STUDIES ON THE PHYSIOLOGY OF THE WHITE BLOOD CELL

The Glycogen Content of Leukocytes in Leukemia and Polycythemia*

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In a previous paper[1] a procedure has been described for determining glycogen in whole blood and isolated white blood cells. By this method, the average of the glycogen content of normal human blood is 5.5 mg. per cent. The average of the glycogen content per million normal total white blood cells is 2.54 γ. Plasma, red blood cells, and blood platelets do not contain any measurable amounts of this carbohydrate.

In the present investigation this method has been applied to the study of the glycogen content of whole blood and white blood cells in cases of leukemia and polycythemia. Cases of leukemia with only one predominant cell form are particularly suitable for studying the biology of the white blood cells as far as their glycogen metabolism is concerned. Polycythemia was included in this study because of its possible relation to leukemia and the large amounts of glycogen detected in whole blood as well as in isolated leukocytes in this disease. Some experiments dealing with the glycogen content of rabbit leukocytes are also included in this study.

TECHNIC

The glycogen determinations in whole blood are carried out on 1 cc. samples according to a micro modification of the Pflüger[2] method. The material for the glycogen determinations in white blood cells is collected in a Cushman[3] tube, analytically weighed, and its glycogen content determined according to the same method as that of whole blood. For the quantitative evaluation of the results a white blood cell count is done on the cell layer, using a white blood cell pipet, and the volume of its first subdivision is determined by calibration with mercury. The dilution for the cell count is 1 to 100. Both the weight of the total white blood cell layer and the weight of its amount in the blood pipet are determined.† The amount of glycogen per million wet white blood cells is then calculated according to the following formula:

$$\text{Glycogen per million white blood cells} = \frac{A \times B}{C \times D \times E}$$

A—Glycogen found in the white blood cell layer (γ).
B—Weight of the white blood cell layer used for the cell count (γ).
C—Volume of the first subdivision of the blood pipet (cmm.).
D—Number of white blood cells in the layer (millions per cmm.).
E—Weight of the white blood cell layer (γ).

RESULTS

White blood cell layers collected in the described manner cannot be considered as homogeneous. They consist of myeloid and lymphoid cells. There is some difficulty

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†This rather complicated procedure is the least time-consuming method for dealing with living white blood cells without losing some of the glycogen as a result of the intense enzymatic activity of the leukocytes.

in evaluating the glycogen content of a white blood cell layer as long as the content of its separate constituents is unknown. In this respect studies on leukemia with one predominant cell type are helpful.

TABLE 1.—*Glycogen Content of Whole Blood and White Blood Cells in Chronic Myelogenous Leukemia*

Exper. Number	W. B. C. per cmm.	Blast Cells	Promyeloc.	Myeloc.	Metamyeloc.	Polys.	Eo.	Baso.	Mono.	Lympho.	Plasma Cells	Glycogen in Whole Blood Determined	Glycogen per Million W. B. C. Determined	Glycogen Content of W. B. C.† Caculated
		%	%	%	%	%	%	%	%	%	%	*mg. per cent*	γ	*per cent*
182	9,450	—	—	8	18	64(23)*	1	—	2	7	—	6.0	45.50	6.10
158‡	10,100	—	—	—	—	45(9)	1	2	32	20	—	6.1	4.16	0.64
84	24,700	—	—	2	9	79(65)	—	2	2	6	—	12.3	3.82	0.51
103	38,250	—	—	2	3	73(27)	4	1	1	16	—	31.5	4.26	0.63
192	38,500	3	3	2	21	54(13)	4	1	2	9	1	19.4	4.70	0.67
16	43,400	4	—	16	—	57(10)	—	19	3	1	—	24.9	3 88	0.51
114	50,000	1	—	19	38	34	—	1	3	4	—	17.6	3.16	0.42
115	60,000	19	12	21	8	32(16)	1	6	—	1	—	37.0	3.87	0.60
185	60,000	—	10	11	10	57(12)	—	3	3	6	—	16.4	2.43	0.32
176	145,300	1	3	13	21	51(20)	2	1	1	7	—	22.9	2.23	0.30
180	355,000	2	7	34	27	27(15)	1	1	—	1	—	48.2	1.98	0.25
179	440,000	1	4	31	28	32(17)	1	—	1	2	—	97.0	1.37	0.17
149	576,000	5	8	43	22	16	2	1	1	2	—	>114.0	2.68	0.36

* The figures in parentheses indicate band forms.

† The figures in this column are calculated on the assumption that the nitrogen content and water content in human leukocytes are the same as in those of rabbits (cf. experiment 198). The figures are calculated by dividing the glycogen content per million W. B. C. by 8.2.

‡ Diagnosis "chronic myelogenous leukemia" confirmed by bone marrow puncture.

TABLE 2.—"*Reducing Substances*" *of Whole Blood and White Blood Cells in Chronic Lymphatic Leukemia*

Exper. Number	W. B. C. per cmm.	Blast Cells	Polys.	Eo.	Mono.	Lympho.	Reducing Substances in the Alcohol Precipitates Following Acid Hydrolysis	
							In Whole Blood Determined	Per Million W. B. C. Determined
		%	%	%	%	%	*mg. per cent*	γ
157	15,000	—	32(2)	1	8	59	4.2	0.12
169	30,000	—	15	3	—	82	6.7	0.75
177	48,700	—	17(4)	—	8	75	2.9	0.46
184	245,000	1	3(2)	—	—	96	8.7	0.24
175	287,280	—	2	—	—	98	4.9	0.07
130	403,000	—	—	—	—	100	10.9	0.37
161	810,000	—	—	—	—	100	14.4	0.22

1. *Glycogen Content of Whole Blood and Isolated White Blood Cells (per Million) in Leukemia.* Tables 1, 2, and 3 contain the experimental data on 25 cases of different types of leukemia. The graphic picture (fig. 1) reveals the relation between the number of white blood cells per cmm. and the concentration of glycogen and

TABLE 3.—*"Reducing Substances" of Whole Blood and White Blood Cells in Acute (Blast Cell) Leukemia*

Exper. Number	W. B. C. per cmm.	Blast Cells		Promyeloc.	Myeloc.	Metamyeloc.	Polys.	Eo.	Baso.	Mono.	Lympho.	Reducing Substances in the Alcohol Precipitates Following Acid Hydrolysis	
		Myelo. blasts	Lympho. blasts									In Whole Blood Determined	Per Million W. B. C. Determined
		%	%	%	%	%	%	%	%	%	%	*mg. per cent*	γ
154	103,000	—	95	—	1	—	—	—	—	—	4	5.2	0.10
92	220,000	—	93	—	—	—	2	—	—	—	5	1.1	0.08
191	17,200	—	95	—	—	—	3	—	—	2	—	<1.0	0.19
159	149,000	96	—	—	—	—	1(1)	—	—	2	1	6.2	0.30
188	158,400	89	—	—	2	4	5(3)	—	—	—	—	24.5	0.80

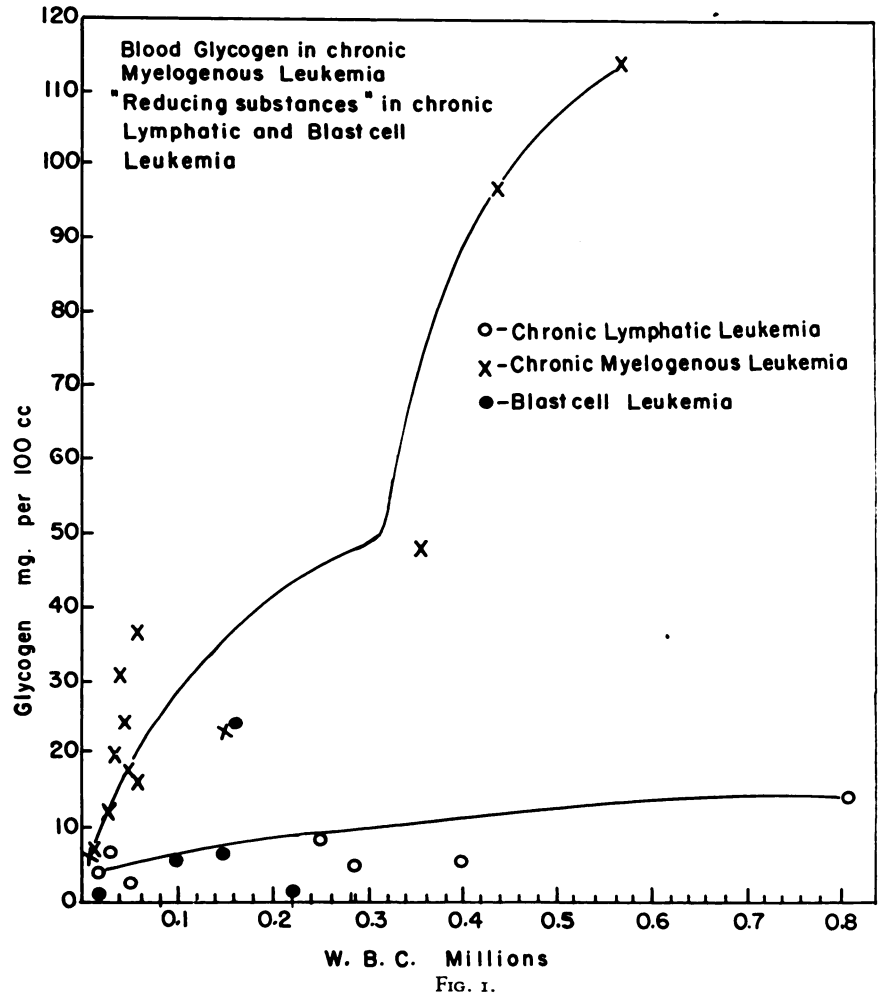

FIG. 1.

"reducing substances"* respectively in mg. per cent for this group. There is practically no increase of "reducing substances" in chronic lymphatic leukemia with increasing number of white blood cells per cmm. In spite of a count of 810,000 W. B. C. per cmm. the "reducing substances" are about on the same level as in normal blood. On the other hand, in chronic myelogenous leukemia there is a considerable increase in almost linear proportionality with the number of cells. The highest value ever observed was found in a case of chronic myelogenous leukemia with 576,000 W. B. C. per cmm. and 43 per cent myelocytes (experiment 149). In 5 cases of acute leukemia, myeloblastic as well as lymphoblastic, the "reducing substances" are again low, in experiments 92 and 191 almost negligible. Only in experiment 188 is the obtained value higher than that in the other acute cases. However, this is the only case of this group where the percentage of more mature elements is relatively higher than in the other 4 cases.

From figure 1 the conclusion is reached that it is evidently the total number of *granulated* leukocytes which determines the polysaccharid content of whole blood. Since in previous studies[1] the blood plasma, the red blood cells and blood platelets were found to be free from glycogen, it was to be expected that glycogen determinations on isolated leukocytes might help to explain the differences of the glycogen content of whole blood in the different types of leukemia. In tables 1, 2, and 3 the concentration of glycogen and "reducing substances" respectively per million W. B. C. is recorded.

In all cases of chronic lymphatic leukemia the content of "reducing substances" per million W. B. C. is below 1 γ (average 0.32 γ). In experiment 175 the lowest value of all observations on white blood cells was found. In all instances of blast cell leukemia the value per million W. B. C. is likewise below 1 γ (average 0.29 γ), approximately within the same range as in lymphatic leukemia. In all cases of chronic myelogenous leukemia the glycogen content is above 1 γ (average 3.21 γ†; experiment 182 excluded).

The conditions under which experiment 182 was carried out may explain the strikingly high value of 45.5 γ. The analysis in whole blood as well as in W. B. C. was done immediately after x-ray treatment. The number of W. B. C. dropped from 374,000 to 9450 cells per cmm. The whole blood glycogen determination was carried out on blood collected from the hematocrit tube after the cells had been resuspended. Some of the glycogen might have been destroyed by this manipulation. The high glycogen content per million W. B. C. may be interpreted as evidence of the capacity of white blood cells to phagocytize glycogen freed from leukocytes following x-ray treatment.

2. *Glycogen Content of Granulated White Blood Cells in the Rabbit (Weight Percentage).* In all of the preceding experiments only the glycogen content per million W. B. C. was determined. Since the weight of the individual white blood cell is not known, it is not possible to come to a conclusion as to the weight percentage of glycogen

*The term "reducing substances" refers to the reducing substances in the hydrolyzates of the alcohol precipitates which are not considered as wholly composed of true glycogen.

Plasma cell leukemia is not included in this investigation, because only 1 case has been studied. However, the concentration of "reducing substances" per million W. B. C. was strikingly high in this instance.

†Assuming 60 per cent granulated leukocytes in normal blood, the average of the glycogen content per million normal granulated W. B. C. is 4.23 γ.

in white blood cells. This would be of interest for comparison with the glycogen content of other tissues, such as liver or muscle. However, this problem can be approached by two kinds of experiments: (a) Granulated W. B. C. of uniform character can be collected from sterile exudates experimentally produced in animals. Glycogen and total nitrogen can be easily determined in the same suspension of leukocytes, since there is no disturbing admixture of platelets present. This procedure is superior to the study of pus cells, since in pus cells the enzymatic activity and its destructive influence on the glycogen content are uncontrollable. Pus cells and living W. B. C. are biologically different as to their glycogen metabolism. (b) Another way of proceeding is to collect white blood cells from normal individuals by the regular procedure in the Cushman tube. One portion of the cells can be analyzed for glycogen without further separation from the remainder of plasma and blood platelets. In another portion, which has to be entirely purified from blood platelets and plasma, the total nitrogen is determined. However, the latter

TABLE 4.—*Glycogen Content of Whole Blood and White Blood Cells in Polycythemia*

Exper. Number	W. B. C. per cmm.	Polymorphonuclear W. B. C.	Glycogen in Whole Blood	Glycogen per Million W. B. C.	Glycogen per Million Polymorphonuclear W. B. C.	Glycogen in Wet Granulated W. B. C. [Glycogen per Million / 8.2]
			Determined	Determined	Calculated	Calculated
		%	*mg. per cent*	γ	γ	%
141	16,600	81	22.4	5.62	6.94	0.85
165	18,000	85(9)	33.4	1.62	1.94	0.24
183	18,850	74	14.3	6.10	8.24	1.00
189	9,500	58	24.3	7.8	13.40	1.64

method offers certain technical difficulties, first of all the necessity of purifying procedures which are indispensable for a correct determination of nitrogen. Therefore the first procedure was chosen with the following results.

Experiment No. 198. The procedure of collecting white blood cells followed the technic of De Haan.[4] A rabbit (not fasting) was injected intraperitoneally with 300 cc. of a physiological saline solution. Twenty-four hours later another 150 cc. were injected; after 1.5 hours the fluid was withdrawn. It represented a homogenous suspension of granulated leukocytes, and this was verified microscopically. In order to prevent clotting the white blood cell suspension was immediately mixed with 0.6 per cent sodium citrate in physiological saline solution. The suspension was then centrifuged on the angle centrifuge at high speed, the cells resuspended in the sodium citrate-sodium chloride solution and made up with it to 5 cc. in a volumetric flask. The suspension contained 3050 cells per cmm.

In 3 cc. of the white blood cell suspension (9,150,000 W. B. C.) 92.5 γ glycogen and in 1.5 cc. (4,575,000 W. B. C.) 120 γ nitrogen were found; this is 10.1 γ glycogen and 26.2 γ nitrogen or 164 γ protein per million W. B. C. Assuming a water content of 80 per cent, granulated leukocytes of rabbits contain 1.23 per cent glycogen..

3. *Influence of Intravenous Administration of Glycogen on the Glycogen Content of Granulated White Blood Cells in the Rabbit. Experiment 196:* After intravenous injection of 3 grams of glycogen "Pfanstiehl" C.P. (15 cc. of a 20 per cent solution) in a rabbit, following the technic of Morris,[5] the glycogen content of the isolated

white cells increased from 1.36 γ per million (0.17 per cent) in fasting conditions before the injection to 7.9 γ per million (0.96 per cent) 30 minutes after the injection.

4. *Glycogen Content of Whole Blood and Isolated White Blood Cells (per Million) in Polycythemia.* In table 4 the experimental material on 4 patients with polycythemia is shown. The glycogen content of the whole blood not only exceeds the normal standards by a considerable amount, but the glycogen content per million W. B. C. is extremely high in 3 of the cases examined, compared with that of normal individuals. Only in glycogen storage disease was a higher glycogen content per million W. B. C. encountered.

DISCUSSION

There is a wide divergence of opinion as to whether or not all of the reducing substances in the alcohol precipitates of blood following acid hydrolysis originate from glycogen. It was shown that the white blood cell is the main carrier of reducing substances of polysaccharid character in whole blood.[1] The studies on leukemic blood give a more precise answer as to which type of white blood cell group contains substances with the properties of glycogen. From the following facts it is evident that at least part of the polysaccharids present in blood and its constituents is glycogen.

1. The general chemical properties of these carbohydrates are the same as those of liver or muscle glycogen. They are precipitable by alcohol and can be hydrolyzed with acids. The breakdown product is fermentable by yeast. They can be extracted with water and determined in the aqueous solution with sufficient exactness if present in large enough amounts, as for instance in chronic myelogenous leukemia.

2. Bridge and Holt[6] identified the polysaccharid isolated from blood in glycogen storage disease as glycogen by its chemical properties, comparing it with a control glycogen which had been reprecipitated from rabbit liver. The small variations from the control were quantitative and not qualitative.

Further proof for the glycogen character of the polysaccharid in blood is its enzymatic breakdown in isolated white blood cells. It shows the same rate of disappearance known to occur in other organs with active glycogen metabolism such as the liver.[1]

In a recent study Verheught[7] expressed the opinion that in blood of normal men no glycogen is present, at least not in quantities that can be measured by Pflüger's method. Our present studies leave no doubt that the polysaccharid found in granulated white blood cells is true glycogen. The linear increase of "reducing substances" in whole blood of chronic myelogenous leukemia with increasing number of white blood cells may be considered as satisfactory evidence for the exclusive presence of glycogen in the granulated leukocytes. The lack of increase in chronic lymphatic leukemia on the other hand rules out the presence of any measurable amount of this carbohydrate in the lymphocytes. The same holds true for blast cells. The studies on isolated white blood cells of one uniform type are consistent with the above assumption. *The "reducing substances" found in lymphocytes and blast cells are not glycogen.*

The exclusive presence of glycogen in the granulated white blood cell explains certain technical peculiarities of the determinations in blood and its constituents. The results of duplicate determinations in normal whole blood often show considerable discrepancies, while determinations in isolated leukocytes show good agreement. There are reducing substances in whole blood which interfere with the exact determination of glycogen. It was shown in previous studies[1] that the ribonucleic acid content of blood platelets explains at least a large part of the reduction obtained after acid hydrolysis of the alcohol precipitates of whole blood. All determinations of whole blood glycogen without yeast fermentation resulted in erroneously high values. In the white blood cell the glycogen is concentrated and the interference of other reducing substances is negligible.

From these investigations on leukemia can be drawn important conclusions as to the physiology of the white blood cells. It is interesting that during the development of the granulated polymorphonuclear leukocyte glycogen appears in considerable amounts as a cell constituent in the myelocytic phase, while the blast forms are still free from glycogen. It might be concluded that the content of a reserve carbohydrate in the granulated white blood cell increases with increasing maturity.* The amount is probably determined by the increasing phagocytic and ameboid activity of the maturing cell. The lymphocytes, on the other hand, representing a biologically different cell type with a different function, are free of glycogen.

In the experiment on the peritoneal exudate of rabbits (experiment 198) it could be shown that the glycogen content of the granulated white blood cells is in the same order of magnitude as that of the striated muscle. With regard to its physiological peculiarities, the tissue of the granulated white blood cells has at least one function in common with the striated muscle—that is, the motor activity. As in the case of the muscle there can likewise be demonstrated a wide range of the glycogen content for the granulated white blood cell.

In human muscle, for instance, the average glycogen content amounts to 0.4 per cent (Moscati[8]). In the muscles of dog a maximum of 3.72 per cent was determined (Schöndorff[9]). Exercise and intake of food exert great influence upon the glycogen content of the musculature. In fasting animals it is 0.1 to 0.4 per cent, after intake of food 0.7 to 1.0 per cent (Böhm[10]).

For comparison an attempt was made at computing the weight percentage of glycogen in human wet white blood cells from the glycogen content per million, assuming the same nitrogen and water content in the human cells as in those of rabbits (cf. last column of tables 1 and 4). Excluding experiment 182, in 7 instances the glycogen content was found to be between 0.42 and 0.67 per cent. In the other 5 instances it was below this value. It may be significant that in the cases with the highest W. B. C. counts the lowest glycogen contents were encountered.

* The appearance of myelocytes in the peripheral blood seems to be of decisive influence on the glycogen content of whole blood (cf. experiments 149 and 176). The highest glycogen value was observed in experiment 149 in the presence of 248,000 myelocytes in contrast to the relatively low value in experiment 176 with only 21.890 myelocytes. The figures for the mature polymorphonuclear leukocytes (92,600 and 74,100) are very close in the two experiments.

While the glycogen content of leukocytes collected from patients with chronic myelogenous leukemia was found to be within the same order of magnitude as that of normal individuals, some variation of this value could be demonstrated under experimental conditions as well as in disease. The increase after intravenous injection of glycogen in the rabbit is evidently the result of phagocytosis and storage (experiment 196). Another example of phagocytosis was previously mentioned. In experiment 182 (table 1) an extremely high glycogen concentration of 45.5 γ per million W. B. C. was encountered following x-ray treatment. In glycogen storage disease we found up to 25 γ per million granulated cells (3.05 per cent). Bridge and Holt[6] calculated for leukocytes in glycogen storage disease a glycogen concentration of from 6 to 10 per cent.

More difficult to interpret are the high glycogen values of leukocytes which we found in polycythemia. They cannot simply be explained by phagocytosis and storage. Other factors may come into play. In view of the intensive enzymatic breakdown of glycogen in leukocytes[1] it is probable that an active synthesis of this carbohydrate likewise takes place within the white blood cell. Willstätter and Rhodewald's[11] experiments on the enzymes of leukocytes may be considered as evidence of such a synthesis. They deny the occurrence of direct glycolysis of glucose in blood and explain the disappearance of blood sugar as the result of glycogen synthesis and glycogenolysis. The granulated leukocyte is equipped with the specific capacity of storing energy in the form of glycogen. It is easy to conceive of glycogen as a reserve carbohydrate, being present in blood in the granulated leukocytes, while its breakdown product glucose must be readily available and can promptly be transported to the tissues of the body wherever there is some immediate need for it. In this way a new function is attributed to the granulated leukocyte in the system of tissues serving the carbohydrate metabolism.

The study of the glycogen concentration in isolated living leukocytes may have further implications as to the physiology and pathology of this cell group and their enzymatic activity. In leukemia it is easy to collect large amounts of material of one uniform cell type. Other cell constituents, such as lactic acid, can be likewise studied by using the same procedure of isolation and quantitative evaluation. Particularly glycolysis ought to be investigated on isolated leukocytes of leukemic blood.

SUMMARY AND CONCLUSIONS

The technic of determining glycogen in isolated white blood cells was applied to the study of the different types of leukemia and of polycythemia, in order to obtain information on the physiology of the white blood cell. From this study it is concluded that the granulated leukocyte is the only carrier of glycogen in whole blood. The "reducing substances" in lymphocytes and blast cells are not considered as true glycogen.

The glycogen content of wet white blood cells in the rabbit amounts to about 1 per cent. In the human being a range of from 0.17 to 0.67 per cent was calculated. In disease higher percentages occur, in polycythemia up to 1.64 per cent and in glycogen storage disease up to 3.05 per cent.

The glycogen concentration of normal white blood cells is within the same range as that of the striated muscle.

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