ONE WAY TO JUDGE the mean age of a population of erythrocytes is the measurement of age-related enzymes. Erythrocyte glucose-6-phosphate dehydrogenase (G-6-PD), erythrocyte hexokinase, and erythrocyte glutamic oxalacetic transaminase (EGOT) have been shown to be related to erythrocyte age. The present study was undertaken to determine the mean erythrocyte age in patients with polycythemia vera through the measurement of these enzymes.

**Materials and Methods**

Normal values for the enzymes were determined by measurement in blood from personnel in our laboratory. The diagnosis of polycythemia vera was made in our patients by the standard criteria. All patients had evidence of a panmyelopathy; the clinical data at the time of diagnosis are presented in Table 1. Patients in remission on busulfan treatment were excluded from the study.

Blood was obtained by venipuncture and collected in tubes containing oxalate for EGOT and G-6-PD determinations, and containing EDTA for hexokinase determination. Erythrocytes were washed free of plasma and buffy coat and hemolyzed by diluting 1:20 in distilled water. For EGOT and G-6-PD the sample consisted of sufficient hemolysate to yield 3.65 mg. of hemoglobin, and for hexokinase the sample consisted of sufficient hemolysate to yield 1.82 mg. of hemoglobin.

Previously reported methods were used to measure EGOT, G-6-PD, and hexokinase. Reagents were obtained from the Sigma Chemical Company, St. Louis, Missouri.

**Results**

The results in the normal patients and patients with polycythemia vera are summarized in Table 2. Since enzyme values are reported in change in absorbance per minute per gram of hemoglobin at a wavelength of 340 (\(\Delta A_{340}/\text{min./Gm. Hb}\)), the presence of hypochromia would have a bearing on the result. Therefore, the final values were corrected to a mean corpuscular hemoglobin concentration of 32 per cent.
ERYTHROCYTE ENZYMES IN POLYCYTHEMIA VERA

Table 1.—Clinical Features of Patients with Polycythemia Vera at the Time of Diagnosis

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Spleen Palpable</th>
<th>Liver Palpable</th>
<th>Hct (%)</th>
<th>WBC's per cu. mm. x 10^9</th>
<th>Reticulocytes (%)</th>
<th>Platelets per cu. mm. x 10^9</th>
<th>LAP Score</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>68</td>
<td>F</td>
<td>Yes</td>
<td>Yes</td>
<td>54</td>
<td>17.7</td>
<td>2.5</td>
<td>560</td>
<td>400</td>
</tr>
<tr>
<td>2</td>
<td>71</td>
<td>F</td>
<td>Yes</td>
<td>Yes</td>
<td>49</td>
<td>30.0</td>
<td>1.0</td>
<td>1,020</td>
<td>400</td>
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<tr>
<td>3</td>
<td>70</td>
<td>M</td>
<td>Yes</td>
<td>No</td>
<td>62</td>
<td>9.8</td>
<td>1.0</td>
<td>630</td>
<td>400</td>
</tr>
<tr>
<td>4</td>
<td>58</td>
<td>F</td>
<td>Yes</td>
<td>No</td>
<td>45</td>
<td>16.6</td>
<td>3.0</td>
<td>595</td>
<td>400</td>
</tr>
<tr>
<td>5</td>
<td>60</td>
<td>F</td>
<td>Yes</td>
<td>Yes</td>
<td>63</td>
<td>14.7</td>
<td>1.5</td>
<td>425</td>
<td>400</td>
</tr>
<tr>
<td>6</td>
<td>55</td>
<td>F</td>
<td>Yes</td>
<td>Yes</td>
<td>54</td>
<td>11.4</td>
<td>2.5</td>
<td>335</td>
<td>125</td>
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<td>7</td>
<td>48</td>
<td>F</td>
<td>Yes</td>
<td>No</td>
<td>40</td>
<td>14.4</td>
<td>3.0</td>
<td>100</td>
<td>400</td>
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<tr>
<td>8</td>
<td>43</td>
<td>F</td>
<td>Yes</td>
<td>Yes</td>
<td>66</td>
<td>12.7</td>
<td>2.0</td>
<td>425</td>
<td>367</td>
</tr>
<tr>
<td>9</td>
<td>63</td>
<td>F</td>
<td>No</td>
<td>No</td>
<td>50</td>
<td>10.0</td>
<td>2.5</td>
<td>365</td>
<td>400</td>
</tr>
<tr>
<td>10</td>
<td>44</td>
<td>F</td>
<td>Yes</td>
<td>No</td>
<td>68</td>
<td>13.5</td>
<td>1.5</td>
<td>419</td>
<td>400</td>
</tr>
</tbody>
</table>

Using the two-tail test of Wilcoxin and White,9 it was determined that each enzyme was significantly increased in the polycythemia patients when compared to the normals (p < .01 for each of the three enzymes).

Table 2 presents hematologic data gathered on the date on which the enzymes were assayed. It can be seen that enzyme levels did not correlate with reticulocyte count or the time of most recent phlebotomy.

COMMENT

In this study erythrocyte glucose-6-phosphate dehydrogenase (G-6-PD), glutamic oxalacetic transaminase (EGOT), and hexokinase (HK) were found to be significantly increased in patients with polycythemia vera. Activity of these enzymes has been shown to be increased in young erythrocytes and to decline with increasing cell age.1'3'4 Enzyme changes, such as the decreased leukocyte alkaline phosphatase in chronic myelogenous leukemia, are seen in association with cell dysplasia. We cannot rule out the possibility that the presence of a dysplastic erythrocyte population accounted for the enzyme changes in our polycythemia patients. However, it seems unlikely that three cell, age-related enzymes would be changed in the presence of a population of erythrocytes of normal age. Therefore, the data are taken to indicate the presence of an erythrocyte population which is younger than normal in these patients.

Previous studies of erythrocyte kinetics have suggested possible mechanisms for the origin of a young erythrocyte population in polycythemia vera. An increased rate of red cell production has been demonstrated.10'12 Normal survival of erythrocytes has been found in some studies.11'13 Sharney et al.13 postulated that increased production with normal survival led to a young erythrocyte population in two of their patients by continued outpouring of cells which did not reach equilibrium. A second possible explanation, suggested by Berlin et al.,14 is that a double population of erythrocytes might be present. The majority of the erythrocytes might live for 120 days, but a small number might be destroyed soon after release into the circulation; these young cells would increase the activities of the enzymes in our assay system. Johnson et al.15 found...
Table 2.—Enzyme Results

<table>
<thead>
<tr>
<th></th>
<th>No. in Series</th>
<th>Mean G-6-PD</th>
<th>Range G-6-PD</th>
<th>Mean EGOT</th>
<th>Range EGOT</th>
<th>Mean Hexokinase</th>
<th>Range Hexokinase</th>
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<tbody>
<tr>
<td>Normals</td>
<td>13</td>
<td>8.4</td>
<td>5.6–11.0</td>
<td>3.6</td>
<td>2.2–4.4</td>
<td>1.5</td>
<td>1.2–2.1</td>
</tr>
<tr>
<td>P.V. patients</td>
<td>10</td>
<td>14.3</td>
<td>10.4–24.7</td>
<td>7.6</td>
<td>3.9–15.6</td>
<td>3.5</td>
<td>2.6–4.4</td>
</tr>
</tbody>
</table>

N.B.—Hematologic results are those obtained on date of enzyme measurements. Enzyme activities reported in ΔA340/min./Gm. hemoglobin.

decreased erythrocyte survival in polycythemics, and this would explain the presence of a young population of erythrocytes. Recently, Pollycove et al.16 reevaluated erythrocyte kinetics in patients with polycythemia vera. Their data indicate that red cell survival decreases as the disease progresses. This appears to be a reasonable explanation of the apparently conflicting results reported in the previous studies.

In conclusion, we found that three erythrocyte age-related enzymes—G-6-PD, EGOT and HK—were increased in a series of patients with polycythemia vera. The data indicate that the majority of these patients have a young population of erythrocytes.

**SUMMARY**

Erythrocyte glutamic oxalacetic transaminase (EGOT), glucose-6-phosphate dehydrogenase (G-6-PD), and hexokinase were found to be increased in patients with polycythemia vera. This suggests the presence of a population of erythrocytes younger than normal in these patients. The pathogenesis of this phenomenon is unclear but several possible mechanisms are suggested.

**SUMMARIO IN INTERLINGUA**

Esseva trovate augmentos de transaminase glutamic-oxaloacetic, de dehydrogenase de glucosa-6-phosphato, e de hexokinase in le erythrocytos de patientes con polycythemia ver. Isto suggestiona le presenta de un population de erythrocytos de etate plus juvene que lo que es normal in iste patientes. Le pathogenese de iste phenomeno non es clar, sed plure mechanismos possibile es suggestionate.

**REFERENCES**

ERYTHROCYTE ENZYMES IN POLYCYTHEMIA VERA

Brief Report: Erythrocyte Enzymes in Polycythemia Vera

HENRY R. BARTOS, JANE F. DESFORGES and Dalia Samanvicius