XL. THE PART PLAYED BY THE DIFFERENT BLOOD ELEMENTS IN GLYCOLYSIS.

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(Received August 26th, 1915.)

When blood is allowed to stand at a suitable temperature an appreciable amount of the sugar present disappears. This glycolysis occurs to a marked extent in human blood; it is also observed in the blood of certain of the lower animals (dog, sheep) [Loeb, 1913], while in other animals (pig) [Melvin, 1912], (goat) [Maclean, 1911], little or no decrease in sugar content appears to take place¹. This difference explains certain divergent statements encountered in the literature. Not only are there differences in the degree of glycolysis in different animals, but great variations may be observed in the same animal under different circumstances. In certain pathological conditions in man, as will be shown later, glycolysis may be greatly increased.

Various observers have demonstrated that the glycolytic power of blood is essentially dependent on the formed tissue elements, and that the fluid constituent plays no part [Rona and Döblin, 1911; MacLeod, 1913]. In general, the power of destroying sugar has been ascribed to the red corpuscles and various theoretical deductions have been drawn, based chiefly on the normal glucose content of the erythrocytes and the relative inability of glucose to penetrate the envelopes and gain access to their interior [Loeb, 1913]. Rona and Arnheim [1913] attempted to separate the cellular elements of blood into erythrocytes and white cells, and found, on estimating the glycolytic

¹ Possibly, this lack of glycolytic power in certain animals is more apparent than real. The presence of a small amount of some sugar-forming substance such as glycogen might supply sugar to replace that destroyed, so that estimation of the sugar content before and after incubation would give no real clue to the glycolytic activity of the blood.

activity of the two constituents, that no glycolysis was produced by the white cells while the red cells were distinctly active. Levene and Meyer [1912], however, showed that leucocytes have the power of destroying glucose, at least in the presence of phosphate mixture. Rona and Arnheim then added phosphates to their white cells, and found that this addition played an important part, the white cells now showing marked glycolytic activity.

So far, the evidence pointed to the red cells as the chief agent in the phenomenon in normal blood, but, since the discovery of Levene and Meyer mentioned above, certain observers have assumed, without any real evidence, that glycolysis depends chiefly or entirely on the leucocytes.

The chief obstacles in the way of settling this question were bound up with the difficulty of separating the blood elements into white and red cells; a suitable method for accurately estimating sugar in small amounts of blood was also needed.

In recent years several methods for this purpose have been published but certain of them are unsuitable. One of the best is Bang's micro-method. In the following experiments, a modification of the method described by Gardner and MacLean [1914] has been adopted. The objects aimed at were to avoid filtration of the cuprous oxide, and to increase the readings given by very small amounts of sugar so that very minute differences could be detected and estimated. The method will be fully described in another paper. It is sufficient to mention here, that such a small amount of sugar as 0.1 of a milligram gave a reading of nearly 1 cc. of a standard thiosulphate solution. From this it is obvious that very small differences in sugar content could be easily detected, and that very small amounts of blood sufficed for the determinations. In each experiment 1 cc. blood was used. Contrary to the results of Rona and Arnheim [1913], we find that the leucocytes play a very important role in glycolysis, being very much more active than the red corpuscles; on the other hand our experiments agree with the results of these and other authors who maintain that the red corpuscles are also capable of causing glycolysis. Though the red corpuscles are active in this respect, each red corpuscle is of little importance when compared with a white corpuscle; the very much greater number of red corpuscles in normal blood, however, tends to correct this predominance of the white so that in reality the red cells do account for a good deal of the total glycolysis of blood. In abnormal bloods, such as that of patients suffering from leucocythaemia, the part played by the white cells is very marked as is shown in the following experiment.

EXPERIMENTS WITH PATHOLOGICAL BLOOD.

Experiment I.

Patient suffering from leukaemia was bled, and to 25 cc. blood 0.1 % potassium oxalate added. The blood was centrifuged and the plasma removed. The upper layer, which contained an excess of leucocytes, was now carefully removed, mixed with plasma, again centrifuged and the upper layer used for glycolysis (*Leucocyte Fraction*). By means of a very fine pipette the lower layers, containing an excess of red cells, were removed mixed with plasma and again centrifuged. The lower layers of this were then taken and used for glycolysis (*Red cell Fraction*).

To each fraction some glucose was added: 1 cc. of each was then taken and the sugar content estimated; both were incubated at 37° for 1.75 hours and the sugar again estimated.

Blood counts of the number of red and white cells in each fraction were made. From the results obtained it is obvious that the fraction containing excess of leucocytes was very much more active than the red cell fraction, which contained but a comparatively small number of leucocytes.

Blood fraction used	Before incubation		After incubation		Sugar destroyed per 100 cc. in g.	Blood counts	
	No. of cc. of N/250 thiosulphate used	Sugar present per 100 cc. in g.	No. of cc. of N/250 thiosulphate used	Sugar present per 100 cc. in g.		Red cells per c.mm.	White cells per c.mm.
Leucocyte fraction	11.75	0.132	4.85	0.054	0.78	6,000,000	231,200
Red cell fraction	12.05	0.134	9.55	0.106	0.28	10,000,000	11,360

Here the activity of the leucocyte fraction was very marked, sugar equivalent to 6.90 cc. N/250 thiosulphate being used up in 1.75 hours, while the red cell fraction, which contained four millions more of cells than the white cell fraction, destroyed sugar only equivalent to 2.50 cc. N/250 thiosulphate.

Part of the original blood was also tested; it contained 3,500,000 red cells and 81,000 white cells per c.mm.

In 1.75 hour it used up sugar equivalent to 3.3 cc. N/250 thiosulphate solution—a greater amount would be found in the case of a normal blood containing five to six million red cells. Similar results to the above were found in other cases. The figures in one more case are given and they will suffice to show that in leukaemic blood the increased leucocyte content plays a marked part in the phenomenon of glycolysis.

Experiment II.

The blood was divided into the two fractions—white and red cells—as in Experiment I. Incubation here lasted for two hours.

Blood fraction used	Before incubation		After incubation			Blood counts	
	No. of cc. of N/250 thiosulphate used	Sugar present per 100 cc. in g.	No. of cc. of N/250 thiosulphate used	Sugar present per 100 cc. in g.	Sugar destroyed per 100 cc. in g.	Red cells per c.mm.	White cells per c.mm.
Leucocyte fraction	5	0.055	none	none	0.055 +	5,000,000	212,400
Red cell fraction	4.5	0.050	1.55	0.017	0.033	10,000,000	17,640

In this experiment the amount of sugar added was too small and the incubation period rather long, for all the sugar was used up in the leucocyte fraction. The numbers obtained for this fraction are therefore too low, for if more sugar had been present more would have been destroyed. While showing the marked effect of the leucocytes these experiments also prove that the red cells take an active part in glycolysis.

They also show that the addition of phosphates is not necessary to bring about this effect. Since the above experiments were done with pathological blood, it might be argued that no deductions applicable to normal blood could be drawn from the results. Nearly twenty experiments, however, have been carried out on normal blood and in every case it has been found that while both red cells and leucocytes are active, the leucocytes are cell for cell very much more active than the red cells. The exact glycolytic power of the white cell compared with the erythrocyte varies very much indeed, but in a general way the experiments suggest that each white cell is from 200 to 1000 times as active in glycolysis as a red cell. Seeing that many delicate manipulations are necessary in arriving at these results it is probable that no great importance is to be attached to the exact figure found for the red to white ratio in any given experiment; it is merely to be regarded as something of the order of the number given. When, however, it is found that in every experiment the glycolytic value for white cells comes out enormously greater than that for the red cells the part played by the leucocytes is abundantly demonstrated. No doubt the destruction of sugar caused by the red cells may depend on certain conditions already mentioned such as the amount of sugar inside the cell, and on other factors: probably also the activity of the white cell varies very much in different cases. If we assume that the white cell is on an average 1000 times as powerful a glycolyser as a red cell, and that normal blood contains about five hundred times as many red cells as white, the leucocytes in a sample of such a blood would account for the destruction of twice as much sugar as the red cells.

EXPERIMENTS WITH NORMAL BLOOD.

We found it quite impossible to separate the blood into two fractions—one containing white cells only, and the other red cells. Invariably our red cell fraction contained leucocytes and our leucocyte fraction had always large amounts of red corpuscles. It is obvious, however, that complete separation into two constituents is not necessary. All that is required in order to determine the relative action of the red and white blood cells on glycolysis is to divide the blood into two fractions in which, though both constituents are present, they differ greatly in number. The sugar in each part is estimated before and after incubation. This at once gives conditions from which the effect of either constituent can be worked out by a simple algebraical equation. The following experiments illustrate these points.

Experiment III.

12 cc. of blood were obtained from a student by inserting a needle in a vein, coagulation being prevented by the addition of 0.1 % potassium oxalate. The blood was centrifuged, the plasma separated and the top layer pipetted off. This layer constituted the leucocyte fraction, while the lower layer represented the red cell fraction. The sugar was estimated in each fraction before and after incubation for 4 hours at 37°.

	Leucocyte fraction	Red cell fraction
Sugar before incubation in g. per 100 cc.	0.037	0.040
Sugar after incubation in g. per 100 cc.	0.009	0.006
Total sugar used in g. per 100 cc.	0.028	0.034
Blood count in cells per c.mm.	{ Red cells 6,000,000 White cells 6,960	{ Red cells 8,000,000 White cells 4,440
Ratio of activity of leucocyte to red cell = 178 : 1.		

Also it is evident that both red and white cells must have been active, for, though the white cell is much more active than the red, if the glycolysis depended only on the white corpuscles, the leucocyte fraction should have destroyed much more total sugar than the red cell fraction.

Experiment IV.

Blood from a patient was treated exactly as in Experiment III with the exception that incubation was restricted to 2 hours at 37°.

	Leucocyte fraction	Red cell fraction
Sugar before incubation in g. per 100 cc.	0.033	0.032
Sugar after incubation in g. per 100 cc.	0.010	0.013
Total sugar used in g. per 100 cc.	0.023	0.019
Blood count in cells per c.mm.	{ Red cells 7,200,000 White cells 15,520	{ Red cells 9,800,000 White cells 2,240
Ratio of activity of leucocyte to red cell = 364 : 1.		

Five other experiments were carried out in exactly the same way except that in certain of them a small amount of glucose was added. The results are given in the table. In No. 8 the red cell fraction first obtained was again centrifuged and the bottom layer of the latter used as the red cell fraction.

No.	Source of Blood	Amount of blood taken in cc.	Sugar added in mgrs.	Time of incubation in hrs.	Sugar of leucocyte fraction		Total sugar destroyed by leucocyte fraction per 100 cc.	Sugar of red cell fraction		Total sugar destroyed by red cell fraction per 100 cc.
					Percent. before incubation.	Percent. after incubation.		Percent. before incubation.	Percent. after incubation.	
V	student	15	none	1.5	0.03	0.009	0.021	0.028	0.01	0.018
VI	patient	15	15	2	0.091	0.061	0.030	0.083	0.058	0.025
VII	student	20	15	3	0.097	0.056	0.041	0.088	0.052	0.036
VIII	patient	15	10	1.75	0.084	0.062	0.022	0.072	0.058	0.014
IX	student	15	10	—	0.062	0.031	0.031	0.063	0.049	0.014

The "blood counts" of the above experiments gave the figures per cubic millimetre.

		Leucocyte fraction	Red cell fraction
No. V	{	Red cells 9,200,000	{ Red cells 12,500,000
	{	White cells 10,440	{ White cells 3,600
No. VI	{	Red cells 4,700,000	{ Red cells 10,700,000
	{	White cells 13,040	{ White cells 3,840
No. VII	{	Red cells 4,500,000	{ Red cells 10,000,000
	{	White cells 12,480	{ White cells 3,840
No. VIII	{	Red cells 8,200,000	{ Red cells 10,000,000
	{	White cells 23,000	{ White cells 1,280
No. IX	{	Red cells 5,000,000	{ Red cells 11,800,000
	{	White cells 29,320	{ White cells 1,000

Ratio of leucocyte activity
to red cell activity

No. V	...	...	863 : 1
VI	...	...	965 : 1
VII	...	...	850 : 1
VIII	...	...	358 : 1
IX	...	...	779 : 1

It is obvious from these experiments that the leucocyte is in every case much more active than the red cell in the production of glycolysis.

GLYCOLYSIS IN HAEMOLYSED BLOOD.

If, according to the observations of Loeb and others, the power of destroying sugar resided entirely in the red cell it is difficult to understand the results obtained by Edelman [1912], who showed that laking of the red cells by saponin does not destroy the glycolytic power. It is known, however, that it is possible under certain conditions to lase the red cells without destroying the leucocytes, and it may possibly be that in these experiments where the red corpuscles were destroyed, the white corpuscles were left sufficiently active partially to destroy the sugar present.

In all the experiments already given, the blood was centrifuged in its own plasma. In some later experiments, some small differences in manipulation were introduced and the corpuscles were washed with isotonic saline solution. Here the results were in agreement with those obtained with unwashed corpuscles except that the ratio of leucocyte activity to that of the red cell was somewhat changed. In all cases clotting was prevented by the addition of 0.1 % potassium oxalate solution.

Experiment X.

The blood was obtained from a student in the usual manner. To this 20 mgrs. sugar were added; after centrifuging, the plasma was removed and the top layer of the solid separated; this was shaken up with the separated plasma and again centrifuged. The top layer of this was mixed with normal saline, again centrifuged and the top layer again pipetted off and used for the experiment (= leucocyte fraction). After the first centrifuging of the blood the layer left behind after removal of the leucocytes was mixed with some normal saline. This was then centrifuged, the lower part of the solid layer formed removed by a fine pipette, again mixed with normal saline, and centrifuged. The lower layer of this, which contained only a relatively small number of white cells, represented the red cell fraction. Incubated 1.5 hours.

	Leucocyte fraction	Red cell fraction
Sugar before incubation in g. per 100 cc.	0.0810	0.0792
Sugar after incubation in g. per 100 cc.	0.0468	0.0567
Total sugar destroyed in g. per 100 cc.	0.0342	0.0225
Blood count in cells per c.mm.	{ Red cells 7,725,000 White cells 23,520	{ Red cells 8,275,000 White cells 840
Ratio of activity of white to red cell = 218 : 1.		

Experiment XI.

This was carried out exactly as in Experiment X. The blood was obtained from a healthy soldier. To 20 cc. blood, 20 mgrs. sugar were added.

	Leucocyte fraction	Red cell fraction
Sugar before incubation in g. per 100 cc.	0.091	0.078
Sugar after incubation in g. per 100 cc.	0.052	0.062
Sugar destroyed in g. per 100 cc.	0.039	0.016
Blood count in cells per c.mm.	{ Red cells 8,225,000 White cells 42,160	{ Red cells 8,625,000 White cells 1,120
Ratio of activity of white to red cell = 325 : 1.		

In these experiments it will be noticed that the activity of the white corpuscle compared with the red cell is low. This however is apparently not due to the action of the saline, as the next experiment shows.

Experiment XII.

20 cc. blood from student treated exactly as above. Incubation 1.75 hours. 20 mgrs. sugar added.

	Leucocyte fraction	Red cell fraction
Sugar before incubation in g. per 100 cc.	0.138	0.091
Sugar after incubation in g. per 100 cc.	0.110	0.078
Sugar destroyed in g. per 100 cc.	0.028	0.013

Blood count in cells per c.mm.	{ Red cells 6,000,000	{ Red cells 10,000,000
	{ White cells 17,600	{ White cells 1,800

Ratio of activity of white to red cell = 1149 : 1.

Several experiments were carried out and always with the same general result, though the figures obtained varied considerably. The results given serve to establish the activity of both red and white corpuscles in the process of glycolysis.

CONCLUSIONS.

- (1) Both the red and white cells of normal blood take part in glycolysis.
- (2) The white cells are much more active than the red; the ratio of activity of the white to the red cell varies roughly from about 200:1 up to 1000:1.
- (3) In leukaemic or other blood containing excess of leucocytes, glycolysis is much more active than in normal blood.
- (4) In normal blood it is probable that the comparatively small number of leucocytes present exert a somewhat greater influence on the total glycolysis than do the red cells which are present in relatively large amount.

This research was carried out as part of an investigation for which a grant has been received from the Medical Research Committee of the National Health Insurance Commission.

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