LXXXI. THE DEGRADATION OF GLUCOSE BY THE BLOOD CORPUSCLE OF THE RABBIT.

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THE experiments recorded in this paper were undertaken in part to discover the relationship between the known impermeability of the normal blood corpuscle of the rabbit to glucose [Irving and Kay, 1926] and its ability to degrade this substance. The fact that the blood sugar in shed blood is rapidly destroyed has been frequently noted. It was first investigated by Claude Bernard, and subsequently at great length by Lèpine, who postulated the existence of an "enzyme glycolytique." Despite many efforts, we are still ignorant of the nature of the agency in question, save that it resides in the corpuscle, and that its intensity is dependent on the integrity and age of this cell. It is inhibited completely by sodium fluoride [Evans, 1922] and partially by potassium oxalate [Macleod, 1913] when used in concentrations sufficient to stop coagulation of the blood. Lèpine [1918, 1, 2] and others have shown that plasma has no power of destroying glucose. Workers appear to differ, however, as to whether haemolysis of the corpuscle affects its glycolytic activity. Macleod [1913] showed that it did not, whereas Kawashima [1923] has stated that after haemolysis the reaction ceases. In none of the papers which the writer has read, have antiseptics been mentioned, though asepsis has been claimed. It thus appeared to be of interest to reinvestigate this phenomenon, using more carefully controlled experiments.

EXPERIMENTAL.

In all the experiments described, the blood of healthy rabbits, drawn as cleanly as possible from the marginal vein of the ear, was employed. Heparin (0.02 %) was used as an anticoagulant, since fluoride and oxalate, in the concentrations required to prevent clotting, were found to slow the reaction. The corpuscles were centrifuged down, the plasma withdrawn, and the corpuscular sediment, which had an average haematocrit value of between 75 and 85 %, was measured in a small measuring cylinder. An equal volume of isotonic saline containing hydroxyquinoline sulphate in a concentration of either 1 in 500, or 1 in 750, was then added, together with a weighed amount

of glucose, and the whole well shaken. The resulting suspension was pipetted into small tubes, which were tightly corked and placed in a water thermostat at 37°. In order to prevent the corpuscles from settling, the tubes were kept slowly oscillating. At intervals the reducing power of the solutions was estimated by Hagedorn and Jensen's micro-method for blood sugar.

All the antiseptics commonly used in enzyme solutions were found to inhibit the reaction very strongly. This was true for toluene, chloroform, borate and thymol. The only antiseptics which did not do this were the flavines and hydroxyquinoline sulphate. The former class possesses the disadvantage that, in the dilution arrived at in the final titration, the fluorescence interferes with the end-point. Hydroxyquinoline sulphate has the disadvantage that it reduces potassium ferricyanide, which is used in the sugar estimations. Control experiments using glucose solutions of varying strengths showed that the values obtained in the presence of hydroxyquinoline sulphate, like those given by glucose alone, gave a straight line when plotted against the actual values for glucose. This antiseptic was found to be stable at 37° for at least 18 hours, and to be unaffected by blood. Nearly all the results obtained in the presence of hydroxyquinoline sulphate were repeated over very short periods in its absence when the risk of bacterial infection was small, and were found to be confirmed. Experiments with bacterial cultures showed that hydroxyquinoline sulphate in a concentration of 1 in 1500 inhibited *B. coli* in broth from destroying glucose for at least 16 hours. It was thus probable that bacterial influences were completely excluded. In concentrations higher than 1 in 1000, hydroxyquinoline sulphate was found to interfere with the glucose degrading system to a certain extent, but in the concentrations employed here such action was very slight.

By removing the plasma it was possible to exclude a large number of unknown factors, including most of the blood sugar, the exact nature of which is still under dispute. Since no enzymes are known to exist in blood which will destroy the non-carbohydrate reducing substances, it would appear to be safe to assume that the loss in reducing power was due to loss of glucose alone.

THE KINETICS OF GLUCOSE DEGRADATION.

The rate of disappearance of glucose was found to be apparently independent of the initial concentration, being linear over a wide range of values. The rate was found to vary but slightly with blood corpuscles from different rabbits, about 18 mg. % per hour being degraded at 37° (Fig. 1). Kanitz [1913] and Fukushima [1924] have found that the values of k as calculated for a monomolecular reaction remained approximately constant. The present writer has found k to have a rising value as the reaction proceeds (Table I), a fact more in accord with a linear rate of degradation than one depending on the law of mass action.

Table I.

Hours	a	$a-x$	k
0	.111	—	—
1	—	.090	.0911
2	—	.073	.0911
3	—	.059	.0915
4	—	.048	.0910
5	—	.034	.1038
6	—	.025	.1079
7	—	.017	.1150

Fig. 1. The rate of degradation of glucose.
1. Intact cells. 2. Haemolysed cells.

As regards the influence of temperature, the only facts so far known are that the rate of the reaction varies with the temperature [Lèpine, 1918, 1, 2, and others].

In more exact experiments, the writer has found that the temperature coefficient between 27° and 37° was equal to 2.1.

THE SITE OF GLUCOSE DEGRADATION.

The majority of workers have stated that the glycolytic mechanism is intracorpuseular. Loeb [1913], making parallel observations on the rate of glucose breakdown and the permeability of the corpuscle membrane to this substance, in several different species found that glucose was destroyed only by those corpuscles into which it could readily enter. He did not, however, include the rabbit among his experimental animals. Fukushima [1924], on theoretical grounds, suggested that the agency in question might be an active surface, since the enzyme was in the cells and the substrate in the plasma. Most other authors have considered the action to be bound up with the vital activity of the corpuscle.

One would expect that, if glucose was destroyed by an enzyme residing in the corpuscle, rupture of the membrane would not diminish and probably increase the rate of the reaction, by setting free the enzyme into its substrate. Experiments with haemolysed blood showed that this was not the case (Fig. 1). A large number of observations were made, the corpuscles being laked by the addition to the centrifugate of an equal volume of distilled water containing hydroxyquinoline sulphate instead of isotonic saline. Contrary to the findings with intact corpuscles, the glycolytic rates were found to be much diminished and to differ considerably from sample to sample, some possessing up to 60 % of that of normal corpuscles, and one or two having lost the power of degrading glucose completely. In all cases, however, the reaction stopped long before all the glucose present was destroyed. The intactness of the corpuscle thus appeared to be an essential factor in this case.

Again, it is difficult to see how added glucose can enter the rabbit's blood corpuscle, as all workers are agreed that this is impermeable to glucose. Several experiments, carried out in conjunction with Dr H. D. Kay, showed that, although, during the drawing of the blood, the corpuscular reducing power increased, further additions of glucose failed to augment their glucose content. The figures in Table II illustrate this.

Table II.

	Blood sugar	Plasma sugar	Haematocrit value	Corpuscle sugar
Preliminary distribution	0.115	0.168	35.5	0.020
After addition of isotonic glucose solution	0.172	0.253	35	0.023

It appeared to be conceivable that, during the course of the reaction, a slow diffusion of glucose into the corpuscles might take place. Several experiments were therefore carried out in which the distribution of glucose during its degradation was followed. The results of one are shown in Fig. 2. A preliminary distribution was calculated from blood and plasma glucose values, and corpuscle volume, and the results are plotted on the ordinate. At the arrow, the corpuscles were centrifuged down as in previous experiments, and suspended in saline-glucose solution. The distribution was then redetermined, and the reaction mixture placed in the incubator, distributions being done at intervals. As will be seen, the increase in the glucose content of the medium had no influence on that of the corpuscles; during the course of the reaction, the reducing substances inside fell slightly in amount, but the bulk of the glucose being degraded appeared to be so changed in the medium. In other experiments, in which the initial corpuscle-glucose value was higher, this was found to fall in a similar way to that outside the corpuscle, but apparently to bear no relationship to it.

In addition, if the reaction took place inside the corpuscle, its rate would be governed by the rate of diffusion through the membrane, as this is very impermeable to glucose. In this case, however, the temperature coefficient would be low, whereas experiment has shown it to be high.

The conclusion arrived at by the present writer is that the bulk of the glucose degraded is destroyed on the surface of the corpuscle. In order

Fig. 2. The distribution of glucose during its degradation by blood corpuscles. At the arrow, the plasma was removed, and glucose saline substituted.

further to test this hypothesis, experiments were carried out in which substances, with strong non-polar groups, were added to the reaction mixture. The blood-corpuscle membrane is supposed, from the recent work of Gorter and Grendell [1925] to be covered by a bimolecular layer of lipoids, and, on the theory of Langmuir [1917], substances with non-polar groups would be adsorbed on this interface. This adsorption would interfere with the degradation of glucose by blocking the membrane to an extent depending on the concentrations and relative "strengths" of the non-polar groups.

The results of experiments, using propyl and ethyl alcohols, are shown in Fig. 3. The former was found to inhibit the reaction completely in a concentration of 6.7 % and the latter in one of 13.3 %. The inhibiting concentration of ethyl alcohol, as would be expected, was far higher than that of propyl alcohol, and the ratio between their inhibiting powers was found to agree with that found by Quastel and Whetham [1925] using these two alcohols while observing the dehydrogenations produced by resting bacteria, and also by Traube [1913] in observations on the respiration of birds' red blood corpuscles.

Usually, when complete inhibition had occurred, haemolysis was fairly far advanced, but was not complete; frequently half the corpuscles could be centrifuged down intact. It was not, however, possible to wash off the alcohol, and restart the action. It is possible that two actions may be occurring simultaneously, adsorption, and an actual destruction of the membrane; this may account for the shape of the ethyl alcohol curve.

In experiments with butyl alcohol, it was not found possible to get quantitative data, owing to its partial miscibility with water, but the inhibiting

Fig. 3. Inhibition of glucose degradation by propyl and ethyl alcohols.

concentration was much lower than that of propyl alcohol. Amytal (*iso*-amylethylbarbituric acid) was also found to have a very distinct inhibiting action in small concentrations, haemolysis not occurring, but owing to its insoluble nature quantitative figures could not be obtained.

DISCUSSION.

Recent work has shown that many biochemical changes appear to be catalysed at surfaces. Warburg [1921] has shown, by means of the charcoal model, that many oxidations produced *in vivo* can be reproduced *in vitro*. According to his work, iron is the responsible catalyst, and the surface of the charcoal is pictured as a mosaic of areas rich and poor in this substance. The surface of the blood corpuscle appears to differ from this in several respects. Since its activity is not at all impaired by KCN in a concentration of $M/500$, it would appear that iron is not one of the factors involved. Nor is the action catalysed by haemoglobin, since this substance can be turned into acid haematin without stopping glucose breakdown. From the work of Slosse [1911], Embden and his co-workers [1912], and Evans [1922], glucose in the presence of the blood corpuscle is changed to lactic acid probably by reactions which do not involve oxidative changes. Whether these changes are connected with organic phosphorus compounds or not will form the subject of a later paper.

It is much more probable that the surface of the blood corpuscle resembles in this respect that pictured for bacteria by Quastel [1926]. In the presence of powerful electric fields which occur in localised areas over the surface, the glucose molecule is activated prior to breakdown into simpler compounds.

In the case of blood haemolysed by osmotic means, one can picture the existence of a suspension of corpuscle surfaces, which have been ruptured, but not completely disintegrated. The curves obtained would suggest that the process of membrane destruction is continually progressing in this suspension, eventually bringing the reaction to a standstill. Substances like toluene and chloroform, however, which when used as antiseptics instantly and completely haemolyse the corpuscle, at once stop glucose breakdown by destroying the surface.

From these facts it would appear that the integrity of the corpuscular surface is essential for the degradation of glucose.

SUMMARY.

1. The rate of the degradation of glucose by the blood corpuscles of the rabbit has been followed. It has been found to have the kinetics of a linear reaction, and a temperature coefficient (Q_{10}) equal to 2.1.

2. From considerations of the permeability of the corpuscular membrane, and the ability of certain substances to inhibit this reaction, the hypothesis is suggested that glucose is degraded on the corpuscle surface.

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