The Cytochemistry of Acute Leukemia: Observations on Glycogen and Neutral Fat in Bone Marrow Aspirates

By John M. Bennett and Thomas F. Dutcher

Examination of bone marrow aspirates using Romanowsky stains, phase preparations and tissue sections is not always sufficient to identify the malignant cell line in acute leukemia. Several histochemical techniques have been explored in attempts to demonstrate characteristic cellular components of primitive cells of the various cell lines. In 1963, Wright demonstrated the absence of glycogen and the presence of neutral fat in the cytoplasm of the primitive lymphoreticular cells of African children with Burkitt's tumor.1 His results have been confirmed in a study of a group of American children and young adults with Burkitt-like tumors.2 The purpose of this paper is to report the findings of a similar study in a series of acute leukemias, evaluated retrospectively.

Materials and Methods

Bone marrow "squash" smears from 44 patients with acute lymphocytic leukemia (A.L.L.), 8 patients with acute granulocytic leukemia (A.G.L.) and 4 patients with chronic granulocytic leukemia in the accelerated phase ("blastic transformation") were studied. These smears were obtained either prior to initial therapy or during a period of relapse. The diagnosis was made following examination of bone marrow smears, tissue sections, peripheral blood smears and occasionally phase microscopy of the marrow aspirate.

The mean age for the A.L.L. cases was 8 years (median: 6 years) and for the A.G.L. cases 24 years (median: 23 years). Six of the 8 patients with A.G.L. had Auer rods recognized in their initial marrow aspirates. The 44 cases of A.L.L. were so designated when the leukemic "blast cells" showed a rather prominent nuclear membrane, less than 2 nucleoli, and a somewhat coarse chromatin pattern with some evidence of aggregation. Cytoplasmic azurophil granules were not present. Cytoplasmic vacuoles were noted in 72 per cent of the A.L.L. cases but only seen rarely in the blast cells of the A.G.L. cases. The use of the term "A.L.L." in our department, therefore, encompassed all leukemic "blasts" that showed any degree of "lymphoid" differentiation and undoubtedly included many cases that are referred to as "acute stem cell leukemia" by others.

For the evaluation of the glycogen and neutral fat content of the leukemic cells, standard histochemical techniques using the periodic acid-Schiff (P.A.S.) with and without diastase digestion and Oil Red O (O.R.O.) stains were performed on air dried bone marrow smears.3

A scoring system for the P.A.S. positivity of lymphocytes similar to those described by Mitus et al.4 and Quaglino and Hayhoe5 had been previously established in our laboratory. The mean normal score is 63 ± 16 (standard error). The leukemia cells in

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the P.A.S. stained bone marrow smears were scored by this system, grading reactive
cells 0 to 3+. Arbitrarily, scores above 100 were considered as elevated.

RESULTS

Acute Lymphocytic Leukemia

Periodic acid-Schiff (P.A.S.). The average score of all 44 smears was 136,
and 27 (61 per cent) of the group were over 100. (Fig. 1). In those with
elevated scores, moderately positive (2+) and strongly positive (3+) leuk-
emtic cells constituted 57 per cent to 96 per cent of the P.A.S. positive cells.
(Fig. 2). In the group which had scores between 50 and 100, 23 per cent
of the positive cells were 2+ or 3+, and in the lowest scoring group (0–50)
an average of 7 per cent of the leukemic cells were 2+ or 3+. Three patients
with no demonstrable P.A.S. positive leukemic cells are included in the lowest
scoring group. Diastase digestion was complete in all but 4 cases.

Oil Red O (O.R.O.) Twenty-seven smears were stained with O.R.O. and
evaluated for neutral fat. Material was unavailable in the remaining 17 cases.
Twenty-two (82 per cent) smears contained leukemic cells that had pink
cytoplasmic globules. Some cells had few such globules, others had several
(Fig. 3). Seven smears contained cells with pink globules within cytoplasmic
vacuoles ("sudanophilic vacuoles"). The number of positive cells ranged from
10–75 per cent of the leukemic cells in any single smear.

coexisted in 21 cases of A.L.L., including the 4 that were diastase resistant
(Table 1). In 2 smears O.R.O. was positive in the absence of P.A.S. In no
instance were both O.R.O. or P.A.S. positive cells absent from a meaningful
percentage of leukemic cells in a case of A.L.L.

Granulocytic leukemia (AGL and cases of "Blastic Transformation")

P.A.S. quantitative scoring was not performed since the vast majority of
"myeloblasts" in all 12 patients were devoid of glycogen. If, however, on
Romanowsky stained smears cytoplasmic granules were identified in leukemic
cells, than a diffuse cytoplasmic staining reaction was observed. With more

Fig. 1.—Level of P.A.S. scores
of lymphoblasts from 44 cases of
Acute Lymphocytic Leukemia.
Vertical bars indicate per cent of
reactive cells in each group.
Fig. 2.—3+ P.A.S. reactive lymphoblasts from a case of A.L.L. Note large blocks of glycogen. A 3+ reactive poly for comparison is present. (X1200).

mature granulocytes (metamyelocytes and polymorphonuclear leukocytes) the reaction became more granular (Fig. 4) and resembled closely that seen in normal polymorphonuclear leukocytes.

In striking contrast to the A.L.L. cases only rare myeloid cells were noted to contain pink globules, when stained with O.R.O. These were restricted to the eosinophil series and maturing granulocytes beyond the myelocyte stage (Table 1).

**DISCUSSION**

The presence of increased glycogen content in leukemic lymphoblasts has been noted by several investigators outside of the United States. In this country, however, only one article has appeared in which Mitus reported no P.A.S. positivity in 4 cases of A.L.L. He postulated that the absence of glycogen in leukemic lymphoblasts was due to the immaturity of the cell, since in "lymphosarcoma cells" the glycogen content was usually high. A review of the morphologic characteristics of our 44 cases of A.L.L. revealed that 35 had a monotonous uniform appearance typical of early lymphoblasts, usually with 1 to 2 barely visible nucleoli. The remaining 9 cases showed a more pleomorphic picture with some "maturation." Four of these had P.A.S. scores above 175, but 5 were below 85, with a mean score of 117. Therefore, no significant difference was recognized between these two arbitrary divisions, i.e., the monotonous and pleomorphic abnormal cell lines.
Fig. 3.—O.R.O. positive globules in lymphoblasts, some within vacuoles and in nuclear fenestrae from a case of A.L.L. (X1200).

Table 1.—Comparison of O.R.O. and P.A.S. in 28 cases of A.L.L. and 12 cases of A.G.L.

<table>
<thead>
<tr>
<th>O.R.O.</th>
<th>P.A.S.</th>
<th># Cases</th>
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<tbody>
<tr>
<td>+</td>
<td>+ or ↑</td>
<td>16 A.L.L.</td>
</tr>
<tr>
<td>+</td>
<td>↓ or —</td>
<td>7 A.L.L.</td>
</tr>
<tr>
<td>—</td>
<td>+ or ↓</td>
<td>5 A.L.L.</td>
</tr>
<tr>
<td>—</td>
<td>—</td>
<td>0 A.L.L.</td>
</tr>
<tr>
<td>—</td>
<td>—</td>
<td>12 A.G.L.</td>
</tr>
</tbody>
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+ = present
— = absent
↑ = increased
↓ = decreased

In the present series approximately two-thirds of the cases of A.L.L. were associated with elevated scores. What was more striking was the finding of a significant number of 2+ and 3+ reacting cells, the latter with large blocks of glycogen, in all but 3 cases.

In contrast, the “blast cells” in granulocytic leukemia were P.A.S. negative. Diffuse reddish staining and the subsequent development of P.A.S. positive granules was noted, however, as the granulocytes matured. In 2 out of the 4 cases of “blastic transformation” approximately 10 per cent of the abnormal myeloid cells revealed a pinkish diffuse tinge and rare fine pink cytoplasmic
Fig. 4.—Rare P.A.S. positive grains (arrows) in leukemic granulocyte precursors. Strongly positive polymorphonuclear leukocyte at the top. (X1200).

granules. This may well have been a reflection of the slight marrow basophilia (mostly immature precursors). These findings confirm the observations made by Hayhoe and by Astaldi.

Considerable controversy exists on the interpretation of “fat stains” in hematopoietic cells. Most investigators have selected either Sudan Black or Sudan IV and have demonstrated neutral fat predominantly in the cytoplasmic granules of granulocytes. Oil Red O, another disazo-dye of the sudan type has seldom been employed to evaluate neutral fat in marrow cells, though Lillie did report positive reactions in polymorphonuclear leukocytes. Indeed, most authors have agreed that utilizing Sudan Black, granulocytes are sudanophilic and lymphoid cells are sudanophobic in bone marrow aspirates.

Our results with O.R.O. indicate an opposite relationship, namely that sudanophilia was present in leukemic lymphoblasts and only rarely was noted in granulocytes. Sudan Black stains phospholipid as well as neutral fat, whereas O.R.O. stains only the latter. The findings of Gottfried that granulocytes contain about twice as much phospholipid as lymphocytes suggest that the difference between the 2 fat stains may result from an unusual avidity of Sudan Black for the phospholipids of developing organelles in granulocytes.

Employing both P.A.S. and O.R.O. provides an even sharper distinction between lymphoblasts and myeloblasts. At least one of the 2 stains was positive in each case of A.L.L. In smears the absence of glycogen and the
presence of sudanophilic vacuoles was of interest since this is the identical histochemical behavior of cells from Burkitt's tumor. The morphology of these 2 cases, however, was not similar to that described for the neoplastic cells of Burkitt's tumor. The myeloblasts from all 12 cases of granulocytic leukemia were not stained by O.R.O. or by P.A.S.

The problem of establishing a specific cellular diagnosis in acute leukemia is emphasized by a recent report that 34 per cent of all childhood leukemias were classified as acute undifferentiated type. A review of our material over the past 2 years indicates that only 4 of 160 cases (2.5 per cent) were so classified, without employing P.A.S. and O.R.O. The application of both of these stains may enable investigators from different centers to classify blast cells more uniformly than previously possible.

SUMMARY
A cytochemical study of 54 cases of acute leukemia utilizing the periodic acid-Schiff reaction for glycogen and Oil Red O for neutral fat was performed on air dried bone marrow smears. The majority of leukemic lymphoblasts revealed significant amounts of glycogen and neutral fat. "Blast" cells in granulocytic leukemia were devoid of glycogen and of neutral fat. The recommendation is made that both of these histochemical stains be employed to attempt to resolve the problem of specific cellular identification in acute leukemia.

SUMMARIO IN INTERLINGUA
Un studio cytochimic de 54 casos de leucemia acute, utilisante le reaction Schiff a acido periodic pro glycogeno e oleo rubie O pro grassia neutre, esseva effectuate in frottis de medulla ossee siccate in le aere. Le majoritate del lymphoblastos leucemic revelava significative quantitates de glyogeno e de grassia neutre. Cellulas "blastic" in leucemia granulocytic esseva disproviste de glycogeno e de grassia neutre. Es formulate le recommendation que le duo mentionate colorantes histochimic es emplete pro resolver le problema del identification specific de cellulas in leucemia acute.

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