CLVII. THE FATE OF THE BLOOD SUGAR AFTER INSULIN INJECTION IN NORMAL ANIMALS.

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INTRODUCTION.

WHAT becomes of the blood-sugar which disappears in such large amounts after the injection of insulin still remains an unsolved problem [Macleod, 1925].

Though it is recognised that the red blood corpuscles play an important part in many physiological functions, no one has expressed the view that they might occupy an equally important rôle in the mechanism of the action of insulin. That this might be so, however, was suggested by the results of certain investigations, to be communicated later, which have been in progress in this laboratory for some time. Briefly stated, our view was that insulin in the presence of the erythrocytes of living blood caused glucose to combine with certain of the other blood constituents, thereby forming a non-reducing complex probably containing both nitrogen and phosphorus, which thereafter found its way to all the tissues of the body, more especially to the liver and muscles, to be utilised or stored as required. This would naturally be a normal physiological process; but on the injection of insulin in a normal healthy animal the reaction would be so accentuated that though the reducing property of the whole blood or of the blood-plasma would be greatly diminished, the blood itself would still contain the greater part of the sugar, which, being now present in a combined state, would only be detected on hydrolysis.

To test the particular point in question a series of experiments was planned, and while these were in progress Scott and Best [1924] published a communication, in which they suggested "that the mechanism for the rapid disappearance of sugar from the blood is to be found in the blood itself." They report, contrary to the observations of McCormick, Macleod, Noble and O'Brien [1923], that while the reducing power of the blood of normal animals is usually only very slightly increased by hydrolysis, that of animals rendered hypoglycaemic by insulin, when similarly hydrolysed, shows a pronounced increase in reducing power, being equal to 70 % of that of the original normal blood. As no details of experimental results or methods have been published by these workers, it is difficult to say whether or not our findings are in agreement.

EXPERIMENTAL.

The majority of the experiments were carried out on rabbits. The animals used were deprived of solid food for 16–20 hours, and were all of approximately the same weight, about 2·9 kg. The blood was removed from the ear vein by means of a 20 cc. Record syringe and transferred immediately to two centrifuge tubes of approximately 10 cc. capacity containing a trace of finely powdered potassium oxalate to prevent coagulation.

A number of capillary tubes, usually four, were then charged with blood for the determination of the relative volume of corpuscles and plasma by the method described by Harvey [1920].

After the normal blood samples were drawn, the animals were injected subcutaneously with comparatively large doses of insulin (15–20 units). Two hours later, or on the appearance of convulsions, samples of blood were again removed from the ear vein as described above.

Determination of sugar content of plasma and corpuscles.

With the minimum of delay the tubes containing the blood samples were centrifuged for 10 minutes at approximately 4000 revolutions per minute. It was found that under this treatment the corpuscles and plasma are satisfactorily separated, as further centrifuging did not alter the relative volumes. By means of a capillary pipette the plasma was then removed carefully and as completely as possible without disturbing the corpuscular layer, following the method of Wu [1922], and the sugar content of the plasma and of the corpuscles determined by MacLean's method [1919].

In connection with the determination of the sugar content of the corpuscles it was found in the preliminary experiments that complete laking of the corpuscles by water previous to the addition of the sodium sulphate solution did not materially affect the results. Consequently in the later experiments the corpuscles were treated exactly as whole blood.

The reducing power of protein-free filtrates after hydrolysis.

At the same time, protein-free filtrates obtained by MacLean's method from the plasma and also from the corpuscles were hydrolysed by heating with sulphuric acid for half an hour under a reflux condenser, 1 cc. of 25 % sulphuric acid being employed for every 20 cc. of filtrate. After cooling, the sulphuric acid was exactly neutralised by the addition of the necessary volume of a concentrated solution of sodium carbonate, and the sugar content then determined by means of MacLean's copper reagent.

It may be well to add that in the first experiments on the determination of the sugar content of protein-free filtrates after hydrolysis, the results obtained were very high. This was traced to the use of an excess of sodium carbonate, so that, although MacLean's process furnishes us with an excellent method for the determination of sugar in whole blood, care must be taken in

applying this and similar methods to other work where the conditions may be somewhat altered. The recent paper by Quick [1925] on the effect of sodium carbonate concentration in the Benedict sugar method is pertinent in this connection.

The reducing power of plasma and corpuscles after hydrolysis.

Samples of plasma and of corpuscles were also hydrolysed directly as follows: 1 cc. plasma (or corpuscles) was added to 99.5 cc. sodium sulphate solution, 5 cc. 25 % sulphuric acid added, and the mixture refluxed for 1 hour. It was then cooled, exactly neutralised with 14.5 cc. sodium carbonate solution, and, after the effervescence had ceased, treated with 5 cc. of "dialysed iron." After thorough shaking, the mixture was filtered through a Whatman No. 40 paper of 9 cm. diameter, and 20 cc. samples of the filtrate used for the sugar estimation.

No difficulty was experienced in obtaining water-clear filtrates from either plasma or corpuscles, but while no irregularity resulted with filtrates from plasma, those from the corpuscles invariably gave slight biuret reactions and consequently contained small amounts of protein material. As this could not be removed by modifying the conditions of the precipitation, it was evidently of the nature of a proteose or peptone—probably derived by hydrolysis of the histone, globin. The determination of the reducing power of the corpuscles after direct hydrolysis is thus rendered unreliable, and it is difficult to estimate how far the presence of, say, peptone, interferes with the process. Tests with Witte's peptone proved that high values would result in determining the reducing power of a solution either by MacLean's or Hagedorn and Jensen's methods [1923], if peptone were present to the extent of 0.03–0.05 %. Consequently this source of error should be guarded against in work of this type.

In order to make clear the discussion which follows, the results of some typical experiments are given in Table I. As already indicated, the majority of the experiments were conducted on rabbits, but similar results were also obtained with rats.

Table I. *Normal rabbit before insulin.*

Animal no.	A		B		C		C - A	
	Plasma %	Corps. %	Plasma %	Corps. %	Plasma %	Corps. %	Plasma %	Corps. %
2	0.136	0.050	—	—	0.136	0.0634	0.0	0.013
3	0.166	0.040	0.170	0.076	0.166	0.096	0.0	0.056

Normal rabbit after insulin.

2	0.044	0.030	—	—	0.045	0.079	0.001	0.049
3	0.036	0.038	0.060	—	0.039	0.125	0.003	0.087
4	0.053	0.039	0.063	0.067	0.058	0.106	0.005	0.066

(A = before hydrolysis. B = after hydrolysis of "protein-free" filtrate. C = after direct hydrolysis.)

Further, as the relative volumes of plasma and corpuscles had been determined in most cases, it was possible to calculate the values for the whole blood. These are given in Table II.

Table II. *Sugar content of whole blood before and after hydrolysis.*

		Before hydrolysis	After hydrolysis	Increase
Animal		%	%	%
1.	Normal rabbit	0.136	0.155	0.019
2.	„ „	0.107	0.112	0.005
3.	„ „	0.122	0.141	0.019
4.	„ „	0.128	—	—
	„ rat	0.093	0.110	0.017
Average increase = 0.015 %.				
2.	Insulin rabbit	0.039	0.056	0.017
3.	„ „	0.037	0.068	0.031
4.	„ „	0.049	0.071	0.022
	„ rat	0.057	0.113	0.056
	„ „	0.025	0.055	0.030
Average increase = 0.031 %.				

DISCUSSION OF RESULTS.

Changes in the whole blood. The results indicate that the sugar content of the blood in the case of the normal rabbit or rat is only slightly increased on hydrolysis. In animals rendered hypoglycaemic by insulin the actual increase in reducing power brought about on hydrolysis is, on the average, about twice as great as that obtained with normal animals. When due account is taken of the usual increased reduction after hydrolysis, apparently 14–20 % of the sugar disappearing from the blood would be accounted for.

Influence of insulin on corpuscle sugar and plasma sugar. Inspection of the results in Table I show that the percentage of free reducing material in the corpuscles is very small and remains practically unchanged before and after the injection of insulin. In fact, this material would seem to correspond very closely to the non-glycolysable and non-fermentable reducing substances recently proved to exist in blood by Hiller, Linder and Van Slyke [1925]. The plasma sugar, on the other hand, has a comparatively high initial value, and on the administration of insulin, falls very nearly to the value of that present in the corpuscles.

Changes on hydrolysis. On hydrolysis the plasma sugar remains practically unaltered in both the normal and insulin-treated animal, whereas the corpuscle sugar increases in both cases. This behaviour is contrary to expectation and points to the fact that if a complex non-reducing compound be formed as the result of the injection of insulin, the compound does not remain in the plasma, but may, to a certain extent, be associated in some manner with the corpuscles.

As only about one-sixth of the vanished sugar can thus be accounted for, our original hypothesis cannot be maintained, and it seems more likely that

a non-reducing sugar complex is formed in the blood and immediately distributed among all the tissues of the body. It is also probable that the different kinds of tissue may be able to hold varying amounts of the complex, so that liver and muscle tissues may take up a greater proportion than other tissues. The red blood corpuscles are thus regarded as a type of tissue, and consequently they would get their share of the glucose which disappears from the blood-plasma.

Whether the results of our experiments support this view is unfortunately rendered doubtful, for at this stage the analyses become unreliable because of the simultaneous production and interfering action of hydrolysed proteins. In the experiments under consideration, however, the error introduced should be of the same order in the case of blood taken in the normal and in the hypoglycaemic condition, and as, so far, greater values have always been obtained in the latter, it seems possible that the difference may be due to the action of insulin. The amount of this difference is not large, and though well beyond the usual experimental error, may still be accounted for in this way; but it must be remembered that if the view indicated above be correct, only a small difference could possibly result. One hesitates, however, to draw the definite conclusion that insulin brings about the conversion of the glucose present in the plasma into a non-reducing complex, which then becomes combined in some manner with the tissues, amongst which are included the red blood corpuscles. The results communicated in this paper, if not conclusive, are, at least suggestive, and the work is being continued in the hope that it may help to solve the mystery of what is the most interesting and fundamental problem in connection with the action of insulin.

SUMMARY.

1. The reducing power of the corpuscles of the blood of the normal rabbit is comparatively low, and is not influenced to any extent by the injection of insulin.

2. The reducing power of the plasma of the blood is comparatively high, and under the action of insulin falls to the level of that possessed by the corpuscles.

3. On hydrolysis, the plasma sugar is little affected either before or after insulin, whereas the reducing power of the corpuscles increases in both conditions.

4. Part of the increase in reducing power of the corpuscles after hydrolysis is due to the interfering action of peptone produced by the hydrolysis; but, as the increase is greater after insulin than in the normal condition, it suggests that a non-reducing glucose complex may be formed which attaches itself in some way to the tissues, including the red blood corpuscles.

5. About a sixth part of the sugar disappearing from the plasma after insulin may be accounted for by the erythrocytes acting in this manner, but no definite conclusion can be drawn.

6. It is pointed out that certain experimental difficulties may arise in applying the usual methods of estimating sugars to "hydrolysed" solutions.

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