Cytochemical Studies of Glycogen Content of Lymphocytes in Lymphocytic Proliferations

By W. J. Mitus, L. J. Bergna, I. B. Mednicoff and W. Dameshek

The results of a number of studies of glycogen content of the lymphocytes and related cells (lymphoblasts, atypical lymphocytes, "lymphosarcoma" cells) indicate that this metabolic component may have both clinical and theoretical importance. Wislocki,1 Storti,2 and, more recently, Astaldi and Verga3 observed an increase of glycogen in lymphocytes of chronic lymphocytic leukemia. One of us (L.J.B.) noted that it was similarly increased in the lymphocytic cells of patients with lymphosarcoma. The present investigation is concerned with further studies of the content of this metabolite in both benign and malignant lymphocytic proliferations.

Method

Air-dried blood smears were stained by the P.A.S. method of Hotchkiss.

Fixation:
1. Absolute methyl alcohol for 15 minutes; rinse in distilled water.
2. 1% aqueous solution of periodic acid* for 7 minutes; rinse in distilled water.
3. Reducing bath* (1 Gm. sodium bisulfite, 8.33 ml. N HCl, 176.66 ml. H2O) for 1 minute; rinse in distilled water.
4. Schiff solution† for 25-30 minutes (in the dark); rinse in distilled water and wash in running water for 10 minutes.
5. Counterstain with celestin blue† for 4 minutes.

Each smear had a control slide which, following the fixation, was exposed to 0.1% malt diastase in normal saline for 1 hour at room temperature.

One hundred lymphocytic cells were counted by two observers and scored in the following way:

- Cells with no red granules..........................................................0
- Cells with 1-9 red granules........................................................1
- Cells with 10 or more red granules.............................................2
- Cells with granules and large blocks of red material......................3

The sum of the scores of 100 lymphocytic cells constituted the "score" as used in this investigation.

Some of the slides were also stained for phosphorylase by the method of Takeuchi.4

The following methods were used in the differentiation of leukemias: phase microscopy; supravital staining (Pynacyanole-Neutral red), according to Schwind; peroxidase; acid phosphatase; Sudan black.

Material

Normal controls consisted of 12 healthy adults, both males and females (physicians, technicians, students).

Abnormal controls consisted of patients suffering from diseases other than...
Table 1.—Glycogen Content of Lymphocytic Cells of Normal Individuals and Patients with Diseases other than Lymphocytic Proliferations

<table>
<thead>
<tr>
<th>Score</th>
<th>Patient</th>
<th>Diagnosis</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 55</td>
<td>1. H.B.</td>
<td>Acute granulocytic leukemia</td>
<td>20</td>
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<tr>
<td>2. 45</td>
<td>2. M.S.</td>
<td>Chronic granulocytic leukemia and infection</td>
<td>20</td>
</tr>
<tr>
<td>3. 42</td>
<td>3. L.T.</td>
<td>Polycythemia vera</td>
<td>28</td>
</tr>
<tr>
<td>4. 40</td>
<td>4. L.R.</td>
<td>Polycythemia vera and phlebitis</td>
<td>42</td>
</tr>
<tr>
<td>5. 36</td>
<td>5. D.B.</td>
<td>Idiopathic thrombocytopenic purpura</td>
<td>28</td>
</tr>
<tr>
<td>6. 36</td>
<td>6. N.D.</td>
<td>Autoimmune hemolytic anemia</td>
<td>26</td>
</tr>
<tr>
<td>7. 34</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. 32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. 32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. 22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. 20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12. 20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Av.: 34</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range: 20–55</td>
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</tbody>
</table>

"lymphocytic proliferations." Six patients with the following conditions were examined: (1) acute granulocytic leukemia; (2) chronic granulocytic leukemia, with lung infection; (3) polycythemia vera with thrombophlebitis; (4) polycythemia vera; (5) idiopathic thrombocytopenic purpura; (6) autoimmune hemolytic anemia.

Lymphocytic proliferations: (44 patients)

1. Chronic lymphocytic leukemia 13
2. Acute lymphocytic leukemia 4
3. Subacute lymphocytic leukemia 1
4. Lymphosarcoma 20
5. Lymphocytosis, Viral 6

The diagnosis was arrived at in each instance after physical examination and examination of peripheral blood and bone marrow. Lymph node aspirations, lymph node biopsies, and splenic aspirations were performed in the more difficult cases. In addition to the above mentioned cytochemical methods, phase microscopy and supravital preparations were utilized in differentiating between various forms of acute leukemia. The patients were considered to be suffering from lymphosarcoma if the process started in one localized area and remained localized for some time. Later, it could become generalized, showing involvement of distant lymph nodes, bone marrow, or blood stream (leukemic phase).

Of the six cases of "viral" lymphocytosis, three were classical cases of infectious mononucleosis with positive heterophile agglutination and absorption tests; the fourth had all the features of infectious mononucleosis, but the agglutination test was negative. The two remaining cases had an increase of normal-appearing lymphocytes in the course of a respiratory infection of unknown etiology. All were of self-limited character and recovered spontaneously.
RESULTS

The results are summarized in tables 1, 2, and 3. The range of the score in 12 normal subjects was 20-55. All 6 abnormal control cases fell within this range and thus within normal limits.

All patients with chronic lymphocytic leukemia had greatly elevated scores. One patient with subacute lymphocytic leukemia gave a high score of 122. On the other hand, all cases of acute lymphocytic leukemia had low scores. Nineteen of 20 patients with lymphosarcoma had high scores. One, however, had a score of 13, well below the normal range and near the scores observed in acute lymphocytic leukemia.

There was no difference in the scores of the lymphosarcoma cases with "lymphosarcoma cells"* in the peripheral blood and those without the cells. The averages in those two groups (ten cases in each) were 96 and 99, respectively. Two patients with lymphocytosis in the course of a probable virus infection had normal scores. However, two patients with infectious mononucleosis and the patient who had "atypical" lymphocytes in the peripheral blood with a negative heterophil agglutination test had elevated scores, while the third case of infectious mononucleosis had a normal score.

DISCUSSION

The glycogen content of lymphocytes of chronic lymphocytic leukemia and of lymphosarcoma is greatly increased. In lymphosarcoma, this increase is not limited to the cases which show the presence of "lymphosarcoma cells" in the peripheral blood (leukemic picture), but is also evident in those whose lymphocytes are morphologically normal. It would thus appear that the cells in the clinical conditions called "lymphosarcoma" and "chronic lymphocytic leukemia" are very similar, at least in respect to glycogen content. This is another point in favor of the concept that these two conditions are closely related, although at some stages in their course they may have differing clinical patterns. The presence or absence of so-called "lymphosarcoma cells" appears to make little if any difference in the total "score." The glycogen increase seems to be related to an over-all production of all lymphocytes and related cells and not to the appearance of a particular or abnormal variety of them. Astaldi and Verga* estimated that the glycogen content of the nucleolated lymphocytic cells was considerably higher than that of the non-nucleolated lymphocytes. In our study, their presence made no difference in the total score. This does not mean that the primitive cells containing nucleoli are less positive than the other cells, but simply that their presence did not appreciably influence the total score.

*The lymphosarcoma cell is an atypical immature cell of the lymphoid series. In the peripheral blood it appears as a large round or slightly elongated cell about 15 microns in diameter, but it may vary considerably in size. The nucleus is frequently slightly indented, notched or kidney shaped, but it also may be round or oval. The chromatin pattern is coarsely reticular (much coarser than in the lymphoblast). One distinct nucleolus is usually present, but occasionally two may be seen. The amount of cytoplasm and the degree of basophilia vary considerably.
GLYCOGEN CONTENT OF LYMPHOCYTES

Fig. 1.—Glycogen-positive lymphocytes in chronic lymphocytic leukemia (1150 X).

Fig. 2.—Glycogen-positive lymphosarcoma cell (1150 X).
### Table 2.—Glycogen Content of Lymphocytic Cells of Patients with Lymphocytic Proliferations

<table>
<thead>
<tr>
<th></th>
<th>A. With lymphosarcoma cells in peripheral blood</th>
<th>B. Without lymphosarcoma cells in peripheral blood</th>
<th>Chronic Lymphocytic Leukemia</th>
<th>Subacute Lymphocytic Leukemia</th>
<th>Acute Lymphocytic Leukemia</th>
<th>Lymphocytosis ? Infectious Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>7. F.B.</td>
<td>70</td>
<td>17. F.W.</td>
<td>87</td>
<td>7. E.H.</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>8. V.S.</td>
<td>58</td>
<td>18. L.M.</td>
<td>84</td>
<td>8. T.L.</td>
<td>90</td>
<td></td>
</tr>
</tbody>
</table>


Range: 13-189

Range: 66-172

Av.: 103

Range: 75-174

*Atypical lymphocytes of infectious mononucleosis type.*
GLYCOPEN CONTENT OF LYMPHOCYTES

Table 3.—Cytochemical Study of Glycogen Content of Lymphocytic Cells in Lymphocytic Leukemia and Lymphosarcoma: Results in Graphic Form

In striking contrast with the chronic cases of lymphocytic leukemia and lymphosarcoma, the four cases of acute lymphocytic leukemia showed low scores. The immaturity of the cell with an associated lack of necessary enzymes for glycogen production can be suggested as the most likely explanation. A study of lymphoblasts for the presence of phosphorylase in a case of acute lymphocytic leukemia gave negative results, while the great majority of cells in chronic lymphocytic leukemia and lymphosarcoma, including “lymphosarcoma cells,” were positive, many of them strongly so.

It is probable that the essential difference between the leukemic lymphoblast and the “lymphosarcoma cell” lies in the greater maturity and atypicity of the lymphosarcoma cell and especially of its cytoplasm. The asynchronism of nucleocytoplasmic maturation is quite characteristic of lymphosarcoma cells, but it is also apparent in other leukemic cells, e.g., “atypical” myeloblasts.7

The glycogen seems to appear relatively early in the process of maturation of the lymphocytic cell. In acute leukemia, one observes an occasional positive cell, but this is rare. In one case of our series, labeled “sub-acute,” which morphologically showed forms between lymphoblast and lymphocyte, the score was very high. It would appear that the very primitive cell of the lymphocytic series contains little or no glycogen and that this substance accumulates rather rapidly as the cell matures.

Generally speaking, the glycogen stain may be a useful method of differentiating acute leukemias and lymphosarcoma. The value of this test is, however, diminished by the fact that one case of lymphosarcoma in our group gave a low score, similar to the cases of acute leukemia and strikingly different from the 19 other cases of lymphosarcoma. This indicates that not all cases of lymphosarcoma are of the same nature nor of the same degree of cellular differentiation. Some of them at least are more closely related to the primitive lymphoblastic proliferations than to the more mature lymphocytic varieties.
It is of considerable interest that the scores of three patients with "atypical" lymphocytes in the peripheral blood were elevated. The patients had nonmalignant conditions closely related to infectious mononucleosis. The small number of these cases does not allow any definite conclusions to be drawn, but the findings suggest that an increase of glycogen content of lymphocytes is by no means limited to the malignant proliferations, and that it is associated to a certain degree with some of the nonmalignant varieties. Heckner\textsuperscript{8} observed such an increase in nonspecific hyperplasias and in infectious mononucleosis.

Astaldi and Verga\textsuperscript{9} suggest that the glycogen increase is in some way related to the leukemic transformation of the cell. In view of our own findings of the increase of glycogen in some nonleukemic conditions, we would like to suggest that this increase is related to the enhanced proliferative activity of the lymphocytic system, both in malignant and in benign proliferations. It seems to be an indication of increased growth and associated increase of metabolism and not of malignancy per se. As a test for differentiating the malignant from nonmalignant lymphocytic proliferations, glycogen estimation of lymphocytic cells may be helpful only in a small degree, and on occasions may be quite misleading.

**Summary**

The glycogen content of the lymphocytes of 44 cases of various lymphocytic proliferations was studied using a semiquantitative cytochemical technic. The lymphocytes of chronic lymphocytic leukemia and of lymphosarcoma (with one exception) showed greatly increased glycogen content. In acute lymphocytic leukemia and in one case of lymphosarcoma the glycogen con-
The presence or absence of lymphosarcoma cells in the peripheral blood in cases of lymphosarcoma made no difference in the total score. The difference between the glycogen-positive “lymphosarcoma cell” and the glycogen-negative lymphoblast is apparently due to the greater maturity of the cytoplasm of the lymphosarcoma cell (which results from an asynchronism of nucleocytoplasmic maturation) and immaturity of the cytoplasm of the lymphoblast.

Three cases of benign lymphocytosis with atypical lymphocytes in the pe-
Peripheral blood gave increased values. One case with atypical lymphocytes had normal values and two cases of lymphocytosis with morphologically normal lymphocytes also had normal values. It is concluded that the increase of glycogen in lymphocytic cells is due to an over-all proliferation of the lymphocytes and is not necessarily related to malignancy per se.

SUMMARIO IN INTERLINGUA

Le contento de glycogeno in lymphocytos ab 44 patientes con varie proliferationes lymphocytic esseva studiate per medio de un semiquantitative technica cytochimic. Le lymphocytos de chronic leucemia lymphocytic e de lymphosarcoma (con un exception) monstrava grandemente augmentate contentos de glycogeno. In acute leucemia lymphocytic e in un caso de lymphosarcoma, le contento de glycogeno esseva basse. Le presentia o absentia de cellulas de lymphosarcoma in le sanguine peripheric in casos de lymphosarcoma effectuava nulle differentia in le resultatos total. Le differentia inter le "cellula de lymphosarcoma" in que glycogeno es presente e le lymphoblasto in que glycogeno es plus o minus absente resulta apparentemente ab le plus avantiate maturitate del cytoplasma in le cellula de lymphosarcoma (effectuare per un asynchronismo del maturation nucleocytoplasmic) e ab le immaturitate del cytoplasma in le lymphoblasto.

Valores augmentate esseva constatate in tres casos de lymphocytosis benigne con lymphocytos atypic in le sanguine peripheric. Un caso con lymphocytos atypic habeva valores normal, e le mesmo esseva constatate in duo casos de lymphocytosis con morphologicamente normal lymphocytes. Es formulate le conclusion que le augmento del contento de glycogeno in le cellulas lymphocytic resulta del proliferation general de lymphocytes e non es relationate necessarimente al malignitate per se.

REFERENCES

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