Erythrocyte Glyoxalase II Deficiency With Coincidental Hereditary Elliptocytosis

By William N. Valentine, Donald E. Paglia, Robert C. Neerhout and Patricia N. Konrad

Hereditary hydroxyacetyl-glutathione hydrolase (Glyoxalase II) deficiency was demonstrated in both homozygous and heterozygous form in kindred in which hereditary elliptocytosis was inherited independently. Homozygotes and heterozygotes for the enzyme deficiency were clearly differentiated by direct enzyme assay and a variety of indirect methods. Four homozygotes exhibited 10 per cent or less of normal activity. Leukocytes do not share the deficiency.

METHYLGLYOXAL (PYRUVALDEHYDE) is converted in accordance with the following reactions to lactic acid\textsuperscript{1,2} by two enzymes\textsuperscript{3} found widely distributed in animal tissues, plants and microorganisms:

\[
\begin{align*}
\text{CH}_3 & \quad \text{Lactoyl-glutathione lyase} & \text{CH}_3 & \quad \text{Hydroxyacyl-glutathione hydrolase} \\
C=O & \quad \text{GSH} & H-C-OH & \quad \text{H}_2O \\
HC=O & & C=O & \quad H-O-C=O \\
S-\text{Lactoyl-glutathione} & & \text{D-Lactic Acid} & \quad \text{GSH}
\end{align*}
\]

The first, lactoyl-glutathione lyase, E.C. 4.4.1.5. (Glyoxalase I\textsuperscript{*}), forms a thiolester\textsuperscript{3} with glutathione (GSH), which is a highly specific coenzyme re-

\textsuperscript{*} For convenience the trivial terms Glyoxalase I and II will be used hereafter in this report.
quirement.\textsuperscript{3,5} It is exceedingly rapid in action. This thiolester is highly stable in neutral or acid solutions at room temperature or in the cold, but undergoes slow decomposition to \(\alpha\)-lactate and GSH in alkali media.\textsuperscript{8} The second, hydroxyacyl-glutathione hydrolase, E.C. 3.1.2.6. (Glyoxalase II\textsuperscript{*}),\textsuperscript{3} catalyses the hydrolysis of the thiolester to the \(\alpha\)-isomer of lactic acid and GSH. Although considerable interest remains in assigning the enzyme a significant physiological function, the demonstration by Lohmann\textsuperscript{4} in 1932 that glycolysis proceeded normally in the absence of glyoxalase activity largely ended a long and fruitless search for a major metabolic role of the reaction. However, urinary excretion of methylglyoxal by thiamine-deficient rats has been reported,\textsuperscript{7} and a possible role of methylglyoxal in the metabolism of fructose, a precursor of glyceraldehyde, has also been postulated.\textsuperscript{8} It has also been stated that methylglyoxal may be formed in certain animal and bacterial systems during the metabolism of glycine,\textsuperscript{9,10} threonine,\textsuperscript{11,12} and aminoacetone.\textsuperscript{13} A summary of a considerable body of literature relative to the glyoxalases may be found in the review by Knox.\textsuperscript{14}

Glyoxalase activity has long been known to be present in blood,\textsuperscript{5,15-20} and in 1961 an extensive survey of red cell and white cell glyoxalase activity in normal subjects and patients with various disease states was reported from this laboratory.\textsuperscript{20} Contrary to earlier studies,\textsuperscript{16-17} while substantial activity was present in both erythrocytes and leukocytes, the activity ratio of WBC:RBC ranged only from 1:1 to 4:1.\textsuperscript{20} The present report describes the first kindred in which erythrocyte Glyoxalase II deficiency has been demonstrated. Both the homozygous and heterozygous deficiency states are documented.

**Materials and Methods**

*General*

Hematologic data were obtained by the usual standard procedures. Autohemolysis tests were performed according to the method of Selwyn and Dacie\textsuperscript{21} as modified by Rudolph and Gross.\textsuperscript{22} Glutathione (GSH) was measured employing 5,5\textquoteright-dithiobis-(2-nitrobenzoic acid) in the method described by Beutler.\textsuperscript{23} Blood typings for erythrocyte antigens were performed by standard methods. Analyses of erythrocyte stromal lipids were performed as previously reported.\textsuperscript{24}

*Enzyme Studies and Glycolytic Intermediates*

The activities of leukocyte and erythrocyte glycolytic and nonglycolytic enzymes were measured by methods previously employed in this laboratory.\textsuperscript{25-31} Glycolytic intermediates were assayed, according to Minakami et al.\textsuperscript{32} on blood deproteinized immediately on withdrawal by addition to perchloric acid solution. The only exception was 2,3-diphosphoglycerate (2,3-DPG) which was determined by a modification of the method of Krimsky.\textsuperscript{33} The activities of the following enzymes were determined: hexokinase, glucosephosphate isomerase, phosphofructokinase, aldolase, triosephosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase, 2,3-diphosphoglycerate mutase, phosphoglycerate kinase, 3-phosphoglyceromutase, phosphopyruvate hydratase (enolase), pyruvate kinase, lactic dehydrogenase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase,

\textsuperscript{*} For convenience the trivial terms Glyoxalase I and II will be used hereafter in this report.

\textsuperscript{†} 2,3-DPG, purified phosphoglycerate mutase, enolase and Glyoxalase I subsequently employed in these studies were obtained from Boehringer Mannheim Corp., Biochemical Division, San Francisco, Calif.
glutathione reductase, glutathione peroxidase, cholinesterase, lactoyl-glutathione lyase (Glyoxalase I), and hydroxyacylglythathione hydrolase (Glyoxalase II).

**Studies on Glyoxalase I and II**

Blood specimens obtained by venipuncture were immediately mixed with 0.25 volume of 5 per cent polyvinylpyrrolidone* containing 2.5 per cent sodium citrate and were allowed to sediment at room temperature or in an ice bath. The leukocyte-rich supernate was continually aspirated off as sedimentation proceeded. All subsequent procedures were performed at 4°C. Separated erythrocyte and leukocyte-rich suspensions were washed 2–3 times in isotonic saline and centrifuged for 10 minutes at 450 × g after each wash. Final preparations were diluted in the case of red-rich suspensions to about 3 × 10^6 cells per μl. and in the case of leukocyte-rich suspensions to about 4 × 10^6 cells per μl. Leukocyte and erythrocyte counts were performed in quadruplicate using a Coulter Model F Counter. Red cell contamination of leukocyte-rich suspensions was determined by hand counting. Enzyme activities of such suspensions must be carefully corrected for red cell contamination. The converse, correction of red cell-rich suspensions for contaminating leukocyte activity, is not usually required because at the usual levels of white cell contamination corrections are negligible.

As a screening procedure, total glyoxalase activity of leukocytes and erythrocytes was measured in terms of methylglyoxal disappearance during timed incubation with cell suspensions as previously described. Glyoxalase I was assayed directly by measurement of the appearance of absorbance due to thiolester formation at 240 μm, employing a Beckman DU spectrophotometer and Gilford Multiple Sample Absorbance Recorder. The assay was conducted at 37°C, using a cuvette with 1 cm. light path, a final volume of 3 ml., and the following final concentrations of reagents: phosphate buffer, pH 6.6, 0.01 M, methylglyoxal 7 μmoles, GSH 4.4 μmoles, and lysates equivalent either to approximately 5 × 10^6 red cells or 2.4 × 10^6 white cells, respectively. All lysates were prepared by freezing and thawing cell suspensions three times. Either redistilled methylglyoxal or dilutions of the commercial 30 per cent solution gave identical results under these assay conditions. The reaction was allowed to continue for 12 minutes and the change in absorbance (A) between the 6th and 10th minutes customarily measured. The high concentration of substrate and the greater activity of the Glyoxalase I reaction render the correction for Glyoxalase II activity essentially negligible over this time interval during which the reaction is linear. The activity is expressed in enzyme units (U), 1 unit representing the formation of 1 μmole of thiolester per minute per 10^10 erythrocytes or leukocytes under the assay conditions.

Glyoxalase II was assayed directly as follows. Thiolester was prepared from methylglyoxal and GSH in phosphate buffer, allowing the reaction to go to completion in the presence of purified commercial Glyoxalase I (free of Glyoxalase II). A perchloric acid extract was then made and neutralized to pH 6.7 with NaOH, destroying all Glyoxalase I activity. Thiolester, 1.25 μmoles, was employed as substrate in a 3-ml. volume reaction mixture, pH 6.7. Lysates were equivalent to approximately 1.5 × 10^7 cells in the case of erythrocytes and 2.5 × 10^6 cells in the case of leukocytes. Activity of Glyoxalase II was recorded in enzyme units (U), 1 unit being equivalent to the disappearance of 1 μmole of thiolester per 10^10 erythrocytes or leukocytes per minute under the assay conditions. Disappearance of theo- lester was followed at 240 μm. The extinction coefficient of the thiolester S-lactoyl-GSH at pH 6.8 according to Racker is 3.37 sq. cm./μmole and this value was employed in all calculations.

**Incubation Experiments**

When S-lactoyl-GSH is incubated with erythrocyte hemolysates containing Glyoxalase II, there should be progressive diminution in absorbance at 240 μm due to loss of thiolester,

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* Plasdone C, Antara Chemicals, General Aniline and Film Corp., Los Angeles, Calif.
† Methylglyoxal, 30 per cent solution (pyruvic aldehyde), K & K Laboratories, Plainview, N. Y.
progressive loss of thiolester as measured by the hydroxamic acid method of Lippmann and Tuttle, and progressive reappearance of GSH as hydrolysis of thiolester, catalyzed by Glyoxalase II, proceeds. To test each, the following incubation was employed. To a series of tubes was added 1 ml. of a thrice frozen and thawed lysate of a 1:5 dilution of erythrocytes (approximately $3 \times 10^8$ cells per ml. prior to dilution). To this was added 2 ml. of thiolester (25 µmoles), pH 6.7, prepared as previously described. The reaction was stopped by the addition of 2 ml. of 10 per cent PCA at times 0, 5, 10, 20 and 30 minutes. Incubation was at 37°C. In experiments where decrease in A at 240 mp (thiolester disappearance) was measured, 200 µl. of clear PCA supernate after centrifugation were added to 2.8 ml. of H₂O and A read directly at 240 mp. Decrease in A with passage of time was plotted with A at time zero as base. In a second similar experiment a sample of PCA supernate was tested for thiolester concentration employing the hydroxamic acid method of Lippmann and Tuttle. Final color development was measured at 540 mp and, again, thiolester concentration residual in each tube was plotted against that present at time zero.

In a third series of experiments, 100 µl. of PCA supernate were assayed for GSH by the DTNB method of Beutler et al. The reappearance of GSH as the incubation proceeded was calculated in terms of the time zero reading. Finally, intact erythrocytes were incubated in a mixture consisting of 1 ml. of erythrocytes ($3 \times 10^8$ cells/ml in saline, 50 µl. of 1 M phosphate buffer, pH 7.4 (final molarity 0.09); and 100 µl. of saline containing 4.2 µmoles methylglyoxal. The reaction was stopped by the addition of 1 ml. of 30 per cent TCA to identical systems at times zero, and at ½, ¾, 1, 1½, 2, 2½, 3, 4, 5, 6, 7, and 8 minutes. Methylglyoxal traverses the erythrocyte membrane with great rapidity. Initially, due to Glyoxalase I reaction, there is rapid complexing of methylglyoxal and GSH to form thiolester. Because this contains no free SH groups and does not produce color in the GSH determination, erythrocyte GSH is rapidly and dramatically reduced as such. With the passage of time and with the hydrolysis of ester by Glyoxalase II, substrate methylglyoxal is shortly consumed, lactic acid is produced and GSH is restored to zero time values. The time sequence of this reaction was followed by determining GSH at the time intervals noted above.

**Case Report**

The proband, a 7½-year-old girl, was first admitted to the hospital at age 7 days for possible congenital heart disease. No definitive diagnosis was established, but it was noted that the spleen, impalpable on admission, was 1.5 cm. below the costal margin by discharge. The hemogram was initially reported as normal. At 3 months the patient was readmitted for failure to thrive. The liver was now 1.5 cm. below the right costal margin and the spleen greatly enlarged down to the iliac crest. Hemolytic anemia was clearly present. The hemoglobin was 9.9 Gm. per cent, the RBC numbered $3.79 \times 10^6$ cu.mm., and the reticulocyte count was 16 per cent. Nucleated erythrocytes were reported on the peripheral blood smear. The Coombs’ antiglobulin test was negative, marrow aspiration revealed normoblastic hyperplasia, the nonincubated erythrocyte osmotic fragility was normal and hemoglobin electrophoresis revealed no hemoglobinopathy. At age 13 months, extensive cardiological studies confirmed the presence of pulmonary stenosis, pulmonary artery coarctation and a probable atrial septal defect. The hemogram again showed prominent reticulocytosis and presence of an active hemolytic process. Lymph node biopsy showed only non-specific lymphoid and reticulum cell hyperplasia.

In the next 2 months the patient’s hematologic status was reinvestigated. Marked splenomegaly, marked hemolysis with reticulocytosis to 25 per cent, and morphological changes compatible with hereditary elliptocytosis were noted. The patient and her mother were seen by Dr. J. V. Dacie at this time, with concurrence in a diagnosis of elliptocytosis in both. Repeated bone marrow aspirations revealed erythroid hyperplasia. Repeated white cell differential studies revealed leukocytosis and undifferentiated cells which gave concern for the presence of leukemia, though this never evolved. At age 13 months, the blood of the proband and her mother were assayed for a variety of glycolytic enzyme activities in this laboratory. In the case of the proband, these were compatible with a young cell popu-
ERYTHROCYTE GLYOXALASE II DEFICIENCY

Table 1.—Kindred G. Hematologic Data and Leukocyte and Erythrocyte Glyoxalase II Activity

<table>
<thead>
<tr>
<th>Subject</th>
<th>PCV ml./100 ml.</th>
<th>Reticulocytes Per Cent</th>
<th>Elliptocytosis</th>
<th>Glyoxalase II* RBC</th>
<th>WBC</th>
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<td>Normals (14)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III- 3 Proband</td>
<td>42.0</td>
<td>0.7</td>
<td>Yes</td>
<td>1.8</td>
<td></td>
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<tr>
<td>III- 1 Brother</td>
<td>42.0</td>
<td>0.5</td>
<td>Yes</td>
<td>0.8</td>
<td>32.7</td>
</tr>
<tr>
<td>III- 2 Brother</td>
<td>43.0</td>
<td>0.4</td>
<td>No</td>
<td>1.5</td>
<td>27.3</td>
</tr>
<tr>
<td>III- 4 Sister</td>
<td>41.0</td>
<td>0.2</td>
<td>Yes</td>
<td>2.3</td>
<td>32.5</td>
</tr>
<tr>
<td>III- 5 Half-brother</td>
<td>44.0</td>
<td>0.6</td>
<td>No</td>
<td>10.9</td>
<td>39.2</td>
</tr>
<tr>
<td>II- 1 Father</td>
<td>47.0</td>
<td>0.3</td>
<td>No</td>
<td>6.6</td>
<td>23.9</td>
</tr>
<tr>
<td>II- 2 Mother †</td>
<td>37.0</td>
<td>2.0</td>
<td>Yes</td>
<td></td>
<td></td>
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<tr>
<td>Maternal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-14 Grandfather</td>
<td>48.0</td>
<td>0.2</td>
<td>No</td>
<td>11.3</td>
<td>40.0</td>
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<tr>
<td>I-15 Grandmother</td>
<td>42.0</td>
<td>0.5</td>
<td>Yes</td>
<td>21.1</td>
<td>29.0</td>
</tr>
<tr>
<td>II- 5 Uncle</td>
<td>48.0</td>
<td>1.3</td>
<td>No</td>
<td>9.6</td>
<td></td>
</tr>
<tr>
<td>II- 6 Uncle</td>
<td>49.0</td>
<td>0.8</td>
<td>No</td>
<td>16.4</td>
<td></td>
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<tr>
<td>I-17 Great aunt</td>
<td>43.0</td>
<td>0.5</td>
<td>Yes</td>
<td>19.0</td>
<td></td>
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<tr>
<td>I-19 Great aunt</td>
<td>42.0</td>
<td>0.6</td>
<td>Yes</td>
<td>20.5</td>
<td></td>
</tr>
<tr>
<td>Paternal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I- 8 Grandfather</td>
<td>48.0</td>
<td>1.0</td>
<td>No</td>
<td>18.3</td>
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<tr>
<td>I- 9 Grandmother</td>
<td>41.0</td>
<td>0.6</td>
<td>No</td>
<td>8.8</td>
<td></td>
</tr>
</tbody>
</table>

* Expressed as enzyme units (U) as defined under Methods.
† Deceased. Hematologic studies were done in 1963.

lation with marked increases in several erythrocyte enzymes, particularly hexokinase. No enzyme deficiency could be found but glyoxalase activities were not measured at that time. The mother, whose blood smear also indicated hereditary elliptocytosis, exhibited no significant anemia, a slight reticulocytosis of 2 per cent, slight elevation of erythrocyte hexokinase activity above normal and elevated erythrocyte cholinesterase.

At age 15 months, the patient was splenectomized and reticulocytosis and anemia subsequently disappeared. At age 17 months the patient experienced pneumococcal sepsis and at 2½ years pneumococcal meningitis. Both were successfully treated. At age 3 years, a peripheral blood chromosome study revealed no chromosomal abnormalities. At the present time, aged 7½ years, the patient is being closely followed for her congenital heart disease which, because of its anatomical nature, is believed due to maternal rubella (though history is lacking), and for her unusual leukocyte abnormalities which remain unexplained but also unchanged. Her mother has since died of cancer. Hereditary elliptocytosis is present in several additional members of the kindred, as well as in the proband, and her mother, as detailed in the following section of this report.

RESULTS

Clinical and Hematologic Features

Table 1 indicates the packed cell volumes, reticulocyte counts, and the presence or absence of hereditary elliptocytosis in all family members studied. None is currently anemic and none has reticulocytosis or other evidence of hemolysis. Elliptocytosis is present in the proband, two siblings, the maternal grandmother, and two maternal great aunts, and was clearly present in the mother (now deceased) when she was studied in 1963. No paternal relatives are affected. Only the proband has exhibited splenomegaly and hemolytic anemia. Osmotic fragility studies were performed on the proband, her sib-
lings, father and all grandparents. The erythrocytes of the proband contain a small population of osmotically more fragile cells evident only in the incubated fragility test. Otherwise, except for a tendency of most family members to have minimally more resistant cells in the incubated fragility test, no abnormality of any moment was observed. Similarly, the autohemolysis test was entirely normal in all the above family members, except the proband prior to splenectomy. The red cells of the latter showed a minimal increase in autohemolysis after 48 hours and this was fully corrected by additives of glucose or adenine. For reasons which have never been clear, the proband exhibits an unusual differential leukocyte formula with a substantial number of early granulocytes being evident on every examination over the past several years. No clinical dyscrasia has thus far developed and, except for previous splenectomy, no current explanation is available for this aberration. There are no significant hematologic abnormalities in those siblings who, like the patient, are homozygous for Glyoxalase II deficiency.

**Lipid Studies**

Quantitative analyses for total lipid, lipid phosphorus, cholesterol, phospholipid fractionation and phospholipid fatty acids were performed on the erythrocytes of the proband, her siblings, father and grandparents. These did not significantly deviate from normal, irrespective of the presence of either elliptocytosis or Glyoxalase II deficiency.

**Enzyme Metabolic Studies Other Than Those for Glyoxalase**

All the enzymatic activities mentioned in the section on Methods were assayed in proband erythrocytes. All are currently normal, including hexokinase (HK), pyruvate kinase (PK), and glucose-6-phosphate dehydrogenase (G6PD), which, in our experience, are elevated in activity in the presence of young erythrocyte populations. There is, therefore, no enzymatic evidence for significant hemolysis at this time. Similarly, in the case of all siblings, the father and all grandparents, assays of HK, PK and G6PD activity were made. In none was there evidence of increased activity compatible with erythrocytes of young mean cell age. Erythrocyte GSH levels were measured in the red cells of all family members shown in Table I. All were entirely normal. The erythrocytes of the mother, now deceased, had been studied metabolically in 1963, as discussed in the Case Report.

**Glyoxalase Activity**

Table 1 indicates erythrocyte Glyoxalase II activity in kindred G. The proband and three siblings (III-3 and III-1, 2, 4, Fig. 3) are homozygous for Glyoxalase II deficiency. The mean red cell activity is 1.6 U. (range 0.84–2.34 U.) and this is 8.4 per cent of mean activity of normal family members. Five subjects (III-5, II-5, II-1, I-9, I-14) have erythrocyte activities indicative of heterozygosity (mean of 9.9 and range of 6.6–11.3 U.). Mean activity in heterozygotes is 51.8 per cent that of normal family members. The remaining five family members have activities entirely comparable to unrelated normal
ERYTHROCYTE GLYOXALASE II DEFICIENCY

Fig. 1.—Comparison of thiolester (S-lactoyl-glutathione) disappearance and glutathione (GSH) appearance during hemolysate incubations with thiolester. Mean values of normal subjects are designated by squares, those of subjects heterozygous for Glyoxalase II deficiency by open circles, and those of homozygously deficient subjects by closed circles. Shaded areas define the range of values in each instance. A for both thiolester disappearance and GSH appearance are in arbitrary units because complete stoichiometry was not achieved under assay conditions as discussed in text. A small, but constant, deficit of GSH is artifactually present for technological reasons.

Fig. 2 shows an experiment in which intact erythrocytes of members of Kindred G were incubated with 4.2 μmoles of methylglyoxal as described in Methods, and GSH measured at short intervals after initiating the reaction. The initial extremely rapid fall in measurable GSH is due to the diffusion of methylglyoxal across the red cell membrane and its almost immediate reaction with intracellular GSH to form thiolester in the very rapid reaction catalyzed

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Fig. 2.—Sequence of GSH disappearance and reappearance when intact erythrocytes are incubated with 4.2 amoles of methylglyoxal as described in the text. All values are expressed as a per cent of initial GSH determinations. A comparison is made for normal subjects and subjects heterozygous and homozygous for erythrocyte Glyoxalase II deficiency. Shaded areas define the time range required in each instance for return of GSH values to 50 per cent of baseline.

by Glyoxalase I. Thiolester is then hydrolysed by Glyoxalase II, GSH is released, but essentially immediately recombined as the thiolester until methylglyoxal has been consumed. When the latter occurs, the remaining thiolester is quickly hydrolyzed in normal cells, and GSH rapidly returns to preincubation values. Again, it can be seen that in all subjects the initial fall in GSH due to the action of Glyoxalase I is comparably rapid. In normal subjects, Glyoxalase II activity rapidly restores initial GSH levels. In heterozygotes, partial deficiency of Glyoxalase II activity delays this restoration, and in homozygotes the delay is marked indeed. If, for comparison, the mean time required to restore GSH to 50 per cent of its preincubation level is computed for each group, this is found to be 40 seconds for normal erythrocytes, 85 seconds in heterozygously deficient cells, and 323 seconds in cells from homozygotes. The ratios are approximately 1:2:8. By any of several methods, homozygosity and heterozygosity for Glyoxalase II deficiency are readily differentiable from each other and from the normal state.

Genetics.

Fig. 3 shows the transmission of erythrocyte Glyoxalase II deficiency and hereditary elliptocytosis in Kindred G. The enzyme deficiency is transmitted as an autosomal recessive trait, both sexes are affected and three generations
Fig. 3.—Genealogy for Kindred G. The proband is indicated by the arrow.

are involved. Elliptocytosis is clearly inherited independently. Only maternal relatives of the affected children are involved and the maternal grandmother, who transmits the abnormality, is not affected with Glyoxalase II deficiency. Elliptocytosis in this kindred as in others is transmitted as a dominant characteristic with variable penetrance. The proband alone had clearcut and substantial hemolytic anemia, though her mother gave equivocal evidence of a possible mild hemolytic state.

DISCUSSION

The proband in Kindred G was initially referred for metabolic studies of the erythrocytes because of a markedly overt hemolytic anemia and splenomegaly. Family studies confirmed dominant transmission of hereditary elliptocytosis through three maternal generations. With the exception of the proband, none of the affected family members had anemia, reticulocytosis, nor elevation in the activity of erythrocyte HK, G6PD or PK. $^{51}$Cr survival studies were not done and subtle shortening of erythrocyte life span cannot be excluded. Indeed, since splenectomy, the proband also fails to demonstrate any signs of overt hemolysis including those of increased red cell enzymatic activities. This has been the general rule where splenectomy has been performed for hemolytic anemia secondary to hereditary elliptocytosis.$^{35-39}$ There is strong evidence that the spleen is the major site of hemolysis when this is present in this disorder. It has been postulated that elliptocytosis may be genetically heterogeneous,$^{40-42}$ and depend upon either of two genes, one of which is linked to the rhesus locus. Although complete blood typings were performed, in the laboratory of Dr. Phillip Sturgeon, on members of the present kindred, the nature of the Rh constitution of family members precluded obtaining any linkage information of value. It has also been postulated that the erythrocytes in hereditary elliptocytosis possess a metabolic defect, possibly in glycolysis.$^{36}$ Studies in our kindred, as in those of the cases of Cutting et al.,$^{35}$ fail to show any discernible deficiency of glycolytic enzymes.

The finding of Glyoxalase II deficiency in both the homozygous and hetero-
zygous form in the erythrocytes of certain members of Kindred G and the demonstration of its transmission as an autosomal recessive trait resulted from an assay of this activity in erythrocytes of referred cases of hemolytic anemia now in use for some 10 years in the laboratory. In this period of time only the above kindred with this deficiency has been detected. It is clear, however, that the deficiency is not associated with discernible clinical disease since three homozygous children are clinically well. Two of these have inherited elliptocytosis and one has not. There is no good evidence that the deficiency has worsened the manifestations of elliptocytosis where the two genetically determined abnormalities have coexisted. Moreover, there is no evidence of glyoxalase deficiency being associated with elliptocytosis. The former is clearly transmitted in paternal and certain maternal members of the kindred who lack the latter. Further, we have studied two additional unrelated cases with hereditary elliptocytosis and both Glyoxalase I and II activities have been entirely normal in both. The fact that the leukocytes do not share the Glyoxalase II deficiency suggests that in this case of this enzyme, as with many others, a varying pattern of isozymes may exist in different tissues. We can find no evidence, however, that erythrocyte vitality is impaired in homozygotes by reductions of activity to 10 per cent or less of normal.

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