Fumarase Activity of Human Leukocytes and Erythrocytes

By Kouichi R. Tanaka and William N. Valentine

ALTHOUGH fumarase was discovered by Einbeck in 1919 and has subsequently been found to have a wide biological distribution, there are no reports to our knowledge of its activity in separated human leukocytes. Fumarase is one of the essential enzymes that catalyze the oxidation of carbohydrates, fatty acids and some amino acids via the citric acid cycle. Specifically, fumarase mediates the establishment of an equilibrium between fumaric acid and l-malic acid. Because of the importance of this enzyme in cellular metabolism and the ease of “biopsying” the blood, it seemed pertinent to assay fumarase activity in the leukocytes in leukemia as well as in other diseases. This paper presents the results of over 400 assays of fumarase activity in separated human leukocytes and erythrocytes in a wide variety of hematologic and non-hematologic disorders.

METHODS AND MATERIALS

A saline suspension of separated human leukocytes in a concentration of approximately 40,000 cells per cu. mm. was prepared as previously described. After standard leukocyte counts and erythrocyte contamination counts had been done in quadruplicate, the suspension was centrifuged, supernatant discarded, and volume restored with glass-distilled water. This suspension was then frozen and thawed six times. Although platelets were not completely eliminated by the differential centrifugation employed in the separation process, assays in diseases with very low or high peripheral blood platelet counts have indicated that results were not significantly altered by the presence of some platelets in the leukocyte suspensions. The erythrocyte contamination ranged up to eight erythrocytes to one leukocyte (usually less than 6:1). Except when the red cell contamination was very low, an hemolysate was prepared in glass-distilled water in a concentration approximately equivalent to the erythrocyte contamination of the leukocyte rich suspension. Freezing and thawing of the red blood cell hemolysate did not appear to enhance or decrease fumarase activity. An assay of erythrocyte fumarase activity was performed simultaneously with the leukocyte assay and appropriate correction was applied.

Fumarase activity was determined by a modification of Racker’s procedure as outlined for the assay of leukocyte aconitase activity, except that in the fumarase assay the substrate was 0.05M l-malic acid, the standard was fumaric acid, and the incubation time was 15 minutes.

The values for fumarase activity are expressed as milligrams of fumaric acid produced from l-malic acid substrate by 10^10 leukocytes or erythrocytes per hour at 37°C. The hemoglobin content, erythrocyte count, packed cell volume, total and differential leukocyte counts were determined on a sample taken from each individual at the time blood was...
drawn for assay. In this report, the term neutrophil includes neutrophilic granulocytes from the myelocyte to mature segmented forms when used in reference to differential counts.

Nine older children (age range 10 to 18, average 15 years) and 40 adults with all types of leukemia (except acute exacerbations of chronic granulocytic leukemia) demonstrating 20 or more per cent blast and "pro" cells in the peripheral blood at the time of study were arbitrarily included in the acute leukemia category according to cell type. Those patients who had acute or subacute leukemia by bone marrow morphology and/or clinical course, but who did not fulfill the above criterion, were therefore excluded. Ten patients with monocytic leukemia whose peripheral blood revealed less than 20 per cent monoblasts and promonocytes at the time of study were grouped as monocytic leukemia, although clinically most of these patients had an acute or subacute course. The above arbitrary categories were used because the fumarase assays were performed on peripheral blood leukocytes, which at the time of study did not always reflect the bone marrow picture or clinical course.

RESULTS

The results, as well as some of the pertinent data, of the leukocyte and erythrocyte fumarase assays are summarized in table 1 according to disease categories. The results of the leukocyte assays in the hematologic disorders are also shown in figure 1 in the form of a scattergram.

Table 1.—Summary of Leukocyte and Erythrocyte Fumarase Assays in Normals and in Hematologic and Nonhematologic Diseases

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>No. of assays</th>
<th>Diagnosis</th>
<th>Mean WBC (mg/hr)</th>
<th>Mean RBC (mg/hr)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>9</td>
<td>Normal</td>
<td>7,732</td>
<td>499</td>
<td>WBC range 273-709, RBC range 21-67</td>
</tr>
</tbody>
</table>

Hematologic Diseases:

- Acute monocytic leukemia: 16
- Monocytic leukemia: 10
- Acute granulocytic leukemia: 22
- Chronic granulocytic leukemia: 27
- Acute lymphocytic leukemia: 11
- Chronic lymphocytic leukemia: 18
- Infectious mononucleosis: 17
- Myeloid metaplasia: 8
- Polycythemia vera: 8
- Lymphomas: 11
- Miscellaneous hematologic diseases: 64

Nonhematologic Diseases:

- Allergic diseases: 10
- Infections and inflammatory diseases: 15
- Neurologic diseases: 6
- Liver disease: 10
- Endocrine diseases: 15
- Collagen diseases: 21
- Miscellaneous nonhematologic diseases: 43

*Expressed as milligrams of fumaric acid produced from l-malic acid substrate by 10⁶ WBC or RBC per hour at 37 C.
Fig. 1.—Scattergram of fumarase activity of leukocytes obtained from normal individuals and from patients with hematologic diseases. Each dot represents a separate assay. Values for fumarase activity are expressed as milligrams of fumaric acid produced from l-malic acid substrate by $10^{10}$ white blood cells per hour at 37 C. Note that the mean values are clearly elevated in acute monocytic leukemia and acute granulocytic leukemia and that the mean value is higher in the acute than in the chronic form of each type of leukemia.
Leukocytes:

A. **Findings in leukemia:** The mean fumarase activity was markedly elevated in acute monocytic leukemia and was moderately increased in acute granulocytic leukemia, but was not significantly elevated in acute lymphocytic leukemia (table 1). Fumarase activity was higher in the acute than in the chronic form of all three types of leukemia. In addition, patients with higher percentages of blast and "pro" cells had higher fumarase values within each type of leukemia. When the peripheral blood became essentially mature during partial remissions of acute leukemia, fumarase values were usually normal. There was no correlation of fumarase activity with the peripheral leukocyte count. No effect of 6-mercaptopurine and/or prednisone therapy on fumarase activity was noted, except that activity decreased if the percentage of immature cells in the peripheral blood decreased with therapy.

B. **Findings in other diseases:** No consistent pattern was noted in a wide variety of hematologic disorders grouped in the miscellaneous category, which included diseases such as paroxysmal nocturnal hemoglobinuria (PNH), iron deficiency anemia, idiopathic thrombocytopenic purpura, hereditary spherocytosis, hemoglobinopathies, pernicious anemia, and refractory anemia.

As shown in table 1, the mean fumarase values in a number of nonhematologic disease categories were not remarkably different from those in normal subjects. Very modest elevations appeared to be associated with the endocrine and collagen disease groups. Most of the endocrine cases were patients with diabetes mellitus in various degrees of control. One patient with myxedema coma had a value of 937, while a single case of hyperthyroidism had a value of 603.

C. **Correlation with cell types:** Blast cells of all types demonstrated greater activity than more mature forms of the same series. Monoblasts were the most active, whereas the lymphoblasts were the least active among the blast cells. Mature neutrophils had about the same activity as lymphocytes on a per cell basis. One patient with chronic granulocytic leukemia in whom 78.5 per cent of the cells were basophils had a fumarase value of 449. On another occasion, when there were 87.5 per cent basophils, the fumarase value was 307. In a subject with a leukocyte count of 400,000 per cu. mm. and 92.5 per cent eosinophils the fumarase level was 611. It appears that eosinophils and basophils also possess this enzymatic activity.

Erythrocytes:

Erythrocytes have about one-tenth the activity of normal leukocytes on a per cell basis (table 1). The mean red cell fumarase values were quite similar in the various disease categories except for slightly elevated values in chronic lymphocytic leukemia (67) and liver disease (73). In the latter two categories the average erythrocyte mean corpuscular volumes were 100.7 and 113 respectively. The slightly increased mean corpuscular volume in chronic lymphocytic leukemia was due to those patients with this disease who had an associated hemolytic anemia. Elevated fumarase values (100 to 130 range) seen in megaloblastic anemias in relapse returned toward normal with therapy. Al-
though markedly elevated fumarase activity was noted in some hemolytic disorders, the results were variable. However, extensive correlative studies were not attempted and thus mean cell age was not known. The highest individual erythrocyte fumarase results occurred in the following: PNH, 150; acquired hemolytic anemia of undetermined etiology, 127; three patients with pernicious anemia in relapse, 127, 118, and 109; and congenital hemolytic anemia with abnormal pigment metabolism,\textsuperscript{a} 109.

**Discussion**

The studies of McKinney and associates\textsuperscript{7} have indicated previously the existence of citric acid cycle activity in human leukocytes. Recently, aconitase, an enzyme in the citric acid cycle, has been demonstrated to be present in human leukocytes.\textsuperscript{2,4} The results which have just been presented furnish evidence for the presence of another citric acid cycle enzyme, fumarase, in human leukocytes. The data also indicate that immature cells of acute monocytic leukemia and acute granulocytic leukemia, and, to a lesser degree, acute lymphocytic leukemia, have greater fumarase activity than mature cells of the same series. The results do not establish whether this difference is also characteristic of immature normal cells or whether this variation is a reflection of the metabolic features of immature leukemic cells. However, leukocytes of normal individuals and mature cell types present in certain leukemias appear to have essentially comparable fumarase activity. No significant pattern, other than that just discussed, was noted among the large number of hematologic and nonhematologic diseases studied.

In 1931 Quastel reported that human erythrocytes are a rich source of fumarase.\textsuperscript{9} However, our data would indicate that leukocytes possess about 10 times greater activity than erythrocytes on a per cell basis. Macrocytes appear to have greater activity in proportion to their increased size and independent of the etiology of the macrocytosis. Rubinstein and associates have shown that reticulocytes of rabbit blood utilize the citric acid cycle whereas mature erythrocytes do not.\textsuperscript{10} Aconitase, a citric acid cycle enzyme, has been reported by Beutler to be increased with reticulocytosis in human red blood cells.\textsuperscript{9} Although some of the highest fumarase values in our study occurred in patients with reticulocytosis, other patients with definite hemolytic anemia did not have elevated fumarase values. More data are necessary to determine whether fumarase activity in human erythrocytes is directly related to cell age or whether the underlying disease process is a modifying factor. Erythrocyte fumarase assays in a wide variety of hematologic and nonhematologic disorders failed to indicate any definite pattern of correlation of enzyme activity with specific disease states.

**Summary**

1. The results of over 400 assays of fumarase activity in separated leukocytes and erythrocytes in a variety of hematologic and nonhematologic diseases are presented.

2. The highest mean leukocyte fumarase values occurred in acute monocytic
leukemia and acute granulocytic leukemia. Blast cells have greater fumarase activity than more mature forms of the same cell type.

3. Fumarase appears to be distributed ubiquitously among the various leukocytes. Cell populations which contained predominantly neutrophils, lymphocytes, monocytes, blasts, eosinophils, and basophils, all exhibited appreciable enzyme activity.

4. Assays of erythrocyte fumarase activity did not reveal a pattern with the exception of elevated values in macrocytic anemias and in some, but not all, cases of hemolytic anemia.

**Summario in Interlingua**

1. Es presentate le resultatos de plus que 400 determinationes del activitate de fumarase in separate leuco- e erythrocytos in diverse statos de anormalitate hematologic e non-hematologic.

2. Le plus alte valores medie de activitate de fumarase occurreva in acute leucemia monocytic e acute leucemia granulocytic. Blastocytos ha plus alte nivellos de activitate de fumarase que le formas plus matur del mesme typo de cellula.

3. Il pare que le distribution de fumarase in le varie leucocytos es universal. Populationes cellular continente predominantemente neutrophilos, lymphocytes, monocytes, blastocytes, eosinophilos, o basophilos exhibiva omnes notable grados de activitate enzymatic.

4. Determinationes del activitate de fumarase in erythrocytos revelava nulle configuration, con le exception del facto que elevate valores esseva incontrate in le anemias macrocytic e in certe (sed non omne) casos de anemia hemolytic.

**References**


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