CLXV. THE DEGRADATION OF GLUCOSE BY THE BLOOD CORPUSCLE OF THE RABBIT. II.

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In a previous communication [Irving, 1926] evidence was adduced on which was based the theory that the glucose which was degraded by blood corpuscles during glycolysis was so changed at the surface of the cell. In the present paper, the writer has further investigated the mechanism involved, and presents data relating to the intervention of organic phosphorus, the influence of ions, and the degradation of some stereoisomers of glucose.

Experimental.

The technique employed was precisely the same as that described in the previous communication, save that in some of the experiments with large quantities of blood, the mixed arterial and venous blood of one or more rabbits was employed. The same precautions as regards bacterial contamination were taken. Very few of the experiments lasted more than four hours.

The intervention of phosphorus.

Martland and Robison [1924] have shown that after haemolysis of the blood corpuscles free phosphorus is rapidly liberated from the pre-existing organic phosphorus compounds in these cells. Piazza [1925], and Morgulis and Barkus [1925] stated that glycolysis had no relationship with this hydrolysis since it was absent or slight when the production of free phosphorus was most marked. The same was found by Martland, Hansman and Robison [1924] and has been confirmed by the present writer.

If the corpuscles were kept in a strictly isotonic medium, the liberation of free phosphorus was very slight and the conditions for glucose degradation were at an optimum, but if haemolysis occurred, free phosphorus was liberated and glycolysis depressed. It appeared that perhaps the "intactness" of the organic phosphorus compounds might be essential for glucose degradation, but since both these phenomena depend on the integrity of the cell for their occurrence, it is more probable that this is the essential factor in both cases.

Bierry and Moquet [1925] stated that during the early stages of glycolysis the free phosphorus in blood fell in amount, and that this indicated the temporary synthesis of an organic phosphorus compound, which was an intermediate step in the reaction. The present writer undertook several experiments in order to test this hypothesis.

Owing to the fact that phosphorus estimations in suspensions of corpuscles during glycolysis are valueless after about the first hour, because of the rapidly increasing liberation of free phosphorus from the preformed organic phosphorus compounds, the experiments described were usually terminated after an hour. The following is typical of several.

A rabbit was killed and the mixed arterial and venous blood collected and centrifuged in the usual way. The plasma was removed and to a given volume of corpuscles an equal volume of a very dilute phosphate mixture in isotonic saline was added. The resulting suspension was divided into two portions, (1) to act as a control, and (2) to which glucose was to be added. The free phosphate and glucose were each estimated in both tubes, the former by the Briggs [1922] technique. A weighed amount of glucose was then added to (2) and both tubes were corked and well shaken. The free phosphorus content of both tubes, and the glucose content of (2) were again estimated. They were then placed in the incubator at 37°, and the free phosphorus content of both tubes and the glucose content of (2) estimated at frequent intervals. The results are shown in Table I.

Table I.

	Tube (1)		Tube (2)		
Hours	mg. P %	Glucose %	mg. P %	Glucose %	Remarks
—	6·77	0·059	6·57	0·059	(Preliminary).
0·0	6·65	—	6·59	0·197	Glucose added to (2)
0·25	7·01	—	6·79	—	
0·5	7·92	—	7·24	—	Incubation at 37°
1·0	—	—	—	0·177	
2·5	—	—	—	0·152	

As will be seen, the addition of glucose to (2) did not alter in any way its free phosphorus content. This remained steady in both tubes till after about half an hour it began to rise under the influence of the corpuscular esterase. Thus the addition of glucose does not seem to lead to the formation of an organic phosphorus compound.

The results of Bierry and Moquet may perhaps be ascribed to the shaking out of CO_2, incidental to drawing, rendering the blood slightly more alkaline and thus starting the synthetic action of the phosphoric esterase mechanism described by Martland [1925]. The present writer has been able to obtain a fall in the free phosphorus by this means. The slight change in free PO_4 in tube (1) in Table I at 0 hours could only be ascribed to shaking out of CO_2.

The influence of initial low concentrations of free phosphorus was now tested. Rabbit's corpuscles were twice washed with isotonic saline and divided

into two portions. One was suspended in an equal volume of 1/750 hydroxyquinoline sulphate, and the other in an equal volume of a dilute isotonic phosphate mixture in presence of antiseptic. Glucose was added to both and the initial free phosphate estimated before incubation. The results of one experiment are shown in Table II.

Table II.

	Tube (1)			Tube (2)		
Hours	Glucose %	Glucose degraded %	mg. P %	Glucose %	Glucose degraded %	mg. P %
0	0·127	—	>0·5	0·108	—	6·85
2	0·097	0·030	—	0·081	0·027	—
4	0·074	0·053	—	0·057	0·051	—

In other experiments, the washing sometimes had a slowing influence on the rate of glucose degradation [cf. Macleod, 1913]; this was not however due to washing away of inorganic phosphorus since the subsequent addition of phosphates did not have any accelerating action.

Experiments in which the action of corpuscles on potassium hexosediphosphate was tested, indicated that, as far as the reducing power of the solution was concerned, this ester was very slowly attacked. It would thus appear improbable that it was an intermediate step in the conversion of glucose to lactic acid by these cells.

The evidence adduced above can therefore be summarised as follows.

The preformed organic phosphorus compounds in the corpuscles have no influence on glycolysis in blood. The addition of glucose to a corpuscular suspension at room temperature or at 37° does not bring about a diminution in the amount of free phosphorus present, nor has the initial concentration of the latter (within the limits of estimation of the methods employed) any appreciable influence on the rate of glycolysis. It appears unlikely that hexosediphosphate is an intermediate metabolite.

The influence of ions.

Hydrogen ion concentration. Several workers have stated that increased acidity retards glycolysis, whereas the addition of alkalis accelerates it. Rona and Wilenko [1914] and Mauriac and Servantie [1922] found the optimal p_H to be approximately 8.

Several experiments were undertaken by the present writer in the following way. The mixed arterial and venous bloods of one or more rabbits were centrifuged and the corpuscles obtained in the usual way. They were suspended in an isotonic solution of 1/750 hydroxyquinoline sulphate and a weighed quantity of glucose was added. The suspension was then pipetted out into a number of flasks. One was kept as a control while to the others varying amounts of isotonic $N/2$ HCl or NaOH were added. About 3 cc. from each flask were then incubated and the glucose content estimated at intervals, while the rest of the suspension was centrifuged and the p_H of the

supernatant fluid determined electrometrically or colorimetrically. The writer is indebted to Dr M. Dixon for carrying out the p_H estimations. Figures typical of those obtained are shown in Table III and Fig. 1.

Table III.

Volume of reaction mixture cc.	cc. $N/2$ NaOH added	cc. $N/2$ HCl added	p_H	Glucose % degraded in 2 hours
10	—	0·3	7·39	0·002
10	—	—	7·73	0·026
10	0·25	—	8·10	0·061
10	0·50	—	8·60	0·068
20	1·50	—	9·38	0·021
20	2·00	—	9·70	0·003

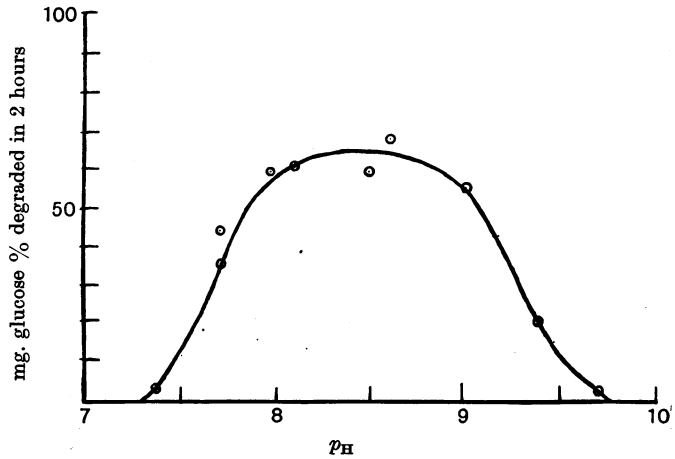

Fig. 1. The influence of p_H on the rate of glucose degradation.

The optimum p_H thus appears to lie between 8 and 9. One interesting fact which emerged from these estimations was the extreme constancy of the p_H of the corpuscular suspension before addition of acid or alkali. This was found to lie between 7·70 and 7·75. Although the addition of alkali caused a certain amount of haemolysis, this was not very marked until the p_H was in the neighbourhood of 9·5, since after centrifuging approximately the same volume of cells was obtained as was present before the addition of alkali. The addition of very small quantities of acid inhibited glycolysis very markedly.

Salts. Several experiments were carried out in which the corpuscles were suspended in isotonic solution of various ions. The following were tried:

$Na^{\cdot}$, $K^{\cdot}$, $Ca^{\cdot\cdot}$, $Mg^{\cdot\cdot}$, $Ba^{\cdot\cdot}$,

Cl', Br', I', F', NO_3', SO_4'', oxalate'', PO_4'''.

None of these was found to have any inhibitory action when in isosmotic concentration save fluoride and oxalate [cf. Macleod, 1913; Evans, 1922]; the former inhibited the reaction completely in small concentrations and glycolysis could not be restarted by either frequent washings or the addition of $CaCl_2$. The action of the fluoride ion in this and other instances is one of

great interest and is not at all understood. Oxalate inhibited in a similar way but to a less degree.

The degradation of other hexose sugars.

Portier [1903] and Griesbach and Oppenheimer [1913] showed that only those hexoses were degraded by blood corpuscles which were more or less closely allied to glucose. The present writer has tested fructose, mannose, galactose and rhamnose. The reducing power compared to glucose was found with pure solutions, and the necessary corrections have been made in all the figures quoted.

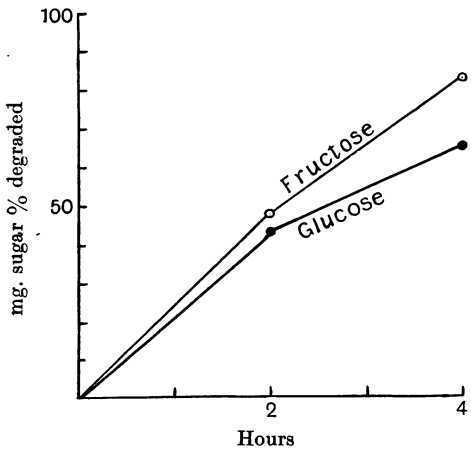

Fig. 2. The degradation of fructose by the adult rabbit's blood cells.

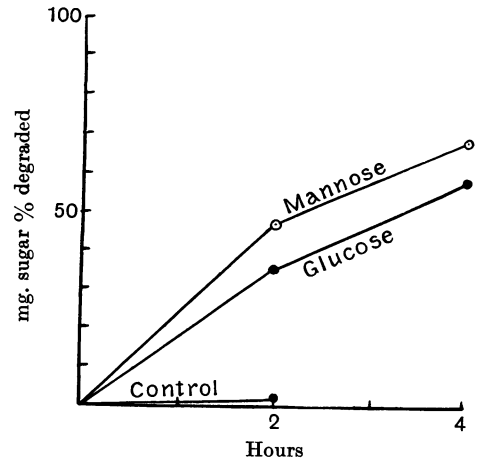

Fig. 3. The degradation of mannose by the adult rabbit's blood cells.

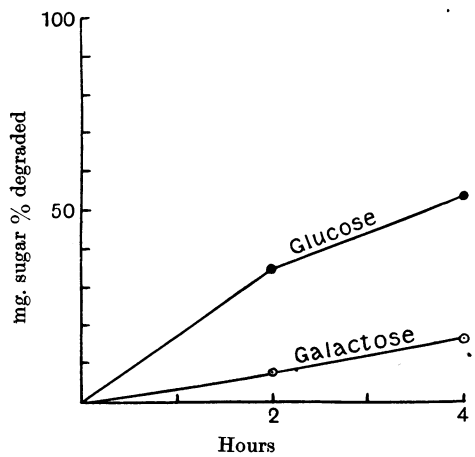

Fig. 4. The degradation of galactose by the adult rabbit's blood cells.

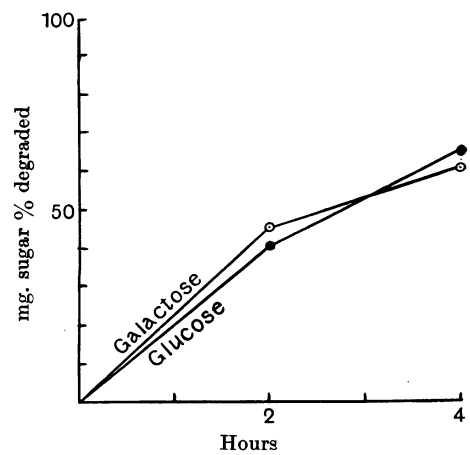

Fig. 5. The degradation of galactose by the infant rabbit's blood cells.

In the following experiments, the corpuscles were washed twice with saline and then divided into three portions: to one glucose was added, to one the sugar which was being tested was added, and the other was kept as

a control on the residual glycolysis. As a glance at Fig. 3 will show, the residual glycolysis was usually small, and in many cases was absent, so that the changes estimated occurred entirely in the added sugar. Figs. 2 and 3 show that fructose and mannose are degraded at about the same rate as glucose, the former usually slightly faster. Galactose (Fig. 4) is much more slowly attacked. Mixtures of glucose and fructose are degraded at the same rate as each alone, showing that the same mechanism is involved in the breakdown of each sugar. Rhamnose is not changed. These facts agree with those found for yeasts by Armstrong [1905] and Slator [1908], and in the main with those recorded by Herring, Irvine and Macleod [1924] in a study of the ability of various sugars to relieve insulin convulsions. These writers, however, found fructose to be relatively ineffective.

It is a matter of interest that the present writer has found that the corpuscles of young rabbits (aged about four weeks), in contrast to those of adults, were able to degrade glucose and galactose at equal rates (Fig. 5). Slator has stated that only those yeasts could ferment galactose which had become acclimatised to it, and perhaps the same occurs in this case.

From the evidence adduced here, there appears to be some fundamental connection between the stereochemistry of the sugar and the ability of the cell surface to degrade it. Until, however, we know more about the structure of the sugars, it will be impossible to postulate any satisfactory explanation.

Summary.

1. The degradation of glucose by the corpuscle of the rabbit does not appear to involve the intervention of organic phosphorus compounds.

2. The optimum p_H is 8·5, but the optimal range is a wide one.

3. Glucose, fructose and mannose are degraded equally fast by the corpuscles of the adult rabbit, but galactose is much more slowly attacked. In the infant rabbit, however, galactose is degraded as fast as glucose.

The writer is indebted to Dr H. D. Kay for a specimen of calcium hexosediphosphate, and to Dr J. H. Quastel for a specimen of rhamnose.

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