Pyruvate Kinase (PK) Deficiency Hereditary Nonspherocytic Hemolytic Anemia

By Kouichi R. Tanaka, William N. Valentine and Shiro Miwa

Although the congenital nonspherocytic hemolytic anemias represent a heterogeneous group of diseases, they have certain features in common, such as lack of spherocytosis, no increase in osmotic fragility of fresh red cells, no abnormal hemoglobin, and little, if any, benefit by splenectomy.1 These disorders were first discussed in detail in 1953 by Dacie and his colleagues.2 In 1954 Selwyn and Dacie3 described further studies on four cases of congenital nonspherocytic hemolytic anemia, and classified them into two types (Type I and Type II) upon the basis of in vitro tests. The most striking difference between these types was the presence of markedly increased autohemolysis, not correctible by glucose, on incubation of sterile, defibrinated blood in Type II cases, whereas Type I cases exhibited normal autohemolysis of whole blood. This was decreased by the addition of glucose, but to a lesser degree than in normal blood.

de Gruchy and associates1 have recently reviewed the literature on these diseases and have presented additional clinical and hematologic data on seven cases (4 of Type I and 3 of Type II). Based upon their review of seven cases conforming to the Type II category collected from the literature and their own three cases, they characterized the Type II cases as showing the following features: a marked uniform macrocytosis with numerous Pappenheimer bodies (postsplenectomy), a normal osmotic fragility of fresh blood, increased autohemolysis, and clinical onset usually, though not invariably, in infancy. The studies of de Gruchy and colleagues1 have demonstrated that in the Type II category the increased autohemolysis is correctible by additions of adenosine triphosphate (ATP).* Although this disorder has been considered to be hereditary, it is to be noted that the family histories in cases classified as Type II have usually been negative or, when positive, disease has been evident in siblings, rather than in parents or offspring.

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*The following abbreviations are used throughout this paper: ATP, adenosine triphosphate; AMP, adenosine monophosphate; ADP, adenosine diphosphate; DPN, diphosphopyridine nucleotide; DPNH, reduced diphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide; GSH, reduced glutathione.
It is significant that both Selwyn and Dacie\(^3\) and de Gruchy et al.\(^4\) have adduced evidence that some disorder of glycolysis exists in the erythrocytes in Type II cases. The former found an abnormally low utilization of glucose; the latter observed a low ATP content in the red cells and the accumulation of abnormally large amounts of certain phosphorylated glycolytic intermediates on incubation with glucose. A defect in glycolysis and impaired energy metabolism with resultant premature erythrocyte death in vivo was therefore postulated.

The present report demonstrates a specific defect in the glycolytic enzyme pyruvate kinase in the erythrocytes of seven patients conforming to the criteria of Type II congenital nonspherocytic hemolytic anemia. In addition, family studies indicate a recessive mode of transmission of the disorder, the heterozygotes having a partial, detectable enzyme deficiency not reflected in clinical disease. A brief report of these findings has been previously presented.\(^5\)

**Materials and Methods**

Fresh blood obtained by venipuncture with sterile precautions was divided into (1) a tube containing polyvinylpyrrolidone\(^*\) as sedimenting agent and citrate as anticoagulant, (2) a bottle with double oxalate for routine hematologic studies, and (3) when applicable, into sterile 125 ml. Erlenmeyer flasks containing 4 mm. glass beads for defibrination. Cover slip smears were also prepared at this time.

Routine hematologic studies were performed by standard methods.\(^6\) Reticulocyte counts were made on dry films after vital staining with new methylene blue. The percentage of reticulocytes in 1000 erythrocytes was determined. The method of Sundberg and Broman\(^7\) was utilized for staining siderocytes. Sickle cell preparations were performed utilizing sodium metabisulfite.\(^8\) Saline suspensions of erythrocytes in a concentration of about three million cells per cu. mm. and leukocytes in a concentration between 30,000 and 40,000 cells per cu. mm. were prepared. Quadruplicate counts for both the red and white cell-rich suspensions and for the contamination of the red cell-rich suspension by white cells, and vice versa, were performed on the Coulter electronic blood cell counter. Hemoglobin determinations and reticulocyte counts were done on each red cell-rich suspension. The small numbers of platelets not eliminated by the differential centrifugation employed in the separation process were disregarded. Appropriate dilutions were made from these original red and white cell-rich suspensions for the various enzymatic assays. All chemicals were reagent grade, other compounds were of the highest purity obtainable from sources stated, and the water used was glass-distilled unless otherwise stated.

In particular reference to the pyruvate kinase assay, the leukocyte contamination in the erythrocyte suspension should be as low as possible, preferably less than 1,500 per cu. mm. Assays performed with higher leukocyte contaminations were not always reliable due to the large correction necessary for the leukocyte contribution. Because the ratio of per cell activity for pyruvate kinase of leukocytes to erythrocytes is approximately 300 to 1, the activity of the white cell-rich suspension does not usually require correction for its erythrocyte contamination, unless the contamination is very high.

Pyruvate kinase was assayed according to the method of Bücher and Pfeiderer\(^10\) with slight modifications. The determination is dependent upon the conversion of phosphoenolpyruvate to pyruvate in the presence of ADP by pyruvate kinase in the assayed homogenate. Pyruvate formed during the reaction is converted by lactic dehydrogenase to lactate in the presence of DPNH. The conversion of DPNH to DPN is followed by measuring change in absorbance at 340 mp \(\times \) 37 C. in the Beckman DU spectrophotometer. The total reac-

\(^*\)Plasdone C (Antara Chemicals, New York, N. Y.).
PK DEFICIENCY ANEMIA

PHOSPHOENOLPYRUVATE

ADP

(PYRUVATE KINASE)

ATP

DPNH DPN

PYRUVATE

(LACTIC DEHYDROGENASE)

LACTATE

Fig. 1.—Diagrammatic representation of glycolytic enzyme steps responsible for the conversion of phosphoenolpyruvate to lactate.

The assay mixture contained the following (final concentrations): triethanolamine-HCl buffer, pH 7.5 (8.3 x 10^{-3}M), KCl (7.5 x 10^{-3}M), MgSO_{4} (8 x 10^{-3}M), ADP (4 x 10^{-4}M), 1000 Baur units lactic dehydrogenase, DPNH (2 x 10^{-4}M), phosphoenolpyruvic acid, trisodium salt (1.5 x 10^{-3}M), 0.3 ml. erythrocyte homogenate* (equivalent to approximately 3.6 x 10^{7} erythrocytes) or 0.1 ml. leukocyte homogenate (equivalent to about 1.6 x 10^{5} leukocytes) and water to final volume of 3.0 ml. in a silica cuvette.

The above conditions have been found to be suitable, and substantial differences in activity may result from variations in molarity of MgSO_{4}, ADP, etc. The blank contained all reagents except cells. The reaction was initiated by adding the substrate, and the change in absorbance was recorded every 2 minutes for 14 minutes. The change occurring between the 4th and 14th minute was arbitrarily used for calculation purposes, since experience indicated that the reaction was linear during this period. Assays for erythrocytes and leukocytes were performed in each case, but on separate determinations, due to differences in initial absorbance.

Values for pyruvate kinase activity are expressed in arbitrary units, one unit being that activity resulting in the conversion of one micromole of DPNH to DPN per minute by 10^{10} erythrocytes or leukocytes at 37 C. under the assay conditions stated.

Other enzymes of the glycolytic cycle from glucose to lactate were assayed by the following methods. Total triose production from either glucose or glucose-6-phosphate as substrate was determined by the procedure of Sibley and Lehninger. The conversion of fructose-1,6-bisphosphate or mixture of triose esters to 1,3-diphosphoglycerate was determined by following the rate of reduction of DPN spectrophotometrically. Slight modifications of methods for 3-phosphoglycerate-1-kinase, 2,3-phosphoglycerate mutase, enolase, lactic dehydrogenase, glucose-6-phosphate dehydrogenase, and 6-phosphogluconic dehydrogenase as reported in the references noted were utilized. Previously

*The following cell mixture was prepared and frozen and thawed three times (in dry ice-propylene glycol methyl ether mixture) just prior to assay: 0.1 ml. red cell or white cell suspension, 0.8 ml. of 0.05 M triethanolamine-HCl buffer, and 1.6 ml. physiologic saline.

DPNH was obtained from Sigma Chemical Co., St. Louis, Mo. Phosphoenolpyruvic acid (trisodium salt) was obtained from California Corporation for Biochemical Research, Los Angeles, Calif. ADP and lactic dehydrogenase (rabbit muscle; Type II if from Sigma) were obtained from either of above sources.
described procedures were employed for assaying the non-glycolytic enzymes arginase,\textsuperscript{19} fumarase,\textsuperscript{20} acid phosphatase,\textsuperscript{21} and glyoxalase.\textsuperscript{22} Cholinesterase was determined according to the method of Michel.\textsuperscript{23} Argininosuccinase was assayed by a modification of the procedure of Ratner and associates.\textsuperscript{24} Autohemolysis of red cells was determined by a modification of the method of Young et al.\textsuperscript{25} and of de Gruchy and associates.\textsuperscript{1} Defibrinated blood was collected according to the procedure of Young et al.\textsuperscript{25} and subsequently transferred in 1 ml aliquots to sterile screw-capped 5 ml bottles as outlined by de Gruchy.\textsuperscript{1} In the first bottle blood alone was placed. To the second bottle had previously been added 0.05 ml of a 10 per cent solution of glucose in sterile 0.85 per cent sodium chloride. To the third and subsequent bottles had been previously added 0.05 ml of a solution of each of the following compounds in sterile 0.85 per cent sodium chloride to give a final concentration of 0.02 M in the whole blood: adenosine, AMP, ADP, ATP, DPN, GSH, and TPN. Whenever coenzyme A\textsuperscript{*} was used as an additive, 1.6 mg of the crude material was added per bottle. For sake of convenience, except for adenosine, these compounds were prepared in solution volumetrically, distributed into the sterile bottles, and kept at $-20$ C. until just prior to use. Determinations were performed in duplicate whenever the volume of blood sample permitted. Pipetting of blood was completed in one set of bottles, a 1 ml sample of blood saved for determination of packed red cell volume (PCV) and control serum, and then pipetting was completed in the duplicate set. The bottles were mixed by rotation and then incubated at 37 C. At the end of 24 hours they were rotated to mix their contents, and at the end of 48 hours the amount of hemolysis was measured by the method of Crosby and Furth.\textsuperscript{26} Appropriate dilutions of the saved fresh serum were used as blank solutions. Corrections for trapped intercellular serum in the hematocrit tubes were applied according to the method of Furth.\textsuperscript{27} The per cent hemolysis was calculated according to the method of Young et al.\textsuperscript{25} and the result is expressed as the mean of the values obtained in the duplicate bottles.

For the glucose utilization studies, defibrinated blood was centrifuged, serum saved, anduffy coat discarded. The red cells were washed twice in saline, theuffy coat being discarded after each centrifugation. After the final centrifugation, the supernatant solution was discarded and original serum added to yield a packed red cell volume of 40 per cent. Sterile glucose was added in the amount of 100 mg. per cent of final volume. The blood was incubated at 37 C. with mixing every 15 minutes. An aliquot was removed initially and at hourly intervals for five hours for determination of blood glucose by the method of Kingsley and Reinhold.\textsuperscript{28}

In regard to the family studies, a blood examination consisting of determination of hemoglobin, red cell count, PCV, reticulocyte count, total and differential white cell count, examination of stained blood film, sickle cell preparation, and determination of ABO and Rh type was performed in all available relatives. Autohemolysis tests and physical examinations were done on some relatives. Pyruvate kinase activity was assayed in both the red and white cells in all the family members studied. The red cells of those individuals considered to be heterozygotes were further assayed for other glycolytic and for non-glycolytic enzymes in most instances.

The patients consisted of four adults and three children. Since these cases have not been previously described, and only ten cases have been reported to date in the literature, case histories of the seven patients are presented.

\textbf{Case 1}

J. L., a 26 year old divorced Caucasian painter, was referred to Wadsworth Hospital on February 1, 1961, for investigation of anemia by his physician.

Studies concerning the patient's anemia had been initiated on May 10, 1955, when he was admitted to a U. S. Army Hospital in Germany for investigation of jaundice which had been noted incidentally during an examination for urethral discharge. He himself had

\*From yeast, 70–75 per cent (Sigma Chemical Co., St. Louis, Mo.).
not noted skin or scleral icterus and was asymptomatic at the time of admission. A diagnosis of mild acquired hemolytic anemia was made at that time. After extensive studies, he was discharged on August 16, 1955. He was readmitted on October 24, 1955, and thence evacuated to Letterman General Hospital in San Francisco on November 14, 1955. He was asymptomatic and there was no history of nausea, vomiting, light colored stools, fever, chills, or itching. Physical examination at that time revealed a well developed but slender Caucasian male with a sallow complexion. There was minimal scleral icterus, but the liver and spleen were not palpated. The remainder of the examination was negative. Laboratory studies included the following: hemoglobin, 11.3 Gm. per cent; PCV, 33 per cent; reticulocytes, 7 per cent; white blood cell count, 9,500 per cu. mm. with a normal differential count; serum bilirubin, 2.5 mg. per cent total with 2.3 mg. per cent indirect reacting; direct and indirect Coombs test, negative; fresh and incubated red cell osmotic fragility, normal; cold and warm hemolysins, acid hemagglutinins, and cold agglutinins, negative; srologic test for syphilis, routine urinalysis, and three LE preparations, negative; liver function tests, gall bladder and chest x-rays, normal; elevated urinary and fecal urobilinogen; urine porphobilinogen, negative. Sternal bone marrow aspiration on January 26, 1956, revealed erythroid hyperplasia. Iron turnover studies using Fe59 on February 28, 1956, showed a plasma clearance time (T½) of 26 minutes (normal 80-120 minutes). An abdominal x-ray examination demonstrated enlargement of the spleen.

On March 1, 1956, a splenectomy was performed. One unit of blood was transfused, the only transfusion ever received by the patient. The spleen weighed 485 Gm. and the histologic description was as follows: "Malpighian follicles are smaller and somewhat less prominent than usual. The sinusoids are distended with large numbers of red blood cells. In some areas the endothelium lining the sinusoids is somewhat more prominent than usual. No iron deposition is apparent within the red pulp. No areas of extramedullary hematopoiesis are seen." The pathologic diagnosis was congestive splenomegaly consistent with acquired hemolytic anemia. Postoperatively, there was no change in his hematologic status. A repeat Fe59 study on March 15, 1956, revealed a plasma clearance time (T½) of 24 minutes. He was asymptomatic and was discharged.

Except for acute gastritis in June, 1956, tonsillectomy in 1957, and right otitis media in 1959, he was quite well and worked regularly, although he had noted some fatigue. During 1959 he was treated with courses of iron and cortisone without hematologic improvement.

Further history obtained at the time of admission to Wadsworth Hospital indicated that he had had an active childhood and had participated in sports. He had entered the U. S. Army in December 1952, had tolerated his 16 weeks of basic training, and had no difficulty until his icterus was noted as an incidental finding in May 1955, as already mentioned above.

Family history: The father, age 59 and of Irish-German ancestry, has had three myocardial infarctions. The mother, age 57 and of partly American Indian ancestry, has had hypertension. Both parents were born in Missouri. They had been studied at Letterman General Hospital in early 1958 and had been told that there was no evidence of hematologic disease. The patient was the eighth child of twelve live births. Two brothers died during infancy of diseases apparently unrelated to that of the patient. The patient's daughter and son, ages 3 and 2, respectively, have been well. Despite a large family, no history of a disorder similar to that of the patient could be elicited in the parents, siblings, children, or other relatives. However, this large and cooperative family was available for investigation. The results are presented under Family Studies.

Physical examination revealed a well developed, but asthenic Caucasian male measuring 71 inches in height and weighing 145 lbs. There was slight pallor and the sclerae were slightly icteric. The liver was palpated 3 cm. below the right costal margin. A well healed splenectomy scar was present. The remainder of the physical examination was unremarkable. Some of the clinical data of this and subsequent patients are summarized in table 1.

Laboratory examinations: The hematologic data at the time of the enzymatic studies are shown in table 2. In a wet preparation most of the red cells appeared to be larger than
Table 1.—Summary of Clinical Data of Seven Patients with Pyruvate Kinase (PK) Deficiency Hereditary Nonspherocytic Hemolytic Anemia

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Pt. Age</th>
<th>Sex</th>
<th>Ancestral Background</th>
<th>Clinical Severity</th>
<th>Onset</th>
<th>Splenectomy</th>
<th>Summary of Clinical History</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>J. L. 26</td>
<td>male</td>
<td>Northern European</td>
<td>moderate</td>
<td>21*</td>
<td>yes</td>
<td>Diagnosis of hemolytic anemia at age 21 as incidental finding while in U.S. Army. Transfused only 1 unit (at time of splenectomy). Leads active life.</td>
</tr>
<tr>
<td>2</td>
<td>R. C. 24</td>
<td>male</td>
<td>British-German</td>
<td>minimal</td>
<td>19*</td>
<td>no</td>
<td>Well compensated at normal PCV values. Crisis at time of episodes of cholecystitis when hemoglobin dropped to 7.6 Gm. per cent.</td>
</tr>
<tr>
<td>3</td>
<td>H. C. 25</td>
<td>male</td>
<td>British-German</td>
<td>moderate “child”</td>
<td>yes</td>
<td></td>
<td>Brother of Case 2. Slightly handicapped childhood and college period. Constant mild icterus at present, but working regularly. Does not require transfusions.</td>
</tr>
<tr>
<td>4</td>
<td>E. T. 31</td>
<td>female</td>
<td>Irish-English</td>
<td>moderately severe</td>
<td>yes</td>
<td></td>
<td>Anemic since birth. Over 160 transfusions, mostly in last 10 years. Severe headaches primary problem at present.</td>
</tr>
<tr>
<td>5</td>
<td>S. H. 5</td>
<td>female</td>
<td>English-Dutch-Scottish</td>
<td>severe</td>
<td>yes</td>
<td></td>
<td>Jaundiced at birth. Decreased transfusion requirements after splenectomy at age of 5 months.</td>
</tr>
<tr>
<td>6</td>
<td>K. H. 2</td>
<td>female</td>
<td>English-Dutch-Scottish</td>
<td>severe</td>
<td>no</td>
<td></td>
<td>Sister of Case 5. Transfused 3 times in first 3 months of life and also subsequently. Intermittent icterus. Spleen at left costal margin.</td>
</tr>
<tr>
<td>7</td>
<td>M. M. 2</td>
<td>female</td>
<td>Mexican</td>
<td>moderately severe</td>
<td>no</td>
<td></td>
<td>Jaundice and splenomegaly noted on second day of life. Requiring transfusions regularly every 6-8 weeks.</td>
</tr>
</tbody>
</table>

*Age when first found to be anemic.

normal in diameter and a few irregularly contracted cells were present. In the stained peripheral blood smear most of the red cells were round, normochromic, and moderately macrocytic with relatively little anisocytosis (fig. 2). Some of the erythrocytes had irregular borders. There was moderate polychromasia. An occasional target cell was present. Nucleated red cells and cells with Howell-Jolly bodies were rare. There was no spherocytosis. The potassium ferrocyanide stain demonstrated siderocytic inclusions in many cells (27.4 per cent). The osmotic fragility of fresh blood and after incubation at 37 C. for 24 hours was interpreted as consistent with his postsplenectomy state. Total serum bilirubin was 3.2 mg. per cent with 2.1 mg. per cent indirect reacting. Serum iron was 106 µg. per cent. The test for Heinz bodies was negative as was the acid serum (Ham’s) test for paroxysmal nocturnal hemoglobinuria. Fecal urobilinogen excretion was 700 mg. per 24 hours over a 3 day period. The 24-hour urinary coproporphyrin excretion was 1490 µg. (normal 100-300 µg.). Red cell survival by the Cr51 procedure was 6.5 days (normal 28-40). The bone marrow aspiration revealed marked normoblastic erythroid hyperplasia.

A steady, partially compensated hemolytic process is indicated by the representative hematologic data of the past five years as shown in table 3. The results of the autohemolysis studies are summarized in table 4.

Case 2

R. C., a 24 year old single Caucasian male, had a largely unhandicapped childhood, participating in football, basketball, swimming, and other activities. He was never jaundiced.
PK DEFICIENCY ANEMIA

during this period. Splenomegaly and reticulocytosis without significant anemia were found at age 19 on medical examination performed at the time his brother's (H. C., Case 3) enlarged spleen and anemia were detected.

In October 1960, he had an episode of acute cholecystitis and jaundice. He was not anemic, but his urine and fecal urobilinogen excretions were increased. Bile was also present in his urine. At the time of his admission in November 1960, for his second episode of cholecystitis, his hemoglobin was 10.9 Gm. per cent and the PCV was 32 per cent. The total serum bilirubin was 4.5 mg. per cent with 3.7 mg. per cent indirect reacting. The osmotic fragility of fresh red cells was slightly decreased.

Physical examination at this time revealed a well developed, well nourished Caucasian male with obvious icterus. Hepatomegaly and splenomegaly (8 cm. below the left costal margin) were present.

During the course of his cholecystitis and cholecystectomy in November 1960, his hemoglobin fell to as low as 7.6 Gm. per cent. Subsequent to surgery there has been a gradual return of the blood values to essentially normal, but with persistence of mild reticulocytosis. It is evident that his illness and subsequent surgery were associated with a temporary exacerbation in his otherwise well compensated hemolytic disease. He has never been transfused.

Ferrokinetic studies employing Fe59 were obtained subsequent to cholecystectomy. They revealed a very short plasma radioiron clearance half-time of 8 minutes (normal 60–120 minutes), and a very rapid maximal appearance of radioiron in circulating erythrocytes. External monitoring over the spleen during the course of ferrokinetic studies raised the possibility of moderate splenic sequestration of red cells.

The hematologic data obtained at the time of the enzymatic studies on February 21, 1961, are summarized in table 2. The results of the autohemolysis studies are shown in table 4. In the peripheral smear the red cells were macrocytic, normochromic, and uniform in appearance (fig. 3). There was slight polychromasia. No Pappenheimer bodies were noted. There were no spherocytes.

The patient was the youngest of three children. An older brother is presented as Case 3. The oldest brother has been examined elsewhere and was said not to be anemic. The parents have been living in Nebraska and have not been available for study, but were apparently well except for Parkinson's disease in the father who was 61 years of age. Their ancestors were from England and Germany.

Case 3

H. C., a 25 year old Caucasian male (older brother of Case 2) has a history of intermittent fatigue, jaundice, and anemia during his childhood. He could not tolerate sports well. While in college in 1954 and 1955, he noted progressive fatigue, drowsiness, and pallor. At this time anemia and splenomegaly were noted and a splenectomy was performed in February 1955. He seems to have achieved considerable subjective improvement from the procedure, though it clearly has not corrected a moderately severe hemolytic process. He is able, however, to lead an essentially normal life at the present time without transfusions. The osmotic fragility of fresh red cells and after incubation at 37 C. for 24 hours was normal. The total serum bilirubin was 2.6 mg. per cent. The hematologic data obtained at the time of the enzymatic studies on February 21, 1961, are summarized in table 2. The results of the autohemolysis studies are shown in table 4. In the peripheral smear the erythrocytes were uniformly round macrocytes and normochromic with a few cells with irregular edges. There was moderate polychromasia. Many red cells contained Pappenheimer bodies. There was no spherocytosis.

Case 4

E. T., a 38 year old Caucasian housewife, has a history of anemia since birth. She received her first blood transfusion at age 6 and has subsequently had over 150 transfusions, most of them in the past 10 years, in order to maintain a hemoglobin between 8 and 10 Gm. per cent. A splenectomy was performed at age 16 without improvement in her hemolytic
Table 2.—Summary of Hematologic Data Obtained at Time of Enzyme Studies in Seven Patients with Pyruvate Kinase (PK) Deficiency Hereditary Nonspherocytic Hemolytic Anemia

<table>
<thead>
<tr>
<th>Case Number</th>
<th>1*</th>
<th>2</th>
<th>3*</th>
<th>4*</th>
<th>5*</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hgb. (Gm. per 100 mL.)</td>
<td>10.8</td>
<td>14.2</td>
<td>12.1</td>
<td>9.9</td>
<td>11.1</td>
<td>8.6</td>
<td>6.5</td>
</tr>
<tr>
<td>RBC (10⁶ per cu.mm.)</td>
<td>2.99</td>
<td>4.57</td>
<td>3.26</td>
<td>2.52</td>
<td>3.04</td>
<td>2.37</td>
<td>1.88</td>
</tr>
<tr>
<td>Packed red cell volume (%)</td>
<td>35</td>
<td>45</td>
<td>38</td>
<td>32</td>
<td>34</td>
<td>25</td>
<td>17</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>32.0</td>
<td>5.2</td>
<td>18.7</td>
<td>34.2</td>
<td>45.3</td>
<td>9.6</td>
<td>7.5</td>
</tr>
<tr>
<td>MCV (c,m)</td>
<td>117</td>
<td>98</td>
<td>117</td>
<td>127</td>
<td>112</td>
<td>105</td>
<td>90</td>
</tr>
<tr>
<td>MCH (µg.)</td>
<td>36</td>
<td>31</td>
<td>37</td>
<td>39</td>
<td>36</td>
<td>36</td>
<td>35</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>31</td>
<td>32</td>
<td>32</td>
<td>31</td>
<td>33</td>
<td>34</td>
<td>35</td>
</tr>
<tr>
<td>WBC (per cu.mm.)</td>
<td>19,100</td>
<td>6,200</td>
<td>18,000</td>
<td>12,900</td>
<td>11,940</td>
<td>10,300</td>
<td>5,270</td>
</tr>
<tr>
<td>Platelets</td>
<td>increased</td>
<td>normal</td>
<td>increased</td>
<td>increased</td>
<td>normal</td>
<td>normal</td>
<td></td>
</tr>
<tr>
<td>Siderocytes (%)</td>
<td>27.4</td>
<td>0.3</td>
<td>48.2</td>
<td>31.7</td>
<td>13.4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Coombs test (direct)</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>Blood group</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>O</td>
<td>B</td>
<td>B</td>
<td>O</td>
</tr>
<tr>
<td>Rh</td>
<td>D positive</td>
<td>D positive</td>
<td>D negative</td>
<td>D positive</td>
<td>D positive</td>
<td>D positive</td>
<td>D positive</td>
</tr>
<tr>
<td>Sickle cell preparation</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
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<td>negative</td>
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<tr>
<td>Hemoglobin type</td>
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<td>AA</td>
<td>AA</td>
<td>AA</td>
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<td>AA</td>
</tr>
</tbody>
</table>

*Splenectomized.
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Fig. 2.—Case 1. Photomicrograph of peripheral blood smear (postsplenectomy) (reduced from x640).

process. Pathologic examination of her spleen revealed hyperplasia of the reticulum with excessive hemosiderin. In 1957 she had three accessory spleens removed, but no remission occurred. She has had frequent episodes of jaundice. At age 28 she had a miscarriage. Menopause occurred at age 35. During the years, she has been treated with adrenal steroids, vitamin B₁₂, folic acid, and folinic acid without improvement. In January 1961, a cholecystectomy was performed for chronic cholecystitis with cholelithiasis. Headaches began about 12 years ago, have become increasingly severe, and at present constitute her primary complaint. She has adjusted very well to her anemia.

Family history: She was of Irish-English-French descent. There was no history of anemia in the family. Her mother, sister, and brother were available for study. Her father was deceased. Her only aunt on her paternal side has no history of anemia or jaundice, but has not yet been available for study.

The patient was a well developed, well nourished Caucasian woman with red hair, fair complexion, minimal pallor, and slight scleral icterus. Her height was 62½ inches and

Table 3.—Summary of Representative Hematologic Data of Patient J. L. (Case 1)

<table>
<thead>
<tr>
<th>Date</th>
<th>Hgb.</th>
<th>RBC</th>
<th>PCV</th>
<th>Reticulocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>11/14/55</td>
<td>11.3</td>
<td></td>
<td>33</td>
<td>7.0</td>
</tr>
<tr>
<td>3/1/56</td>
<td></td>
<td>(Splenectomy, splenic weight 485 Gm.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4/1/56</td>
<td>11.0</td>
<td></td>
<td>38</td>
<td>13.2</td>
</tr>
<tr>
<td>9/13/56</td>
<td>11.8</td>
<td>2.7</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>3/10/59</td>
<td>11.3</td>
<td></td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>3/23/59</td>
<td>10.0</td>
<td></td>
<td>33</td>
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<tr>
<td>4/29/59</td>
<td>9.0</td>
<td></td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>2/6/61</td>
<td>11.1</td>
<td>2.82</td>
<td>35</td>
<td>30.2</td>
</tr>
<tr>
<td>2/14/61</td>
<td>10.8</td>
<td>2.99</td>
<td>35</td>
<td>32.0</td>
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<tr>
<td>5/10/61</td>
<td>9.7</td>
<td>2.72</td>
<td>33</td>
<td>23.8</td>
</tr>
</tbody>
</table>
Table 4.—Results of Autohemolysis Studies in Seven Patients with Pyruvate Kinase (PK) Deficiency Hereditary Nonspherocytic Hemolytic Anemia and in Subjects Considered to be Heterozygous for the PK Defect

<table>
<thead>
<tr>
<th>Additive</th>
<th>Final Conc.</th>
<th>Number Studied</th>
<th>Mean</th>
<th>Case 1</th>
<th>Case 2*</th>
<th>Case 3</th>
<th>Case 4</th>
<th>Case 5</th>
<th>Case 6</th>
<th>Case 7</th>
<th>Heterozygotes</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>16</td>
<td>1.7</td>
<td>13.1</td>
<td>1.8</td>
<td>9.0</td>
<td>17.2</td>
<td>44.0</td>
<td>11.2</td>
<td>4.4†</td>
<td>16</td>
<td>2.7</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.026M</td>
<td>17</td>
<td>16.7</td>
<td>1.5</td>
<td>3.4</td>
<td>3.9</td>
<td>48.2</td>
<td>8.1†</td>
<td>5.0†</td>
<td>16</td>
<td>0.2</td>
</tr>
<tr>
<td>Adenosine</td>
<td>0.02M</td>
<td>17</td>
<td>18.5</td>
<td>0.6</td>
<td>6.9</td>
<td>1.0†</td>
<td>34.3</td>
<td></td>
<td></td>
<td>14</td>
<td>0.2</td>
</tr>
<tr>
<td>AMP</td>
<td>0.02M</td>
<td>12</td>
<td>1.4</td>
<td>0.5</td>
<td>1.9</td>
<td>0.6†</td>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>0.3</td>
</tr>
<tr>
<td>ADP</td>
<td>0.02M</td>
<td>15</td>
<td>0.7</td>
<td>0.1</td>
<td>1.2</td>
<td>0.4†</td>
<td>4.0†</td>
<td></td>
<td></td>
<td>11</td>
<td>0.2</td>
</tr>
<tr>
<td>ATP</td>
<td>0.02M</td>
<td>17</td>
<td>1.2</td>
<td>0.2</td>
<td>1.0</td>
<td>0.5</td>
<td>1.9</td>
<td>1.0†</td>
<td>0.4†</td>
<td>12</td>
<td>0.2</td>
</tr>
<tr>
<td>DPN</td>
<td>0.02M</td>
<td>12</td>
<td>1.8</td>
<td>0.3</td>
<td>1.6</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>0.3</td>
</tr>
<tr>
<td>Coenzyme A†</td>
<td>5</td>
<td>0.2</td>
<td>3.6</td>
<td>1.1</td>
<td>2.0</td>
<td>1.9†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSH</td>
<td>0.02M</td>
<td>8</td>
<td>8.7†</td>
<td>0.4</td>
<td>2.6*</td>
<td>1.2†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>TPN</td>
<td>0.02M</td>
<td>3</td>
<td>4.8</td>
<td></td>
<td></td>
<td>0.5†</td>
<td>5.6†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Our normal values are shown for comparison. The per cent hemolysis in the patients is the average of the values obtained in the duplicate bottles unless otherwise indicated.

*Patient not anemic at time of study, due to compensation of mild hemolytic process.
†Single bottle.
‡1.6 mg. per bottle of coenzyme A from yeast, 70–75% (Sigma Chemical Co.).
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Fig. 3.—Case 2. Photomicrograph of peripheral blood smear. This patient has not been splenectomized. (reduced from x640).

weight was 118 lbs. There was no significant lymphadenopathy. The liver was palpable 7 cm. below the right costal margin.

Her hemoglobin has ranged between 8 and 10 Gm. per cent with transfusions. There has been a slight leukocytosis (postsplenectomy), but the differential count has been normal. Reticulocytes have usually ranged between 10 and 45 per cent. The platelets have been increased (postsplenectomy). Hematologic data obtained at the time of the enzymatic studies are summarized in table 2. The red cells on the stained peripheral smear were moderately macrocytic and normochromic with slight anisocytosis. There was moderate polychromasia. A few target cells were present. Many of the cells contained Pappenheimer bodies. An occasional irregularly contracted red cell was present. Normoblasts were rare. The red cell osmotic fragility was normal. The total serum bilirubin was 1.6 mg. per cent with 1.3 mg. per cent being indirect reacting. Serum iron was 216 μg. per cent with a total iron binding capacity of 233 μg. per cent. Bone marrow aspirations have revealed marked erythroid hyperplasia and hemosiderosis. The acid serum (Ham’s) test for paroxysmal nocturnal hemoglobinuria was negative.

The patient’s own red cells tagged by the Cr51 method exhibited a markedly shortened survival time of three days. The plasma clearance of Fe59 (T1/2) was 38 minutes. Incorporation of Fe59 into red cells reached a sharp peak of 61 per cent on the fourth day, followed by a rapid fall, consistent with marked hemolysis. The results of the autohemolysis studies are summarized in table 4.

Case 5

S. H., a 5 year old Caucasian girl, had been jaundiced at birth, requiring an exchange transfusion four hours after birth. There was no ABO or Rh incompatibility. In early infancy, she required further transfusions about once a month in order to maintain her hemoglobin above 4 to 5 Gm. per cent. At the age of 5 months, a splenectomy was performed for hemolytic anemia, with a decrease in her transfusion requirements, although her hemoglobin has remained in the range of 7 to 9 Gm. per cent. She has not been transfused during the past 11 months.
Her father was of English-Dutch ancestry, and her mother was English-Scottish, with no history of anemia on either side of the family. The hemograms of the father and mother were normal. Both had hemoglobin patterns of AA by paper electrophoresis and fetal hemoglobin within the normal range. The patient's only sibling, a young sister, is described below as Case 6.

Physical examinations have not been remarkable except for intermittent scleral icterus. With the exception of the first few months of life, she has maintained a hemoglobin level between 7.1 and 9.0 Gm. per cent, a marked reticulocytosis between 27 and 64 per cent, and normal platelet counts. The hematologic values obtained at the time of the enzymatic studies and some of the other significant data are summarized in table 2. The results of the autohemolysis studies are shown in table 4. In the peripheral blood smear the red cells were round, normochromic, and slightly macrocytic with some anisocytosis. There was marked polychromasia. A moderate number of normoblasts and cells with basophilic stippling were present. A few irregularly contracted cells of various sizes were also noted. An occasional cell contained Howell-Jolly bodies. Pappenheimer bodies were present in many of the red cells. There was no spherocytosis.

Case 6

K. H., a 2 year old Caucasian girl (born July 6, 1959, and younger sister of S. H., Case 5), was transfused three times during the first three months of her life, and has subsequently required three more transfusions. Her last transfusion (100 ml.) was given on April 7, 1961, and our enzymatic studies were performed on May 31, 1961.

Physical examinations during the past two years have revealed intermittent icterus, no hepatomegaly or lymphadenopathy, and a spleen occasionally palpable at the left costal margin.

Her hemoglobin values have ranged between 4.3 and 7.7 Gm. per cent during the past year, the reticulocyte counts have been between 6.9 and 20.6 per cent, and white blood counts between 13,800 and 15,200 per cu. mm. The hematologic values obtained at the time of the enzymatic studies are summarized in table 2 and the results of the autohemolysis studies are shown in table 4. The erythrocytes in the peripheral smear were of variable size, ranging from normocytes to macrocytes. This was probably due to her recent blood transfusion. There was moderate polychromasia. A few nucleated red cells and erythrocytes with basophilic stippling were present. There was slight poikilocytosis. No spherocytes or cells containing Pappenheimer bodies were noted.

Case 7

M. M., a 2 year old Mexican girl, was born on June 2, 1959, with a birth weight of 6 lbs. 4½ ounces. The following day she became jaundiced and the spleen was palpable 1 cm. below the left costal margin. Laboratory studies demonstrated the following: hemoglobin, 11.6 Gm. per cent; PCV, 40 per cent; serum bilirubin, 21.8 mg. per cent; Coombs test negative; and 226 normoblasts per 100 white blood cells. The bilirubin decreased to 3.8 mg. per cent on June 8, 1959. She received her first blood transfusion on July 9, 1959, and has subsequently been transfused about 10 times. At present she is requiring transfusions every 6 to 8 weeks. She has been treated elsewhere with parenteral iron, vitamin B₁₂, and folic acid without hematologic response.

The family history was noteworthy. Her parents and grandparents were Mexican with no mixture with European stock. The first husband of the patient’s mother had died in Mexico at age 34 of an unknown cause. There was no history of anemia in the parents, maternal grandparents, five older siblings, and two half-siblings, or in the numerous other relatives. Complete hematologic studies and enzymatic assays were performed on both parents, all the siblings, and some of the other relatives, and these are mentioned later under Family Studies.

Physical examination revealed no icterus or lymphadenopathy. At age 2½ years, she weighed 25 pounds and was 33½ inches in height. The liver was palpable 2 cm. below the right costal margin and the spleen was palpable 3 cm. below the left costal margin.
Laboratory studies have included the following: hemoglobin values have ranged between 3.7 and 7.1 Gm. per cent and PCV between 15 and 19 per cent when determined at intervals not immediately following transfusions. Reticulocyte counts have been between 3.0 and 9.4 per cent, with most values around 4 per cent. Erythrocyte osmotic fragility, white blood counts, and platelet counts have been normal. Bone marrow examination has shown normoblastic erythroid hyperplasia. The hemoglobin pattern was AA by paper electrophoresis. Fetal hemoglobin was 1.8 per cent. The hematologic values obtained at the time of the enzymatic studies are summarized in table 2 and the results of the autohemolysis studies are shown in table 4. These studies were performed 53 days after her last transfusion. In the peripheral smear the red cells were generally normocytic but with macrocytes also present. Moderate anisocytosis and slight poikilocytosis and polychromasia were present. No cells containing Pappenheimer bodies were noted. There were no spherocytes.

RESULTS

1. Summary of Data on Patients

A summary of some of the clinical data of the seven subjects with pyruvate kinase (PK) deficiency hereditary nonspherocytic hemolytic anemia is shown in table 1. The significant hematologic data obtained at the time of the enzyme assays are included in table 2.

2. Autohemolysis Studies

The results are summarized in table 4. Our normal mean is given for each type of addition compound utilized. It is to be noted that in contrast to the normals there is moderate to marked autohemolysis in all the patients except one (R. C.), who was not anemic at the time of the study. The increased autohemolysis was not definitely corrected by glucose or adenosine, but was corrected by ATP as well as by AMP and ADP. Glucose, however, did decrease autohemolysis in Cases 3 and 4 to some extent. The corrective effect of adenosine, though not optimal, in Cases 2 and 4 is to be noted. DPN, coenzyme A, GSH, and TPN also decreased autohemolysis by variable degrees. In the extreme right column are shown the results (mean values) of the studies in subjects (relatives of patients) considered to be heterozygous for pyruvate kinase deficiency. It is evident that the heterozygotes have normal autohemolysis studies except for the possibility of minimally increased autohemolysis of whole blood alone.

3. Pyruvate Kinase Assays

a. Results in normal subjects: Assays were performed on 40 normal individuals (34 males, 6 females) ranging in age from 4 to 65 years. The mean, standard deviation, and range of pyruvate kinase activity for both erythrocytes and leukocytes are shown in table 5. The results of two apparently normal subjects were excluded from this series and are mentioned below in section c.

b. Results in seven patients clinically conforming to Type II congenital nonspherocytic hemolytic anemia: The results of the pyruvate kinase assays (performed in duplicate in all cases) are summarized in table 5. It is apparent that all patients possess very little pyruvate kinase activity in their red cells compared to normal subjects. No detectable activity was observed in the red cells of J. L. and S. H. under our assay conditions. It is to be noted that the leukocytes have normal activity. Patients J. L., R. C., H. C. and S. H. had not
### Table 5.—Summary of Pyruvate Kinase Assays on Erythrocytes and Leukocytes of Normal Individuals, Patients with Pyruvate Kinase (PK) Deficiency Hereditary Nonspherocytic Hemolytic Anemia, and Subjects Considered to be Heterozygous for the PK Defect

<table>
<thead>
<tr>
<th>Subject</th>
<th>RBC</th>
<th>WBC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number Studied</td>
<td>Mean</td>
</tr>
<tr>
<td>Normals</td>
<td>40</td>
<td>2.65</td>
</tr>
<tr>
<td>Heterozygotes</td>
<td>34</td>
<td>1.19</td>
</tr>
<tr>
<td>Case 1 J. L.</td>
<td>0.00</td>
<td>729</td>
</tr>
<tr>
<td>Case 2 R. C.</td>
<td>0.18</td>
<td>697</td>
</tr>
<tr>
<td>Case 3 H. C.</td>
<td>0.14</td>
<td>627</td>
</tr>
<tr>
<td>Case 4 E. T.</td>
<td>0.47</td>
<td>966</td>
</tr>
<tr>
<td>Case 5 S. H.</td>
<td>0.00</td>
<td>670</td>
</tr>
<tr>
<td>Case 6 K. H.</td>
<td>0.81</td>
<td>621</td>
</tr>
<tr>
<td>Case 7 M. M.</td>
<td>0.83</td>
<td>638</td>
</tr>
</tbody>
</table>

*Expressed in terms of arbitrary units. One unit = that activity resulting in the conversion of one micromole of DPNH to DPN per minute by 10¹⁰ erythrocytes or 10¹⁰ leukocytes at 37 C. under the assay conditions of these experiments.

†S.D. = Standard deviation.

‡Value of single assay shown, but two or more assays were performed in each case.

§Except for single value of 0.35.

been transfused for at least 11 months prior to the time of study. Patient E. T. had received a series of transfusions 90 to 100 days prior to assay. Patient K. H. had been transfused 54 days prior to assay, and hence her true pyruvate kinase activity is probably lower than 0.81. This is also the situation in patient M. M. with a red cell PK value of 0.83. She has been transfused regularly, her last transfusion being 53 days prior to assay. It is significant that the lowest pyruvate kinase values occurred in those patients who were known to be free of transfused cells.

c. Results in heterozygous subjects: In the course of family studies, 34 subjects (21 males, 13 females, ranging in age from 2 to 59 years) had red cell pyruvate kinase values ranging from 0.63 to 1.73, except for a single value of 0.35. The mean was 1.19 (table 5). It is to be noted again that the leukocytes have normal pyruvate kinase values.

These red cell PK values appear to be consistent with heterozygosity for the defect, when considered together with family data to be discussed later. The highest value of 1.73 in this group was arbitrarily selected on the basis that it represented the highest value among those who presumably must be heterozygous, that is, parents or children of the patients, or parents of children with unquestionably low PK values. Employing this upper limit for heterozygotes, three members of PK deficiency kindreds were arbitrarily excluded from classification as heterozygotes, although they had values of 1.77, 1.82 and 1.82, and these were below our lower limit (2.00) of normal range. During the determinations of PK values in normal subjects, two of 42 apparently normal individuals had values in this border zone. They may represent the lower limit of normal range, but were also arbitrarily excluded from our normal series. Family studies in one such case revealed segregation of family members
PK DEFICIENCY ANEMIA

into those with apparently normal PK values and those with border zone values. At the present time, a clear distinction between heterozygosity and low normal values is not possible with precision in certain instances. The status of subjects with borderline values is therefore unclear.

Although three of the patients had pyruvate kinase activities greater than 0.35, these three patients were the only ones not entirely free of transfused red blood cells. While surveying a variety of diseases (over 200 patients), we have encountered a patient with acute lymphoblastic leukemia with a red cell PK value of 1.66 and a patient with acute monocytic leukemia with a red cell PK value of 1.44, and who thus appear to have heterozygosity for PK deficiency as an incidental finding.

d. Results in hematologic diseases: Pyruvate kinase assays were performed in over 100 patients with a variety of hemolytic as well as other hematologic disorders. In every case the red cell pyruvate kinase value was normal or increased. (The only exceptions have already been mentioned above.) In fact, the values were usually moderately to markedly elevated in the patients with a hemolytic process such as hereditary spherocytosis (presplenectomy), sickle cell anemia, acquired hemolytic anemia, etc. Representative data are shown in table 6. The leukocytes were always assayed, but results are not shown.

e. Results in nonhematologic diseases: Pyruvate kinase activity was normal or increased in the erythrocytes from patients with a variety of nonhematologic diseases, such as diabetes mellitus, myxedema, essential hypertension, cerebral thrombosis, hypogonadism, pancreatitis, Laennec’s cirrhosis, duodenal ulcer, and rheumatic heart disease. Pyruvate kinase activity was increased in cord blood.

4. Results of Other Glycolytic and Non-Glycolytic Enzyme Assays in the Red Cells

a. In the seven patients: Total triose production from either glucose or glucose-6-phosphate, the rate of conversion of fructose-1, 6-diphosphate or triose esters to 1,3-diphosphoglycerate, and activity of 3-phosphoglycerate-1-kinase, 2,3-phosphoglycerate mutase, enolase, lactic dehydrogenase, glucose-6-phosphate dehydrogenase, and 6-phosphogluconic dehydrogenase were normal or increased (usually the latter) in the red cells of all seven patients. The non-glycolytic enzymes, cholinesterase, arginase, argininosuccinase, fumarase, acid phosphatase, and glyoxalase were assayed in most of the patients and the activity of all these enzymes was found to be normal or increased. Erythrocyte cholinesterase values, for example, were 189, 133, 155, 143, 186 and 127 per cent of normal for Cases 1 through 6 respectively.

b. In the heterozygotes for PK deficiency: The red cells of 15 presumably heterozygous individuals from four kindreds for PK deficiency were assayed for other glycolytic and non-glycolytic enzymes as listed above for the patients. The results were within normal limits except for one individual with concurrent iron deficiency anemia, who had increased activity in most of the glycolytic enzymes. The erythrocytes of 19 other heterozygotes were assayed for some, but not all, of the glycolytic enzymes and a few of the non-glycolytic enzymes. The results were within normal limits.
Table 6.—Representative Data of Red Cell Pyruvate Kinase Assays in Hematologic Diseases

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>PK activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normals (mean and standard deviation)</td>
<td>2.65 ± 0.34</td>
</tr>
<tr>
<td>Hereditary spherocytosis: Case 1</td>
<td>5.68</td>
</tr>
<tr>
<td>Case 2</td>
<td>3.48</td>
</tr>
<tr>
<td>Case 3</td>
<td>4.12</td>
</tr>
<tr>
<td>Case 4†</td>
<td>2.67</td>
</tr>
<tr>
<td>Case 5†</td>
<td>2.20</td>
</tr>
<tr>
<td>Sickle cell anemia: Case 1</td>
<td>6.75</td>
</tr>
<tr>
<td>Case 2</td>
<td>4.79</td>
</tr>
<tr>
<td>Sickle cell hemoglobin C disease</td>
<td>3.22</td>
</tr>
<tr>
<td>Paroxysmal nocturnal hemoglobinuria</td>
<td>5.84</td>
</tr>
<tr>
<td>Nonspherocytic hemolytic anemia due to G-6-PD deficiency</td>
<td>6.48</td>
</tr>
<tr>
<td>Nonspherocytic hemolytic anemia, unknown type†</td>
<td>5.13</td>
</tr>
<tr>
<td>Acquired hemolytic anemia</td>
<td>3.43</td>
</tr>
<tr>
<td>Hereditary elliptocytosis, non-anemic</td>
<td>2.92</td>
</tr>
<tr>
<td>Negro with G-6-PD deficiency, non-anemic</td>
<td>2.43</td>
</tr>
<tr>
<td>Thalassemia minor</td>
<td>3.68</td>
</tr>
<tr>
<td>Iron deficiency anemia</td>
<td>4.54</td>
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<tr>
<td>Nutritional megaloblastic anemia</td>
<td>6.90</td>
</tr>
<tr>
<td>Pernicious anemia in relapse</td>
<td>6.97</td>
</tr>
<tr>
<td>Polycythemia rubra vera: Case 1</td>
<td>3.90</td>
</tr>
<tr>
<td>Case 2</td>
<td>2.46</td>
</tr>
<tr>
<td>Agnogenic myeloid metaplasia</td>
<td>2.95</td>
</tr>
<tr>
<td>Chronic granulocytic leukemia</td>
<td>3.40</td>
</tr>
<tr>
<td>Acute myeloblastic leukemia</td>
<td>2.39</td>
</tr>
<tr>
<td>Chronic lymphocytic leukemia</td>
<td>2.74</td>
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<tr>
<td>Refractory anemia</td>
<td>2.60</td>
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<tr>
<td>Hodgkin’s disease</td>
<td>3.09</td>
</tr>
</tbody>
</table>

*See table 5 for definition of unit of pyruvate kinase activity.
†Splenectomized.
‡Glucose-6-phosphate dehydrogenase.

5. Special Studies on Erythrocytes of Patient J. L.

a. Repetitive sampling: Pyruvate kinase assays on six separate blood specimens obtained over a three month period consistently demonstrated no activity in the erythrocytes. The leukocytes had normal activity on each occasion.

b. Addition of crystalline pyruvate kinase: When crystalline rabbit muscle pyruvate kinase* was added in increasing amounts to our usual assay system containing J. L.’s hemolysate, corresponding increments in PK activity were measurable. Addition of about 0.3 Bücher unit of pyruvate kinase produced activity approximately equivalent to normal erythrocytes, while 0.5 Bücher unit yielded activity above normal. Comparable increases in PK activity occurred when crystalline pyruvate kinase was added to assay systems containing hemolysates from normal individuals. These experiments demonstrate that the lack of activity with J. L.’s hemolysate is correctible by the addition of crystalline pyruvate kinase and no inhibitory substance could be demonstrated by this

*Obtained from California Corporation for Biochemical Research, Los Angeles, Calif.
procedure. Similar results were obtained with cells of R. C., H. C., and S. H.

c. Incubation experiments: Incubation for 20 hours at 37 C. of normal red cells with J. L.'s serum and J. L.'s red cells with normal serum did not affect the respective cellular enzyme activities.

d. Mixture experiments: Mixtures (without preincubation or with incubation for 30 minutes at 37 C. prior to assay) of varying proportions of J. L.'s hemolysate with the hemolysate of a normal individual or with the hemolysate of a patient with sickle cell anemia and elevated erythrocyte PK activity yielded activities which were essentially the algebraic sum of the activities of the separate specimens. These experiments provide no evidence for the presence of an inhibitor of PK in J. L.'s red cells. Similar results were obtained with cells of R. C.

e. Freezing and thawing: Rapid freezing and thawing of J. L.'s red cells, as well as the erythrocytes of his mother and father (homozygotes), for as many as 25 times did not alter the subnormal enzyme activity. Similar results were obtained with cells of R. C. and H. C. The PK activity of normal red cells or of cells with high activity was unchanged by this procedure.

f. Heating of erythrocytes: J. L.'s red cells were heated at 56 C. for one hour. Subsequent hemolysates of these cells on mixture with normal hemolysates did not affect the PK activity of the latter. In similar experiments, the PK activity of J. L.'s fresh hemolysate was not affected by the addition of a hemolysate prepared from heated red cells of a patient with sickle cell anemia.

g. Change in assay conditions: Assays of J. L.'s red cells employing various concentrations of ADP, MgSO4, and KCl did not increase the red cell PK activity.

6. Other Special Studies

a. Assays on top and bottom layers of cells: After centrifugation of J. L.'s red cell-rich suspension at 2000 g for 30 minutes at 4 C., theuffy coat was discarded, the top and bottom thirds resuspended in physiologic saline and processed as previously described in regard to red cell, white cell, and reticulocyte counts. Normal red cells and red cells with increased PK activity (sickle cell anemia) were similarly prepared. As would be expected, reticulocyte counts were higher in the top layer of packed cells in all three individuals (table 7). The PK activity, however, was still zero in J. L.'s top layer cells, whereas considerably higher activity was present in the top layer cells of the other two individuals (table 7). These results indicate that PK activity is higher in the normal young red cell or in young cells of such diseases as sickle cell anemia, but even the youngest cells of J. L. did not demonstrate measurable PK activity in our dilute hemolysate system. However, similar experiments in patients R. C. and H. C. demonstrated that their top layer cells have greater PK activity than bottom layer cells, though all the values were still very low. In similar experiments with the red cells of J. L.'s father, mother, daughter, son, and brother R. (heterozygotes), the top layer of cells had greater activity than the bottom layer or the usual red cell suspension. However, the PK values of the top layer of cells in the heterozygotes were still below the lower limit of normal range.
b. **Glucose utilization studies:** The red cells of patient J. L. with 15.2 per cent reticulocytes utilized glucose at the same rate as normal blood with 0.2 per cent reticulocytes. In the same experiment, cells of a patient with sickle cell anemia with 6.9 per cent reticulocytes utilized glucose much more rapidly. In similar experiments, the cells of patient H. C. with 12.3 per cent reticulocytes utilized glucose at about the same rate as the control normal blood with 0.9 per cent reticulocytes. Patient R. C. with 4.5 per cent reticulocytes utilized glucose slightly better than his brother H. C. These results indicate that these red cells deficient in pyruvate kinase activity were unable to utilize glucose at the normal rate.

7. **Family Studies**

Forty-three members of the large family of J. L. were studied (fig. 4). Complete blood counts, including reticulocyte counts, were normal in the family members, except for the finding of iron deficiency anemia in J. L.'s oldest sibling, who had had a subtotal gastrectomy for peptic ulcer. As may be seen in figure 4, J. L.'s father (PK value of 1.23) and mother (1.41) and J. L.'s two children (1.47 and 1.36) had PK values consistent with heterozygosity for the defect. Altogether there were 22 members who appeared to be heterozygotes and 12 blood relative members who apparently had normal PK activity in their erythrocytes. The mean, standard deviation, and range of PK values of these two groups are shown in table 8 in comparison with the PK values of apparently normal persons from the general population. Two individuals in this kindred had PK values of 1.77 and 1.82, which were considered to be in the borderland between apparent heterozygotes and normals. Thus they were not included in the tabulation above.

None of the heterozygotes demonstrated any clinical evidence of disease. The results of the assays for other enzymes and autohemolysis studies performed in many, but not all, of the heterozygotes were normal, except for the results in one subject with concomitant iron deficiency anemia as already mentioned. All six non-blood relative members of kindred L. (spouses of siblings of J. L.) had normal PK values ranging from 2.02 to 2.89 with a mean of 2.50.

Members of kindred C. (fig. 4) were not available for study, but the father, mother, and older brother of the patients (R. C. and H. C.) apparently had no disease similar to that of the patients and were said not to be anemic.

As portrayed in figure 4, family members of Kindreds H., T., and M. were studied. Persons who were found to have PK values consistent with heterozygosity for the defect were clinically in good health and had normal hemograms.
Fig. 4.—Graphic representation of erythrocyte pyruvate kinase deficiency in five kindreds. The propositi have pyruvate kinase (PK) deficiency hereditary non-spherocytic hemolytic anemia. The squares represent males; the circles, females.

in all instances, except for one subject with slight iron deficiency anemia. Assays for glycolytic enzymes (other than PK) and for nonglycolytic enzymes as well as autohemolysis studies, when performed, were within normal limits in these heterozygotes. There was considerable variation in PK values among the heterozygotes. For example, in kindred H., the heterozygote PK values were 0.91 and 0.84; in kindred T., 1.51 and 1.55; and in kindred M. the father and mother had PK values of 1.22 and 1.24, respectively, but the PK values among the five heterozygous children ranged from 0.63 to 1.18 with a mean of 0.80. In addition, the uncle of the patient had a very low value of 0.35.

It may be seen in figure 4 that PK deficiency hereditary nonspherocytic hemolytic anemia appears to result from homozygosity for an autosomal defective gene, with heterozygotes having an enzymatically detectable but a
Table 8.—A Comparison of Pyruvate Kinase Activity in the Erythrocytes of Members of Kindred L. Believed to be Heterozygous for Erythrocyte Pyruvate Kinase Deficiency with That of Apparently Normal Blood Relative Members of Kindred L. and Normal Persons in the General Population

<table>
<thead>
<tr>
<th>Subject</th>
<th>Number Studied</th>
<th>Pyruvate Kinase Activity*</th>
<th>% of Normal Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normals</td>
<td>40</td>
<td>2.65 ± 0.34</td>
<td></td>
<td>2.00–3.40</td>
</tr>
<tr>
<td>Heterozygotes (kindred L.)</td>
<td>22</td>
<td>1.32 ± 0.26</td>
<td>49.8</td>
<td>0.73–1.73</td>
</tr>
<tr>
<td>Normals (kindred L.)</td>
<td>12</td>
<td>2.89 ± 0.34</td>
<td>109.1</td>
<td>2.16–3.51</td>
</tr>
</tbody>
</table>

*See table 5 for definition of unit of pyruvate kinase activity.  
†Standard deviation.

clinically undetectable abnormality. There was no history of consanguinity in any of these kindreds.

DISCUSSION

Clinical and Hematologic Features

The clinical and hematologic features of Type II congenital nonspherocytic hemolytic anemia have been recently reviewed by de Gruchy et al. who collected seven cases from the literature and added three cases. Our seven cases generally conform to this pattern, but illustrate certain facets of this disease which merit emphasis or review. The age range and sex incidence of our patients (2 to 38 years; 3 males, 4 females) are similar to these cases previously reported (3 to 42 years; 5 males, 5 females). In spite of a moderately severe hemolytic anemia in most instances, survival to adulthood apparently is common. Although the majority of our patients had their onset of disease in infancy or childhood, two patients had minimal, if any, symptoms prior to the discovery of an anemia or icterus as an incidental finding at ages 19 and 21. Previous cases of congenital nonspherocytic hemolytic anemia have occurred mostly in persons of British or Northern European stock. Six of our seven patients had such an ancestral background. Our Case 7, however, appears to be the first patient with a Mexican ancestry.

There was considerable variation in the severity of the hemolytic process, degree of anemia, and transfusion requirements. Three of our four adult patients are leading active lives without transfusions at present, while Case 7 requires frequent transfusions to maintain a packed red cell volume above approximately 15 per cent. Case 1 has had only one transfusion (at time of splenectomy), whereas Case 4 has required over 150 transfusions to date. Our Case 2 has never been transfused and has a compensated hemolytic process at present (PCV, 45 per cent), but apparently had what might be termed a crisis during two attacks of cholecystitis, when his hemoglobin fell to as low as 7.6 Gm. per cent.

Icterus was an inconstant feature, but splenomegaly of slight to moderate degree was the rule. Splenectomy in four cases has not significantly abated the
hemolytic process, though some benefit may have been achieved in two individuals. Unfortunately, detailed reports of the histology of the spleens were not available, since the splenectomies were performed elsewhere. However, in Case 1 the histologic features were considered consistent with congestive splenomegaly while in Case 4 the spleen apparently revealed "reticulum cell hyperplasia with excessive hemosiderin."

Hematologically, our patients generally demonstrated a fairly uniform macrocytosis of moderate degree (consistent with reticulocytosis) without spherocytosis. The cells were normochromic with only slight anisocytosis and poikilocytosis. It should be noted that only one of the ten previously reported cases in the literature has had a presplectectomy description of the red cell morphology. Certain features such as numerous Pappenheimer bodies and siderocytes, Howell-Jolly bodies, and target cells previously described appear to be the result of splenectomy. There were no striking morphologic features in the erythrocytes of our three patients who have not been splenectomized. The degree of reticulocytosis varied widely in our patients. The white blood counts and platelet counts were either normal or compatible with postsplenectomy changes. The blood types of the patients were consistent with the incidence of the respective types in the general population. Red cell survival as determined with Cr was markedly shortened (6.5 days in Case 1, 3 days in Case 4).

**Autohemolysis Studies**

The autohemolysis studies are of interest. The degree of autohemolysis varied considerably and appeared to correlate to some extent with the clinical severity of the disease. It is of interest that the only patient with normal autohemolysis also was not anemic at the time of study. Although the increased autohemolysis of Type II cases is usually not correctible by additions of glucose or adenosine, two of our patients showed some decrease in autohemolysis with glucose and one of these two patients also demonstrated correction with adenosine. (One of de Gruchy’s Type II patients also showed correction of autohemolysis with adenosine.) The data emphasize the fact that incubation studies, while valuable, may exhibit variability in different patients with the same fundamental disorder.

As reported by de Gruchy et al., ATP essentially eliminated autohemolysis in all our subjects. It is of interest that ADP and AMP were virtually as effective as ATP in this regard. Furthermore, other compounds such as DPN, TPN, GSH, and coenzyme A also partially correct the increased autohemolysis. The mechanism by which any of these compounds reduce hemolysis in the red cell autohemolysis test is not certain. However, it is possible that they act in some way as an energy source as suggested for ATP by de Gruchy and associates. Since ATP does not easily penetrate the erythrocyte membrane, the added ATP probably first undergoes hydrolysis to ADP by the action of the phosphatases of the plasma. The ADP thus formed may then be converted in the erythrocyte membrane to ATP and AMP by adenylate kinase. Some of the ATP enters the cell and reconstitutes, at least in part, the ATP sup-
ply of the cell and thus may reduce autohemolysis. This explanation would be consistent with the results obtained by our additions of ATP, ADP, and AMP, and with our finding of pyruvate kinase deficiency in the erythrocytes of these patients. It may be recalled that the pyruvate kinase step results in the regeneration of ATP.

Pyruvate Kinase Assay and Other Studies

The data clearly demonstrate for the first time a specific defect in the glycolytic enzyme pyruvate kinase in the erythrocytes of patients conforming to Type II congenital nonspherocytic hemolytic anemia. This enzyme catalyzes the conversion of phosphoenolpyruvate to pyruvate. In sharp contrast to the normal red cell PK values, all seven patients had very low red cell pyruvate kinase activity. No detectable activity was observed in the red cells of two patients under our assay conditions. No absolute correlation between the severity of anemia and the precise activity of erythrocyte PK in the assay procedure was evident. The low values obtained by our assay conditions probably preclude such a correlation. Also, other variables such as differences in capacity for compensation together with unknown factors may be of importance in this regard.

It has been possible by the PK assay to differentiate individuals with normal red cell PK activity from those persons who are clinically well and have normal hemograms, but who demonstrate subnormal PK activity in their erythrocytes. This finding emphasizes the increasing importance of the need to refine our methods of disease detection. Among these heterozygous individuals, there was considerable variation in activity, but the mean was about one-half that of normal subjects. In our series there was no overlap at the lower end of the heterozygous range, if transfused blood is taken into consideration in the patients. However, some of the apparent heterozygotes demonstrated moderately low PK values, and yet had no reticulocytosis or other evidence of disease. The data do not delineate the critical PK level at which hemolysis may occur.

It is of interest that the leukocytes in the patients (as well as heterozygotes) possess normal pyruvate kinase activity. The data in other diseases appear to indicate that leukocyte PK activity varies primarily with cell type and maturity. The leukocyte values at the lower range of normal in some of the patients are most likely due to an increased percentage of lymphocytes, which have lower activity than segmented neutrophils.

It is apparent from the data in a variety of hemolytic disorders that young red cell populations have increased, rather than decreased, PK activity. The presence of increased activity of many enzymes in erythrocytes of young mean cell age is well recognized. Patients with hereditary spherocytosis, for example, demonstrated increased activity if presplenectomy, but normal activity after splenectomy. Patients with nonspherocytic hemolytic anemia not conforming to Type II of Dacie have shown increased PK activity. The PK assay data to date in a variety of hematologic and nonhematologic disorders would appear to indicate that homozygosity for PK deficiency occurs only in patients
conforming to Type II of Dacie. However, it is not certain whether all patients in this category will be shown to have a PK defect, since the group may not represent a single entity.

The activity of hexose monophosphate shunt enzymes, glucose-6-phosphate and 6-phosphogluconic dehydrogenases, was increased (six cases) or normal (in one who had been transfused recently). These studies clearly exclude the present cases from those congenital nonspherocytic hemolytic anemias associated with glucose-6-phosphate dehydrogenase deficiency. A patient with the latter diagnosis exhibited increased red cell PK activity. Studies of the enzymatic steps involved in the Embden-Meyerhof pathway from glucose to lactate, as well as a number of non-glycolytic enzymes, have failed to disclose any abnormality other than pyruvate kinase deficiency in our patients. These data lend support to the premise that the PK defect is specific and is pathogenetically related to the disease, as will be discussed later.

Nature of Pyruvate Kinase Defect

Although no detectable PK activity was present in the red cells of two of the patients, these results cannot be literally interpreted to mean total absence of the enzyme since (1) dilute homogenates are employed for assay and 2) the experimental conditions may not be optimal. Furthermore, the data do not exclude the presence of an inhibitor or the absence of an activator, although several experiments to check these possibilities were negative. Also, the subnormal erythrocyte PK activity may be the result of one or more factors, such as destruction of the enzyme, reduced production, or synthesis of a qualitatively abnormal enzyme, in addition to the possibilities mentioned above. The pyruvate kinase defect was present in immature, as well as mature, erythrocytes of the patients, but to a lesser degree in the younger red cells. The heterozygotes have about one-half the normal activity and demonstrate greater activity in their immature cells (though still less than normal) than in their mature cells. Although we have not studied other body tissues, the fact that the leukocytes possess normal activity is another point in favor of a specific red cell enzyme defect at the molecular level.

Genetics

Previous data have been inadequate to establish definitely whether this disorder is genetically determined. de Gruchy and associates1 have suggested that a recessive mode of transmission was likely since the family histories in cases classified as Type II in the literature have usually been negative or, when positive, disease has been noted in siblings rather than in the parents or offspring. Our family studies have demonstrated the following: (1) PK deficiency anemia always occurred as a single case in a family or as multiple cases in siblings, but was never observed in parents or children of affected patients; (2) all (of those studied) parents and children of the patients exhibited erythrocyte PK activities well below those of the lowest normal subjects studied, and about one-half of the normal mean; (3) all 69 family
members studied could be separated into normals, apparent heterozygotes, and homozygotes (if transfused red cells were taken into consideration) on the basis of the erythrocyte PK assays, with the exception of three individuals whose PK values were in the borderland between heterozygous and normal values; (4) all members with PK values within the heterozygous range were parents or children of patients, or children of parents, one or both of whom had PK values within the heterozygous range; (5) none of the children of parents with normal PK values had PK values in the heterozygous range; (6) the mean pyruvate kinase activity of individuals regarded as presumably heterozygous was about one-half the normal mean; (7) all persons considered to be heterozygous for PK deficiency were clinically well and had normal hemograms (except for two subjects with iron deficiency anemia), including reticulocyte counts. These data provide strong evidence for a genetically determined disorder and suggest, of course, an autosomal recessive mode of transmission. The heterozygotes have a partial, but detectable enzyme deficiency not reflected in clinical disease. The patients presumably represent the homozygous state.

The family data clearly demonstrate that an heterozygous-homozygous relationship exists. However, it should be recognized that the upper limit of PK value for heterozygotes was arbitrarily selected, as defined earlier in this report, and that there is no assurance that a complete or accurate separation of some normals at the lower PK range from heterozygotes has been made or is possible by the enzyme assay technic utilized. At the present time, with our assay method, it appears that PK values in the range of approximately 1.70 to 1.90 constitute the border zone where there may be an overlap between normal and heterozygous values. It is also possible that an overlapping zone may exist between heterozygous and homozygous values.

It is to be noted that there are considerable variations in PK values in the apparent heterozygotes among families as well as within families. The mechanism underlying these differences is not clear.

It would appear from the literature that pyruvate kinase (PK) deficiency hereditary nonspherocytic hemolytic anemia is a rare disorder, only ten cases that conform to this type having previously been reported. The frequency of both the homozygous and heterozygous state, however, remains to be determined.

Pathogenesis

The data substantiate the previously suspected glycolytic defect and are in accord with the observation of Selwyn and Dacie of deficient glucose utilization by the erythrocytes of two of their cases. The data are also compatible with the demonstration of low erythrocyte ATP by Pranker and by de Gruchy and associates, and the accumulation during blood incubation studies of abnormally large amounts of certain phosphorylated intermediates of glycolysis reported for certain of their cases by de Gruchy and his colleagues.

It is generally recognized that the mature human red cell depends primarily upon glycolysis as its energy-producing mechanism. This energy is essential...
Fig. 5.—Schematic outline of the Embden-Meyerhof glycolytic pathway.

for the maintenance of its structural integrity and for a normal life span of the erythrocyte. For purposes of orientation, the Embden-Meyerhof glycolytic pathway is schematically depicted in figure 5. Since pyruvate kinase catalyzes a glycolytic step responsible for the regeneration of two moles of ATP from ADP for each mole of glucose traversing the step, it can be surmised that a pyruvate kinase deficiency of major degree should result in a serious disturbance in energy metabolism and impose a critical metabolic handicap on the affected erythrocytes. This can presumptively be considered to be related to their premature destruction in vivo. The disorder described would appear to represent another instance in which clinical disease results from a metabolic error, definable at the molecular, enzymatic level.

Classification of the Congenital Nonspherocytic Hemolytic Anemias

The term “congenital nonspherocytic hemolytic anemia” has been applied to a heterogeneous group of congenital hemolytic disorders. A precise classification has not been possible due to a lack of understanding of the pathogenesis of these diseases. The classification of Selwyn and Dacie into Type I and Type II on the basis of the autohemolysis test serves a useful purpose. However, it is anticipated that Type I cases, already recognized as a heterogeneous group, will be subdivided in the future when specific differences, possibly enzymatic in nature, are elucidated. Our patients with pyruvate kinase deficiency conform in all major respects to those previously reported in the literature as Type II, which appears to be a more homogeneous group. It is, of course, not certain that all cases in the literature having clinical and in vitro findings of Type II are necessarily homogeneous until these are specifically studied. Therefore, in the light of current knowledge, it is suggested that congenital nonspherocytic hemolytic anemias be classified as Type I or Type II according to the autohemolysis test, but that new catego-
ries be established as specific defects are discovered. In the latter group would be considered at this time (1) those patients with a deficiency of the erythrocytic enzyme, glucose-6-phosphate dehydrogenase, who have chronic hemolysis in the absence of drug therapy or ingestion of fava beans,[36-37] and (2) pyruvate kinase (PK) deficiency hereditary nonspherocytic hemolytic anemia. It is expected that this list will be enlarged in the future as the discovery of specific defects will allow more groups to be removed from the "waste basket" category of nonspherocytic hemolytic anemias.

**Summary**

1. The erythrocytes of seven patients conforming to the criteria of Type II congenital nonspherocytic hemolytic anemia have been demonstrated to have a specific deficiency in the glycolytic enzyme pyruvate kinase. Other glycolytic enzymes, glucose-6-phosphate and 6-phosphogluconic dehydrogenases, and certain non-glycolytic erythrocyte enzymes are normally active. The leukocytes of these patients possess normal pyruvate kinase activity.

2. Although no inhibitors were detected, the exact nature of the enzymatic defect remains to be elucidated.

3. Family studies provide strong evidence for a genetically determined disorder and are consistent with an autosomal recessive transmission of the defect. A partial enzyme deficiency, not reflected in clinical disease, is present in heterozygotes. The symptomatic disease, though variable in severity, appears to be due to homozygosity for the defect.

4. It is suggested that the enzyme deficiency is pathogenetically related to the premature demise of the red cells in vivo.

5. The name "pyruvate kinase (PK) deficiency hereditary nonspherocytic hemolytic anemia" is proposed for these patients.

**Summario in Interlingua**

1. Esseva demonstrate que le erythrocytos de septe patientes con anemias satisfacent le criterios del typo II de non-spherocytic anemia hemolytic hereditari esseva characterisate per un carentia specific del enzyma glycolytic cinase de pyruvato. Altere enzymas glycolytic—dishydrogenase de glucose-6-phosphato e dishydrogenase 6-phosphogluconic—e certe non-glycolytic enzymas erythrocytic manifestava un activitate normal. Le leucocytos de iste patientes manifestava un normal activitate de cinase de pyruvato.

2. Ben que nulle inhibitores esseva detegite, le exacte natura del defecto enzymatic remane a elucidar.

3. Studios familial supporta fortemente le these que il se tracta de un geneticamente determinate disordine. Lor resultatos es in congruentia con le postulato de un transmission recessive autosomal. Un partial carentia enzymatic, non reflectite in morbo clinic, es presente in subjectos heterozygotic. Le morbo symptomatic, ben que variabile in su severitate, pare esser causate per homozygotismo pro le defecto.

4. Es formulate le these que le carentia de enzyme es relationate pathogeneticamente con le morte prematur del erythrocytos in vivo.
5. Es proponite designar le morbo de iste patientes como “non-spherocytic anemia hemolytic hereditaria a carencia de cinase de pyruvato.”

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Pyruvate Kinase (PK) Deficiency Hereditary Nonspherocytic Hemolytic Anemia

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