hexosediphosphate + 0.1 cc. <i>M</i> /50 lactate	27

This shows that an amount of lactic acid much greater than the experimental error in the estimation of lactic acid produces only a relatively small acceleration in the rate of reduction of methylene blue.

The experiments on the mixture of hexosediphosphate and lactate were carried out, since the glucose enzyme preparation also contains an enzyme capable of oxidising hexosediphosphate, and Meyerhof and Lohmann [1927] have given evidence that in the lactic acid production in muscle extract with glucose, one molecule of hexosediphosphoric acid is formed with every two molecules of lactic acid. Mixtures of hexosediphosphate and lactate in the ratio of one molecule of the former to two of the latter in concentrations of $M/50$ and $M/10$ were found to be oxidised much more slowly than glucose. These results, coupled with the fact that no lactic acid could be detected on incubating the enzyme with glucose anaerobically, show that the oxidation of glucose by the enzyme is not due to the formation and oxidation of lactic acid either alone or with hexosediphosphoric acid. Indirect evidence in favour of this conclusion is furnished by the fact that neither sodium fluoride nor iodoacetic acid, both of which inhibit lactic acid formation in muscle, produces any inhibition in the rate of reduction of methylene blue by glucose *plus* the enzyme. (The experiments were carried out with $N/100$ sodium fluoride and with $N/1000$ sodium iodoacetate, both at p_H 7.6.)

A mixture of glucose and hexosediphosphate, the latter being above its optimum concentration, reduced methylene blue more quickly than either of the substrates in the same concentration by themselves. By the line of reasoning previously employed in proving the non-identity of the citric and hexosediphosphate enzymes [Harrison, 1931], this indicates that the oxidation of glucose and hexosediphosphate is brought about by two different enzymes, rather than by the same enzyme acting on two different substrates.

Properties of the enzyme.

In studying the effect of p_H on the activity of the enzyme, the dialysed enzyme free from ammonium sulphate was used, and glucose was added at its optimum concentration. No buffer was added, since the protein in the solution possesses a large buffering power. It was found that at p_H 6–8, the activity at 37° remained approximately constant. At p_H 9–9.5 the activity was apparently greater, but this was found to be due, at least in part, to the increased reducing power towards methylene blue of the enzyme solution alone. In still more alkaline solutions, glucose by itself slowly reduces methylene blue. In phosphate buffer of p_H 8.0, glucose by itself showed no reduction of methylene blue even after 10 hours at 37°.

The glucose dehydrogenase is completely precipitated, together with all the pigments, by saturation with ammonium sulphate. Attempts to precipitate the enzyme without precipitating the pigments were not very successful, as the enzyme is only partially precipitated even by 75% saturation with ammonium sulphate. Owing to the difficulty of obtaining the enzyme free from ammonium

sulphate, a number of other possible precipitants were tried without success. The precipitates obtained with 50 %, 75 %, and 90 % acetone showed little or no activity even on carrying out the precipitation with ice-cold acetone. Similarly, the product obtained by precipitation with 90 % alcohol keeping the mixture below 0° was inactive.

The enzyme may be filtered without loss through kieselguhr, whereby some pigment is removed and freedom from insoluble cell material is ensured. The enzyme may also be filtered through a fine porcelain candle (Pasteur-Chamberland L 5) without loss of activity. With the concentrated enzyme, filtration may be slow unless the solution is first filtered through kieselguhr. The dilute solution obtained after dialysing and centrifuging the extract of acetone-liver, however, filters through the candle readily and can then be saturated with ammonium sulphate yielding a preparation free from cell debris and giving a perfectly clear solution in water.

A number of attempts were made to purify and concentrate the enzyme by adsorption methods. Most of these experiments were carried out with the dialysate from acetone-sheep liver before precipitation with ammonium sulphate. It was found difficult to standardise the conditions since different enzyme preparations contained different amounts of protein. Treatment of 20 cc. of dialysate (p_H 5.7) five times with 0.5 g. of charcoal (Merck's Medicinal), stirring for a minute and filtering under suction each time, followed by precipitation with ammonium sulphate usually gave an enzyme containing much less pigment and with its activity unimpaired. By continuing the charcoal treatment, more pigment is removed, but the enzyme is also adsorbed from the solution. The enzyme does not appear to be adsorbed from the untreated dialysate by kaolin or kieselguhr either in acid or alkaline solution. If, however, part of the protein be first removed by treating about five times with charcoal, the enzyme can be adsorbed from acid solution by kaolin. Thus, by stirring 10 cc. of the charcoal-treated dialysate three times with 0.5 g. kaolin at p_H 4.5 or 5.5, about two-thirds of the enzyme was removed from the solution. Treatment of the kaolin with $N/10$ K_2HPO_4 followed by precipitation of the eluted enzyme with ammonium sulphate gave a solution with some activity towards glucose, though much of the enzyme was lost.

The effect of temperature on the destruction of the enzyme depends on the nature of the enzyme preparation. For example, the presence of ammonium sulphate appears to protect the enzyme. A solution of the ammonium sulphate-precipitated enzyme, after heating at 52° in nitrogen at p_H 7.3 for 5 minutes and centrifuging off the precipitate of inactive protein, was found to have lost little of its activity. On the other hand, the enzyme dialysate before precipitation with ammonium sulphate, warmed under the same conditions, lost nearly half of its activity. The ammonium sulphate-precipitated enzyme solution at p_H 7.3 lost about one-third of its activity after heating at 45° for half an hour. The enzyme is rapidly destroyed at 60° even in presence of ammonium sulphate.

The reduction of methylene blue by glucose in presence of the enzyme was not inhibited by the presence of neutralised $M/300$ KCN. The addition of 0.048 mg. (= 1 unit) of insulin hydrochloride (British Drug Houses) in a total volume of 2.1 cc. produced no effect on the rate of reduction of methylene blue. The enzyme is not destroyed by toluene, and several preparations were made by grinding liver with toluene, extracting with water and precipitating the enzyme with ammonium sulphate.

The aerobic oxidation of glucose.

A large number of experiments were carried out in the Barcroft apparatus to determine whether the system glucose-oxidising enzyme *plus* glucose was capable of taking up oxygen directly. The results have been rather inconclusive, for while with a considerable number of preparations of the enzyme, the oxygen uptake has been somewhat greater in presence of glucose than in its absence, this extra uptake being unaffected by KCN, yet with many other preparations of the enzyme, no oxygen uptake with glucose has been obtained. Most of these experiments were carried out with the ammonium sulphate-containing preparation, but in those experiments in which the dialysed ammonium sulphate-free enzyme was used, it is noteworthy that in no case was an oxygen uptake observed. It was also noted that, with most enzyme preparations, at the end of the experiment the solution containing glucose was definitely more acid than at the beginning, and more acid than the control without glucose. These observations suggested the following explanation as possibly accounting for the apparent oxygen uptake observed in some cases. The glucose, while not being directly oxidised by oxygen, may possibly be able to use as a hydrogen acceptor some reducible substance in the enzyme preparation, for example, a reducible pigment. The formation of an acid oxidation product of glucose oxidised in this manner would account for the development of acidity in the solution. (Formation of lactic acid by glycolysis has already been excluded.) At the beginning of all the experiments, the solution was definitely alkaline (p_H 7.4 or 7.6). When the ammonium sulphate-containing preparation was used, free ammonia would thus be present in the gas phase. During the course of the experiment, the development of acidity in the solution would cause the absorption of this ammonia gas, which would be read on the manometer as an apparent uptake of oxygen.

When methylene blue was added to the solution in the Barcroft apparatus, an oxygen uptake with glucose was always observed, the methylene blue being alternately reduced by glucose and oxidised by molecular oxygen. It seemed therefore that in the oxidation of glucose with glucose dehydrogenase, molecular oxygen cannot act as a hydrogen acceptor. It was thus of interest to see whether the aerobic oxidation could be brought about in presence of active oxygen.

*Oxidation of glucose in presence of the cytochrome and
indophenol oxidase system.*

Keilin [1929] has shown that cytochrome is oxidised by oxygen activated by indophenol oxidase and that it is reduced by dehydrogenase systems. Cytochrome is believed to act as a carrier between the oxygen activators and the hydrogen activators of the cell. Keilin [1930] has shown that the oxidation of cysteine is greatly accelerated by indophenol oxidase in presence of cytochrome, the latter acting as a carrier between the activated oxygen and the sulphhydryl group. The oxidation of an actual dehydrogenase system by cytochrome *plus* indophenol oxidase has not hitherto been experimentally brought about. One of the difficulties is that the indophenol oxidase which is prepared from heart muscle, itself contains certain dehydrogenases. Preliminary experiments with methylene blue having shown that the indophenol oxidase prepared from heart muscle does not contain any glucose dehydrogenase, an attempt was made to build up the complete oxidising system using glucose dehydrogenase, that is, to find whether glucose *plus* glucose dehydrogenase would take up oxygen in presence of cytochrome *plus* indophenol oxidase. The following experiments and the curves in Fig. 2 show that in presence of the complete system there is a rapid oxygen uptake.

A fresh solution of cytochrome *c* was prepared from 2 lbs. of Delft yeast by the first method of Keilin [1930]. The precipitate obtained with SO_2 was well washed, suspended in water, brought to p_{H} 7.4 with NaOH and made up to 7 cc. Indophenol oxidase was prepared by a method similar to that of Keilin by grinding fresh sheep's heart muscle with fine washed sand, using 50 cc. of dry sand to 20 g. of muscle. The very fine suspension in water was centrifuged, washed twice with distilled water and evenly suspended in phosphate buffer, 14 g. of wet pulp being made up to a volume of 50 cc. and brought to p_{H} 7.4. This very strong suspension was used, since during the experiment the particles become aggregated on the sides of the vessel, the enzyme surface being thereby much reduced. The glucose dehydrogenase solution was an ammonium sulphate-free preparation prepared as already described. At 37° , 0.4 cc. (equivalent to 1 g. of dry liver) + 0.6 cc. p_{H} 7.4 buffer reduced 0.5 cc. methylene blue in 10 minutes in presence of 0.025 cc. 2*M* glucose and in 30 minutes without glucose. Four Barcroft differential manometers were used, the right-hand bottle of each receiving 1.7 cc. of glucose dehydrogenase. The first received in addition 0.8 cc. p_{H} 7.4 phosphate buffer; the second, 0.8 cc. buffer and 0.2 cc. 2*M* glucose; the third, 0.8 cc. indophenol oxidase and 0.3 cc. cytochrome; the fourth, 0.8 cc. oxidase and 0.3 cc. cytochrome and 0.2 cc. 2*M* glucose. Water was added to make the volume in each bottle up to 3.0 cc. The left-hand bottles each contained 3.0 cc. buffer. The side-tube of all bottles contained 5% NaOH soaked on a roll of filter-paper to absorb CO_2 . The apparatus were shaken in a water-bath at 37° .

The oxygen uptakes after 110 minutes were as follows:

	mm. ³	Final p_H
1. Dehydrogenase	83	7.2
2. Dehydrogenase + glucose	85	7.2
3. Dehydrogenase + cytochrome + oxidase	189	7.0
4. Dehydrogenase + cytochrome + oxidase + glucose	352	6.7

The course of the oxygen uptake is shown in the corresponding curves in Fig. 2.

Fig. 2. Oxygen uptake of glucose in presence of dehydrogenase, cytochrome and indophenol oxidase.

It is clear that while no oxidation of glucose was brought about by the dehydrogenase itself, a large oxidation of glucose was induced by the dehydrogenase in presence of the oxygen-activating system, cytochrome and indophenol oxidase. Incidentally, the greater uptake in 3 compared with 1 shows that the cytochrome-oxidase system also increased the oxygen uptake of the residual reducing systems in the dehydrogenase preparation.

This experiment has been repeated a number of times with different preparations of the enzymes, and similar results have always been obtained. In spite of the highly buffered solutions, the p_H at the end of the experiment was always definitely lower in the solution in which oxidation of glucose had taken place. It appears that the oxidation product of glucose is an acid.

The following experiment was carried out to show that the complete system, glucose dehydrogenase, cytochrome and indophenol oxidase, is necessary in order to bring about the oxidation of glucose; *i.e.* that neither cytochrome nor the oxidase alone will induce the oxidation. The cytochrome, oxidase and dehydrogenase solutions were the same as used in the previous experiment, stored overnight at 0°. Five Barcroft apparatus were used, the solutions in the two bottles of any one apparatus being the same except that each right-hand bottle contained glucose while each left-hand bottle contained an equal volume of water. The bottles of No. 1 each contained 1.7 cc. glucose dehydrogenase and 0.8 cc. p_H 7.4 phosphate buffer; No. 2 contained 1.7 cc. dehydro-

genase and 0.8 cc. indophenol oxidase; No. 3 contained 1.7 cc. dehydrogenase, 0.3 cc. cytochrome and 0.8 cc. buffer; No. 4 contained 1.7 cc. dehydrogenase, 0.3 cc. cytochrome and 0.8 cc. oxidase; No. 5 contained 0.3 cc. cytochrome, 0.8 cc. oxidase but no dehydrogenase. After adding 0.2 cc. 2*M* glucose to each of the right-hand bottles, sufficient water was added to make the volume in each bottle equal to 3.0 cc.

The oxygen uptake after 170 minutes at 37° was as follows:

	mm. ³
1. Dehydrogenase + glucose	19
2. Dehydrogenase + glucose + oxidase	0
3. Dehydrogenase + glucose + cytochrome	7
4. Dehydrogenase + glucose + cytochrome + oxidase	101
5. Glucose + cytochrome + oxidase	18

It is clear that the only solution showing an appreciable oxygen uptake is that containing the complete system, glucose with its dehydrogenase, cytochrome and indophenol oxidase. (In some experiments, an oxidation of glucose has been induced by indophenol oxidase without added cytochrome. Keilin has found, however, that the heart-muscle indophenol oxidase always contains a small amount of cytochrome. The oxidation of glucose in presence of oxidase *plus* added cytochrome has always been much greater.)

In order to exclude the possibility that the results in this experiment might be due to glycolysis of glucose to lactic acid by the indophenol oxidase preparation, followed by oxidation of the lactic acid, the following experiment was carried out. The manometric experiment was performed exactly as before except that the undialysed dehydrogenase preparation was used and the temperature was 30°. At the same time, to each of four small conical flasks were added 3.5 cc. dehydrogenase and 1.65 cc. indophenol oxidase. 0.41 cc. 2*M* glucose + 0.62 cc. water were added to two flasks *A* and *A'*, while duplicate control flasks *B* and *B'* received 1.03 cc. water but no glucose. (The concentrations are the same as those used in the manometric experiments.) The flasks were quickly filled with nitrogen, stoppered and incubated for 3½ hours at 30°. 14 cc. of water and 6 cc. 20 % trichloroacetic acid were then added to each flask, and after removal of glucose by the copper-lime method, lactic acid was estimated by the method of Friedemann, Cotonio and Shaffer [1927]. The results were as follows:

	Lactic acid mg.
<i>A</i> with glucose	0.47
<i>A'</i> with glucose	0.47
<i>B</i> without glucose	0.42
<i>B'</i> without glucose	0.47

It is evident that no measurable quantity of lactic acid has been formed from the glucose. The manometric experiment which was carried out simultaneously at 30°, gave similar results to that described above, namely no appreciable oxygen uptake except where the complete system, glucose with its dehydrogenase, cytochrome and indophenol oxidase, was present. This shows

therefore that the oxygen uptake is due to the direct oxidation of glucose rather than to the oxidation of lactic acid formed by glycolysis of the glucose.

DISCUSSION.

This appears to be the first time that a soluble glucose dehydrogenase has been isolated from the cell. The only reference in the literature to the extraction of an enzyme which oxidises glucose appears to be that of Müller [1929] who obtained a glucose oxidase from *Aspergillus niger*. This enzyme is entirely different in its properties from the enzyme which I have described. Müller's enzyme does not bring about the oxidation of glucose in presence of methylene blue and does in presence of oxygen. Like tyrosinase, therefore, it is an oxidase and not a dehydrogenase. The resistance of glucose dehydrogenase to toluene suggests that it is different from the glucose-oxidising enzyme which is present in suspensions of certain bacteria; Quastel and Wooldridge [1927] have found that in "resting" *B. coli* the enzyme which brings about the oxidation of glucose is completely inactivated by treatment with toluene.

I have not been able to find any example in the literature of an oxidising enzyme which can be filtered through kieselguhr and a fine porcelain candle without loss of activity. The lactic dehydrogenase prepared by Stephenson [1928] from *B. coli* could be filtered through kieselguhr but was completely inactivated by passing the solution through a porcelain candle. The observation that glucose dehydrogenase may be filtered through a fine porcelain candle is of interest as indicating that the activity of the enzyme is associated with molecules in true colloidal solution. The molecules constituting the enzyme or to which the enzyme is attached are doubtless large and extremely complex, but the enzyme activity is evidently not to be ascribed to a suspension of microscopic remnants of the broken cell. It has been shown that glucose dehydrogenase with glucose does not take up oxygen to any appreciable extent. Also, the indophenol oxidase preparation from washed heart muscle does not contain any glucose dehydrogenase. These two facts rendered the glucose dehydrogenase a particularly suitable enzyme for testing the ability of cytochrome *plus* indophenol oxidase to induce the aerobic oxidation of a substrate activated by an anaerobic dehydrogenase. The experiments described above, showing that in presence of glucose dehydrogenase, cytochrome and indophenol oxidase, glucose is oxidised by oxygen, afford the first direct demonstration of the co-operation of the oxidase and cytochrome with a dehydrogenase in bringing about the aerobic oxidation of the substrate of the last of these.

It is premature to speculate on the function in the body of glucose dehydrogenase until more is known about the distribution of the enzyme and until the oxidation product of glucose has been identified. The acid formed by the oxidation of glucose is now being investigated.

SUMMARY.

1. An enzyme (glucose dehydrogenase) has been obtained by saturating the water extract of acetone-liver with ammonium sulphate. The enzyme has been extracted from the liver of the ox, sheep, cat and dog.

2. The enzyme is obtainable in a perfectly clear, cell-free solution which can be filtered through a porcelain candle or through kieselguhr.

3. The enzyme brings about the oxidation of glucose in presence of methylene blue as hydrogen acceptor.

4. This anaerobic oxidation of glucose is not inhibited by KCN, NaF, toluene or iodoacetic acid.

5. Glucose is not oxidised in the presence of oxygen, an oxygen activator apparently being necessary.

6. The system glucose and glucose dehydrogenase has been combined with Keilin's cytochrome-indophenol oxidase system in presence of which the aerobic oxidation of glucose readily occurs, cytochrome acting as a carrier between active oxygen from the oxidase and active hydrogen from glucose *plus* its dehydrogenase.

7. The oxidation product of glucose has not yet been determined, but it appears to be an acid. (See Note below.)

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It has now been found that the oxidation product of glucose is gluconic acid. This has been isolated as the characteristic calcium salt, from which the pure phenylhydrazide has been prepared. The latter melted at 199°, the melting-point being unchanged by mixing with an authentic specimen. The cinchonine salt of the acid melted at 186° after recrystallisation from 95% alcohol. Gluconic acid appears to be the main, if not the sole, product of the oxidation. It is hoped shortly to publish these results in detail.