THE GLYCOGEN CONTENT OF HUMAN LEUKOCYTES IN HEALTH AND IN VARIOUS DISEASE STATES [1]

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INTRODUCTION

Studies by Wagner (1) on the quantitative estimation of glycogen in human blood disclosed the average glycogen content per 100 ml. to be 5.5 mg. In separated leukocytes an average of 2.54 micrograms of glycogen was found per 10^6 total leukocytes, or 4.23 micrograms per 10^6 granulocytic leukocytes. No detectable glycogen is present in human plasma or erythrocytes. It has been both denied (2) and claimed (3) on the basis of histochemical data that lymphocytes and platelets contain small amounts of glycogen. The work of Wagner (1, 4), however, would indicate that the granulocytic leukocyte is probably the only significant representative of the white cells in the peripheral blood which contains glycogen. Studies on leukemic leukocytes (4) indicate that the blast cell contains no measurable amount of glycogen. Both histochemical studies (5) and the quantitative observations of Wagner (1, 4) suggest that glycogen first appears in the granulocytic leukocytes at the myelocytic stage and may increase with increasing cell maturity. Wagner (4) has observed a normal content of glycogen per 10^6 leukocytes in chronic myelocytic leukemia. In this laboratory, recent studies (6) have shown that whereas total leukocyte glycogen is frequently within the normal range, *unit myeloid cell leukocyte glycogen* is low. This is not explainable solely in terms of cell immaturity in the leukemic blood analyzed. In polycythemia vera of the type associated with leukocytosis and leukemoid reactions, the unit myeloid cell glycogen is substantially above normal (6). Wagner (4) has observed increased leukocyte glycogen values in polycythemia vera and Wachstein (5) has made similar qualitative observations on the basis of histochemical staining techniques. The leukocyte glycogen content may be remarkably increased in Von Gierke's disease (1, 7).

It is the object of this study to present further observations on the glycogen content of human leukocytes in health and in various disease states employing an adaptation of the anthrone technique (8, 9) to glycogen measurements in white blood cells.

METHODS

Since the myeloid leukocytes are apparently the only significant source of blood glycogen (1) the following method has been employed in the estimation of leukocyte glycogen. Blood was collected as for the phosphatase determination (10) in a freshly prepared saline solution of bovine fibrinogen (Fraction I), Armour, and the erythrocytes allowed to settle. Since this process is relatively rapid and satisfactory separation nearly invariable by thirty minutes, it was possible within this space of time to remove the practically red-cell-free but white-cell-rich supernatant to a separate container. This leukocyte-platelet suspension was thoroughly mixed and 1 ml. aliquots pipetted into triplicate boiling tubes containing NaOH in final concentration of 30 per cent. Total leukocyte counts were made in triplicate on the mixed suspension subjected to analysis, and in duplicate on a simultaneously removed specimen of venous blood. Blood smears were also obtained from the ear at the time of venepuncture and 200 cell differential leukocyte counts made. The remaining leukocyte suspension was then centrifuged and 1 ml. of the *cell-free supernatant* pipetted into each of two tubes containing NaOH in the same manner as the unknowns. These served as (*1*) a blank and (*2*) a standard after addition of a known amount of glucose since their composition was identical with that of the unknown except for the contributions of the cells. All material was digested by boiling for 45 min. in a water bath, the tubes being covered with glass marbles to prevent evaporation. At the end of 45 min. sufficient 95 per cent alcohol was added to each tube to bring the final alcohol concentration to 60 per cent. The glycogen which precipitates at this concentration of alcohol was allowed to settle and the precipitate centrifuged. The precipitate was then washed twice with 60 per cent alcohol and, after removal of the bulk of the final supernatant, dried at approximately 100° C in a drying oven. Each of the triplicate unknowns was suspended in 4 ml. of distilled water and to this was added

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TABLE I

Glycogen content of separated leukocytes in normal subjects

Subject	W.B.C. per cu. mm.	Glycogen content		
		Per 10^{10} leukocytes *mg.*	Per 10^{10} myeloid leukocytes *mg.*	Per leukocytes of 100 ml. of blood *mg.*
A. L.	5,250	40.9	66.7	2.13
H. F.	6,250	60.5	118.6	3.78
C. E.	4,850	39.9	79.8	1.93
A. L.	8,700	43.2	69.2	3.75
A. S.	8,150	50.2	85.0	4.08
E. C.	6,075	48.8	76.3	2.96
R. H.	7,750	42.0	81.5	3.24
V. H.	9,375	66.0	85.0	6.20
C. C.	8,625	60.0	93.8	5.17
M. H.	7,425	41.6	73.8	3.08
H. F.	6,325	31.8	53.0	2.02
J. O.	8,075	49.2	65.2	3.97
G. S.	10,300	57.0	79.3	5.86
E. A.	6,350	34.8	51.2	2.21
J. B.	8,250	44.7	70.0	3.69
A. A.	8,650	62.4	99.0	5.37
H. K.	7,125	74.0	86.0	5.27
F. C.	6,450	43.0	66.2	2.77
N. W.	6,400	38.0	47.2	2.43
H. H.	10,250	35.7	49.0	3.68
M. L.	7,325	48.6	81.0	3.56
Mean:		48.2	75.1 ± 17.4*	3.61
Range:		31.8–74.0	47.2–118.6	1.93–6.2

* Standard deviation.

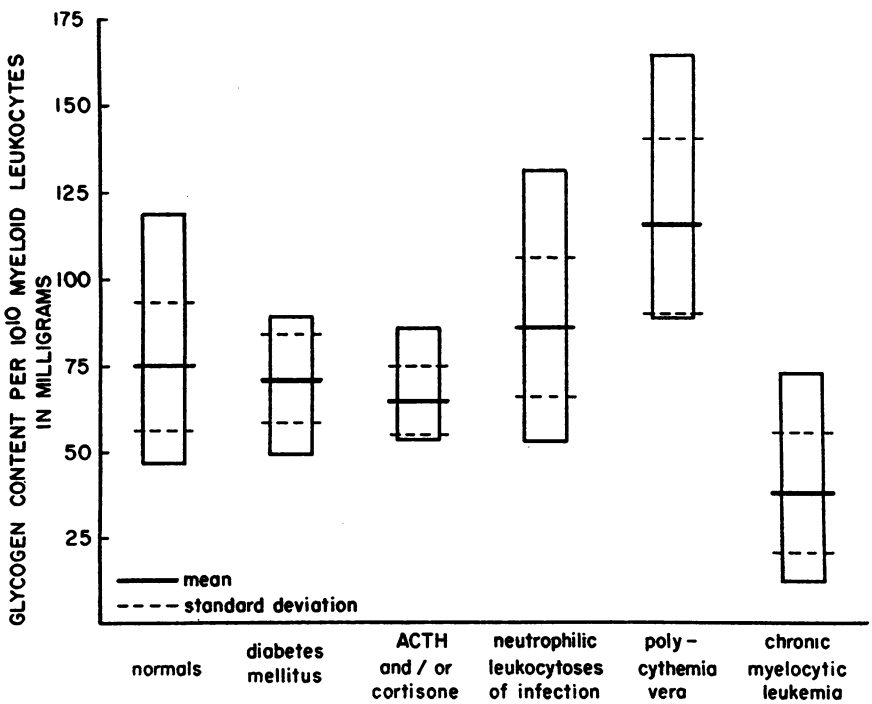

FIG. 1. MEAN, STANDARD DEVIATION, AND RANGE OF GLYCOGEN CONTENT PER 10^{10} MYELOID LEUKOCYTES

The data in diabetes mellitus have excluded a single, unexplainably high value as discussed in the text. The data for polycythemia vera refer *only* to cases of this syndrome with concomitant leukocytosis and/or leukemoid reaction.

8 ml. of the anthrone reagent (8, 9) (0.2 per cent anthrone in concentrated H_2SO_4). The blank was identically treated and the standard was prepared by adding 4 ml. of distilled water containing 50 micrograms of glucose to the second blank material. The color developed was read after 10 minutes at wave length 620 using the Beckman Model B spectrophotometer. The determination was related to the glucose standard and the glycogen content of the unknowns computed by the standard formula:

$$\text{micrograms of glycogen in aliquot} = \frac{50 \times V}{1.11 \times S}$$

V—optical density of unknown,

S—optical density of 50 microgram glucose standard,

1.11—factor of conversion of glucose to glycogen.

To our knowledge the anthrone method (9) has not previously been employed specifically for leukocyte glycogen. It has the advantage that hydrolysis of the purified glycogen is not necessary. The anthrone reagent must be prepared fresh, the sulphuric acid of high purity, and care must be taken to prevent contamination with lint or other material which will react with this reagent.

RESULTS

Normal subjects. Table I and Figure 1 show the leukocyte glycogen values in 21 normal subjects. The latter were ostensibly healthy individuals admitted to the hospital for such conditions as hernia repair and all samples were analyzed prior to any corrective procedures. All had total and differential leukocyte counts within the normal range. By the anthrone technique mean leukocyte glycogen per 10^{10} total leukocytes was 48.2 mg. with a range of 31.8 to 74.0 mg. The more significant mean value of glycogen content per 10^{10} myeloid cells (since the only significant leukocyte carrier of glycogen appears to be the granulocytic cells) was 75.1 mg. and the range 47.2 to 118.6 mg. The mean glycogen content of the leukocytes separated from 100 ml. of blood was 3.61 mg. and the range 1.93 to 6.20 mg. These values for separated leukocytes are somewhat higher than those of Wagner (1) but more closely approximate the theoretical expectancy on the basis of whole blood glycogen values. Wagner explained his less than expected values in terms of glycogen degradation during the separation procedure. The difference in separation technique and the short interval between the time of blood withdrawal and the introduction of leukocytes into the alkali digestion mixture may explain the higher values here obtained.

TABLE II

Effect of fasting and food ingestion on leukocyte glycogen

Subject	Glycogen per 10^{10} leukocyte mg.	Remarks
J. C.	157.0*	Fasting
	149.5	105 min. p.c.
H. L.	53.0	Fasting
	46.3	105 min. p.c.
D. H.	71.6	Fasting
	68.9	105 min. p.c.
B. F.	67.2	Fasting
	73.1	90 min. p.c.
R. P.	43.2	Fasting
	51.0	75 min. p.c.

* Polycythemia vera with leukocytosis responsible for high values.

Effect of fasting and food intake on leukocyte glycogen. Table II shows a comparison of leukocyte glycogen values determined on the same day during the fasting state and 1¼ to 1¾ hours after a routine breakfast in subjects with various diseases. It can be readily seen that the postprandial values are essentially the same as those obtained before ingestion of food. It has, therefore, not been deemed necessary to employ fasting blood only in these determinations.

Diabetes mellitus. Table III summarizes the findings of 16 determinations on 15 diabetic subjects. All but two were receiving insulin. In twelve instances the studies were performed on fasting blood on which blood sugar and carbon-dioxide combining power determinations were simultaneously made. In all subjects the diabetes was insufficiently controlled in terms of fasting blood sugar values and/or urinary sugar. In the 12 instances in which simultaneous blood sugar determinations were made they were found to range from 138 to 608 mg. per cent. One subject, J. J., was in coma. The blood sugar was 608 mg. per cent and the carbon-dioxide combining power less than 5 volumes per cent. Despite the diabetic state and high blood sugar levels at the time of the studies, with one exception the mean glycogen per 10^{10} myeloid leukocytes was within the normal range. The mean for the entire group was only slightly higher than normal and this could be attributed entirely to the unexplainably high value obtained in a single patient (J. R.). In Figure 1

TABLE III

Glycogen content of leukocytes in poorly controlled diabetes mellitus

Subject	Glycogen content: Per 10^{10} leukocytes *mg.*	Glycogen content: Per 10^{10} myeloid leukocytes *mg.*	Glycogen content: Per leukocytes of 100 ml. of blood *mg.*	Insulin	Blood sugar *mg. %*
G. S.*	47.7	82.2	1.85	yes	
A. P.*	43.4	61.4	1.84	yes	
W. S.*	80.1	85.0	24.83	yes	
P. I.*	50.0	65.0	5.10	yes	
I. I.†	54.1	64.0	14.00	no	608
P. I.	48.6	77.0	4.34	yes	228
J. R.	117.5	163.0	10.20	yes	185
J. D.	57.7	88.7	4.55	yes	460
J. B.	42.7	88.4	3.12	yes	150
L. S.	62.9	69.5	8.70	yes	330
J. H.	38.8	76.8	2.32	no	190
A. M.	37.8	49.7	3.04	yes	214
S. L.	30.0	58.9	1.29	yes	200
C. S.	37.3	54.8	2.56	yes	138
J. C.	68.3	84.7	7.13	yes	276
H. C.	56.8	82.3	3.18	no	190
Mean:	54.6	78.5 ± 26.9‡	6.13		
Range:	30.0–117.5	49.7–168.0	1.29–24.83		

* In poor control but blood sugars not measured at time of glycogen determination.
† In coma. CO_2 combining power of blood less than 5 volumes per cent. All blood sugar determinations made on sample of blood analyzed for glycogen.
‡ Standard deviation. Large standard deviation is chiefly result of inclusion of unexplainably high value obtained on patient J. R.

the range and mean glycogen values per 10^{10} myeloid cells are shown *with the exclusion of patient J. R.* It appears that neither the metabolic defect of diabetes mellitus nor the high sugar environment of the leukocytes in these subjects is reflected in any deviation of leukocyte glycogen from the normal range.

Subjects receiving cortisone and/or ACTH. ACTH and cortisone therapy are well known to have profound effects on carbohydrate metabolism and glyconeogenesis. It was, therefore, considered of interest to investigate the glycogen content of separated leukocytes in subjects receiving large doses of these agents. Table IV and Figure 1 show the findings in nine such subjects. Seven subjects were receiving cortisone alone in doses ranging from 75 to 200 mg. a day. One subject (L. H.) was receiving 150 mg. of cortisone a week

TABLE IV

Glycogen content of leukocytes in subjects receiving cortisone or ACTH

Subject	Glycogen content: Per 10^{10} leukocytes *mg.*	Glycogen content: Per 10^{10} myeloid leukocytes *mg.*	Glycogen content: Per leukocytes of 100 ml. of blood *mg.*	Therapy
C. H.	38.9	53.2	2.06	ACTH 75 mg. daily and cortisone 75 mg. daily
W. T.	54.2	70.4	8.33	Cortisone 75 mg. daily
S. L.	60.0	86.3	7.06	Cortisone 75 mg. daily
P. Y.	43.5	60.0	5.22	Cortisone 75 mg. daily
F. N.	46.8	73.8	5.33	Cortisone 100 mg. daily
L. H.	39.4	54.3	3.22	Cortisone 150 mg./week
A. D.	51.8	62.8	7.06	Cortisone 200 mg. daily
O. C.	59.4	68.7	8.80	Cortisone 150 mg. daily
P. C.	45.0	56.5	6.36	Cortisone 150 mg. daily
Mean:	48.8	65.1 ± 10.8*	5.94	
Range:	38.9–60.0	53.2–86.3	2.06–8.80	

* Standard deviation.

and one (C. H.) was receiving 75 mg. of ACTH and 75 mg. of cortisone daily. The underlying disease process in these patients included atopic dermatitis, rheumatoid arthritis, and scleroderma. Although the mean glycogen content per 10^{10} myeloid leukocytes was somewhat lower than that of the larger group of normal subjects, all values were well within the normal range. Only one value was above the normal mean.

Neutrophilic leukocytosis of infection. Twenty-eight determinations of leukocyte glycogen content were made in 24 subjects with neutrophilic leukocytosis of infection. In two determinations recorded, the leukocyte count was within the normal range but one day previously had been elevated on the basis of an infectious process. The subjects studied had pneumonia, tonsillitis, meningitis, urinary tract infection, surgical complications, acute appendicitis, tuberculous pericarditis, and bacteremia of various types as a cause of leukocytosis. The mean glycogen content per 10^{10} myeloid leukocytes was 86.7 mg., substantially above the mean for normal subjects. Comparison with other disease states and with normal subjects is indicated in Figure 1. Twenty-one of twenty-eight determinations were above the normal mean but only two above the highest normal value obtained. However, almost 40 per cent of the values were above 90 mg. while only 14 per cent of values in normal subjects were in this range. It may be concluded that the glycogen content of myeloid leukocytes is appreciably elevated on the average in presence of neutrophilic leukocytosis of infection. These elevated leukocyte counts and high unit cell glycogen values, of course, are reflected in marked elevations in total circulating leukocyte glycogen.

Determinations were also made in eight cases with neutrophilic leukocytosis associated with such non-infectious processes as decompensated liver disease and metastatic carcinoma. All values per 10^{10} myeloid leukocytes were within the normal range and only one above 88 mg. This subject had metastatic carcinoma and marked leukocytosis ranging from 60,000 to 78,000 per cu. mm. On successive determinations unit cell glycogen was well above that of the highest normal subject, being over 130 mg. per 10^{10} myeloid leukocytes. In this subject the leukocyte glycogen content per 100 ml. of blood was as high as 102 mg., more than 14 times the highest value obtained in any normal subject.

Chronic myelocytic leukemia, polycythemia vera with leukocytosis and/or leukemoid reactions, and other "myeloproliferative syndromes." The data obtained on leukocyte glycogen in these conditions has been incorporated in a separate study (6) reported elsewhere and will only be summarized here. In chronic myelocytic leukemia, while the glycogen content per 10^{10} leukocytes of all types is usually within the normal range, the more significant value per 10^{10} myeloid leukocytes was consistently low in 14 subjects with this disease. The mean value was 37.9 mg., approximately one-half that of normal subjects. In sharp contrast, myeloid leukocyte glycogen in polycythemia vera of the type associated with marked leukocytosis and leukocyte immaturity was higher than in any other disease state studied. The mean content per 10^{10} myeloid leukocytes was 116.2 mg. in 15 subjects. The differences are not explainable in terms of therapy or the differential leukocyte picture and must be considered as evidence of a marked metabolic difference in the two conditions. This is true even in cases which clinically may be suspected of having undergone so-called leukemic transition. In other "myeloproliferative syndromes" (11) thought not to be either polycythemia vera or chronic myelocytic leukemia, but with similar leukocyte pictures, the pattern of glycogen content has not been consistent. Some have exhibited high glycogen content of myeloid leukocytes; others have shown low or normal values. These findings have been discussed previously in more detail (6). For purposes of comparison, the values in chronic myelocytic leukemia and in polycythemia vera are incorporated in Figure 1.

Miscellaneous disease states. In acute blastic leukemias and in chronic lymphocytic leukemias the glycogen content per 10^{10} leukocytes of all types was very low. Since in most instances some cells of the myeloid series were also present, it cannot be categorically stated that blast cells and lymphocytes are completely glycogen-free. In a single instance of chronic lymphocytic leukemia in which a 200 cell differential leukocyte count revealed 100 per cent of the cells to be lymphocytes, no measurable glycogen was found, however. Moreover, in two cases of infectious mononucleosis in which mononuclear cells constituted a higher than normal per-

centage of the total leukocytes, low glycogen values per 10^{10} leukocytes were also observed. It may be concluded that the data support the concept that myeloid cells beyond the blast stage of maturity are the only significant carriers of glycogen among the circulating leukocytes.

DISCUSSION

It can be presumed that the glycogen within the myeloid leukocytes serves as a storehouse of reserve energy conveniently available to meet metabolic needs in carrying out normal physiological functions. The leukocytes of the blood are freely floating cells in fluid environment containing glucose readily available to the entire cell surface. It is of interest that the glycogen content remains relatively unchanged during the immediate postprandial period when blood glucose is known to rise and that it is also within the normal range in poorly controlled diabetic subjects with marked elevation in blood sugar and the metabolic handicap of the diabetic state. Likewise, leukocyte glycogen does not appear to be particularly sensitive to cortisone and ACTH therapy, although the mean value per 10^{10} myeloid leukocytes in the relatively small group of patients receiving these medications was somewhat below the normal mean.

On the other hand, the low glycogen content of myeloid leukocytes in chronic myelocytic leukemia appears to be a consistent metabolic aberration in this disease while in polycythemia vera of the type with associated leukocytosis and/or leukemoid reaction, even when the blood picture is indistinguishable from chronic myelocytic leukemia, very high glycogen values are consistently observed. Dempsey and Wislocki (12) have commented upon the possible interrelationship of alkaline phosphatase and glycogen within cells. Observations in this laboratory (6, 10, 13) have indicated a certain parallelism between glycogen content and alkaline phosphatase activity of leukocytes. In conditions where blast cells or lymphocytes are the predominating cell type, both glycogen content and alkaline phosphatase activity of leukocytes are low. In chronic myelocytic leukemia a similar situation exists whereas in polycythemia vera with leukocytosis and/or leukemoid features, both values tend to be well above the normal range. The parallelism is not absolute, however. On the average, the highest unit cell leukocyte alkaline phosphatase values are found in the neutrophilic leukocytoses of infection. Glycogen content is also elevated above normal in this situation, but not quite so strikingly as in polycythemia vera with leukocyte abnormalities where the highest values are observed. In this connection, it should be mentioned that in polycythemia without concomitant leukocyte abnormalities, both unit leukocyte alkaline phosphatase and glycogen are usually normal. Whether this parallelism between glycogen content and alkaline phosphatase activity is fortuitous or represents a metabolic interrelationship cannot be stated with certainty.

SUMMARY

Data are presented on the glycogen content of separated human leukocytes in health and in various disease states.

Leukocyte glycogen has been observed to remain relatively unchanged during the postprandial rise in blood sugar, in poorly controlled diabetes mellitus and in the presence of massive cortisone therapy.

Unit cell myeloid leukocyte glycogen tends to be substantially low in chronic myelocytic leukemia and high in polycythemia vera with leukocytosis and/or leukemoid features. It also tends to be substantially above normal in the neutrophilic leukocytoses of infection.

The data confirm previous observations that lymphocytes and blast cells are either glycogen-free or extremely poor in glycogen content.

The parallelism between unit leukocyte glycogen and alkaline phosphatase activity is discussed.

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