Causal Mechanisms of Multiple Acquired Red Cell Enzyme Defects in a Patient With Acquired Dyserythropoiesis

By Axel Kahn, Dominique Cottreau, Catherine Boyer, Joelle Marie, Colette Galand, and Pierre Boivin

A patient with an unclassified form of acquired dyserythropoiesis was found to have multiple defects in erythrocyte enzyme activity, involving especially pyruvate kinase (PK), glucose phosphate isomerase (GPI), and phosphofructokinase (PFK). The PK activity defect was associated with a normal concentration of PK-related antigen, and the enzyme could be reactivated during the procedure of partial purification of the enzyme. The concentration of GPI-related antigen was as reduced as the GPI enzymatic activity, and the defect was not improved by any treatment (cross-incubation of red cells or treatment of the hemolysate by SH reagents); the residual enzyme had a normal stability to heat, and a normal electrophoretic and electrofocusing pattern. The PFK activity defect was not improved either by cross-incubation of red cells or by treatment with SH reagents. Immunologic data with antimuscle and antileukocyte antisera seemed to indicate that the defect involved especially the muscle-type subunit of erythrocyte PFK. In agreement with this assumption was the fact that deficient PFK was markedly more inhibited by ATP than normal enzyme. Changes similar to those of deficient PFK herein studied were noted for PFK of unfractionated erythrocytes from premature newborns or of “old” erythrocytes from full-term infants. It appeared that each of the three enzyme defects detected in the patient could be due to a different mechanism, involving post-translational changes, decreased synthesis, and possible reversion of the genetic regulation mechanisms of the abnormal erythroid precursors toward a fetal type. The possible relationships between these various phenomena and the nature of a hypothetical common underlying cause are discussed.

ACQUIRED DEFECTS in erythrocyte enzyme activity have been described in various blood disorders, especially in acute myeloid leukemias, smoldering leukemias, refractory anemias with or without abnormal sideroblastosis, dyserythropoiesis, and erythroleukemias.2 These defects involve mainly pyruvate kinase (PK), glucose phosphate isomerase (GPI), phosphofructokinase (PFK), adenylate kinase (AK), 2,3-diphosphoglycerate mutase, and glyceraldehyde-3-phosphate dehydrogenase.1,4 The mechanisms of such acquired enzyme defects remain a subject of discussion, and several hypotheses have been put forward to explain them, i.e., mainly partial reversion to a fetal form of erythropoiesis,5 the existence in some blood diseases of several qualitative and quantitative disturbances in gene expression,1,3,6,7 and, finally, the post-synthetic alteration of some enzymes in the malignant cells.8–11

From the Unité 24 de l’INSERM, ERA 573 du CNRS, Département d’Hématologie et Laboratoire Central d’Immuno-Hématologie, Hôpital Beaujon, Clichy, France.
Submitted April 24, 1976; accepted July 12, 1976.
Supported in part by Grant C.R.L. 75.4.164.2 from INSERM and a grant from the University of Paris-VII.
Address for reprint requests: Dr. A. Kahn, INSERM U24, Hôpital Beaujon, 92110 Clichy, France.
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This report describes the observation of a woman with an unclassified form of acquired dyserythropoiesis in whom a defect in the activity of three erythrocyte enzymes has been proved. By means of immunologic, electrophoretic, and kinetic methods we are able to suggest that each of the defects is due to a different direct mechanism. It has not been possible, as yet, to detect the common underlying phenomenon, if any, responsible for these enzyme abnormalities.

MATERIALS AND METHODS

Materials

The substrates and intermediate enzymes were furnished by Boehringer-Mannheim or Sigma CC. The ion exchangers came from Pharmacia, ampholines from LKB, acrylamide and bis-acrylamide from Eastman Kodak, Starch gel from Connaught, and agarose from l’Industrie Biologique Française. The gelatin solution used for the isolation of the leukocytes was Plasmagel, from Roger Bellon Laboratories.

The enzymatic assays were performed at 30°C in a Zeiss PMQ II spectrophotometer connected to a Servogor recorder.

Methods

Blood samples. Venous blood obtained from normal healthy volunteers and from the patient, and cord blood obtained from premature or full-term newborn babies, was anticoagulated with heparin. Leukocytes and platelets were removed by filtration through a mixture of sulfoethylcellulose-Sephadex G-25. In some experiments, erythrocytes from adults and infants were fractionated in ‘‘young’’ and ‘‘old’’ cells by centrifugation in microhematocrit tubes, according to Herz et al. Reticulocyte count was as follows: in adults (n = 10), 6.65 ± 2 in young cells and 0.22 ± 0.14 in old cells; in infants (n = 8), 24.8 ± 8.8 in young cells and 0.73 ± 0.061 in old cells. In order to avoid all contamination by leukocytes, the ‘‘young fractions’’ of erythrocytes were further filtrated through a cellulose column, according to Beutler. Isolation of leukocytes was performed by the usual methods of sedimentation in isotonic gelatin solution and differential centrifugations.

Enzyme activity assays. In the present study, the enzyme activities were measured according to the methods summarized by Beutler; the results were expressed in international units per gram of hemoglobin at 30°C. The erythrocyte enzyme activities determined in 1971 were measured according to other methods summarized in Refs. 2 and 4; the results were expressed in IU/100 ml of red blood cells at 25°C. In the partially purified preparations, pyruvate kinase activity was expressed in IU/mg of proteins; proteins were measured according to Lowry et al. Studies of the influence of incubation and dialysis upon enzyme activity. Attempts to restore the activity of the deficient enzymes were made by incubating the patient’s red cells in isologous plasma from a healthy donor for 4 hr at 37°C and by incubating or dialyzing the patient’s hemolysate under various conditions: for PK, incubation for 2 hr at 37°C in the presence of 10 mM dithiothreitol and 0.1 mM 1-6 fructose diphosphate and dialysis for 2 hr at 4°C against a 50 mM Tris-Cl buffer, pH 8, containing 500 mM sucrose, 10 mM β-mercaptoethanol, 1 mM EDTA, and 1 mM ε-aminocaproic acid. For PFK and GPI, incubation of hemolysate was performed in the presence of 10 mM β-mercaptoethanol 1 mM AMP, and 0.1 mM fructose 6-phosphate; the dialysis was performed against a Tris-Cl buffer, pH 8, containing 10 mM dithiothreitol 1 mM AMP, 0.1 mM fructose 6-phosphate, 1 mM EDTA, and 1 mM ε-aminocaproic acid. Enzyme activities and hemoglobin concentrations were measured again after these various treatments.

Immunologic titrations. Pyruvate kinase-antigen concentration was measured in the whole hemolysate by immunoneutralization, as previously described. After partial purification the molecular specific activity (i.e., the ratio of enzymatic activity to immunologic reactivity) was also determined by electroimmunodiffusion with specific staining of the immunoprecipitate peaks for pyruvate kinase activity. Erythrocyte PFK was studied by immunoneutralization with antisera against rabbit muscle and human leukocyte phosphofructokinase, as reported elsewhere. Molecular specific activity of GPI from whole hemolysates was measured by electroimmuno-diffusion.
Kinetic studies. Heat stability of GPI was appraised in crude hemolysates, as previously described.¹⁹

For the study of the inhibition of PFK by ATP, 1:20 hemolysates were dialyzed 3 hr in 40 mM Tris-Cl buffer, pH 8, containing 0.1 mM F-6P, 10 mM β-mercaptoethanol, 1 mM EDTA, and 1 mM ε-aminocaproic acid. In order to eliminate ammonium sulfate, the commercial intermediate enzymes used for the enzymatic reaction were extensively dialyzed against the buffer described above.

The reaction was measured at 30° in 40 mM Tris-Cl buffer, pH 8, containing 2 mM NADH, 0.5 mM (NH₄)₂SO₄, 20 mM fructose 6-phosphate, 1 mM EDTA, 10 mM β-mercaptoethanol, 0.5 IU/ml aldolase, 0.4 IU/ml glycerol 3-phosphate dehydrogenase, 1 IU/ml triose phosphate isomerase; 25 µl of dialyzed 1:20 hemolysate were preincubated for 10 min at 30° with the reaction mixture, and the reaction was started with various concentrations of ATP-MgCl₂ in the ratio 1:10. The final volume of the reaction mixture was 1 ml.

Electrophoresis and electrofocusing. Pyruvate kinase was focused in slab gels of 3%, acrylamide containing 1.33%, ampholines of pH range 5-8, 0.66%, ampholines of pH range 7-9, and 10% sucrose as described elsewhere.²⁰ Glucose phosphate isomerase was subjected to isoelectric focusing in a column of 5%, acrylamide, 1%, ampholines of pH range 9-11, and to starch gel electrophoresis according to Detter et al.²¹

CLINICAL OBSERVATION

The patient, a 61-yr-old woman, had no particular pathologic antecedents until she was admitted to the hospital in April, 1970, for the investigation of an anemic syndrome.

On physical examination only a palpable spleen tip was noted. Blood examination showed hemoglobin, 9.2 g/dl; RBC, 2.18 × 10¹²/liter; PCV, 0.28; MCV, 130 fl; MCHC, 32.4 g/dl; reticulocytes, 10⁶; peripheral erythroblasts, 22 per 100 leukocytes; WBC, 3.2 × 10⁹/liter (51% neutrophil polymorphonuclears, 40%, lymphocytes, 9%, monocytes); platelets, 31 × 10⁹/liter. Total bilirubin was 7 mg/liter, serum iron 25 μmoles/liter; total iron capacity binding 57.2 μmoles/liter. A bone marrow examination showed erythroid hyperplasia with macrocytosis, and only minor morphological abnormalities. No “ring sideroblast” was found. Ultrastructural examination confirmed the absence of major dysmorphism of the erythroid cells. Bone marrow biopsy showed no abnormality of the cells and no myelofibrosis. Karyotype was normal. The Coombs test was negative, and the hemoglobin electrophoresis was normal (97.4%, Hb A1, 1.8%, Hb A2, 0.8%, Hb F). Serum folate and serum B₁₂ were normal. The percentage of agglutinability by anti-“i” sera was markedly increased (68%, instead of 24% ± 9% in controls). The agglutinability by anti-I, anti-H, anti-A, and anti-A₁ sera was normal (blood group of the patient was A₁, Rh[+]). Half-lives of autologous ⁵¹Cr-labeled erythrocytes were 21 days (normal: 24-28 days).

In the course of a search for a primary malignant tumor, an asymptomatic villous adenoma of the upper rectum was discovered. Surgical excision of the lesion was performed, and, during the same operation, the spleen was removed. Splenectomy resulted in marked hematologic improvement, and, a few weeks later, blood examination showed hemoglobin, 12.5 g/dl; RBC, 3.27 × 10¹²/liter; PCV, 0.406; MCV, 125 fl; MCHC, 32.4 g/dl; reticulocytes, 2.1%; peripheral erythroblasts, 30 per 100 leukocytes; WBC, 5.8 × 10⁹/liter; platelets 163 × 10⁹/liter. Until 1976 the hematologic features remained unchanged, characterized by chronic macrocytosis and peripheral erythroblastosis without major dysmorphism of the erythroid cells in bone marrow. The half-lives of autologous ⁵¹Cr-labeled RBC were 24 days, and an investigation of erythropoiesis with ⁵⁹Fe showed only a slowing down of incorporation of radioactive iron into red cells (half incorporation, 4.5 days instead of 2.8 ± 0.3 in controls). The plasma ⁵⁹Fe T½ (70 min) and the RBC uptake (90%) were normal.

RESULTS

Enzyme Titration

In 1971, before splenectomy, enzyme assays showed that, despite the high reticulocyte count, several erythrocyte enzymes showed reduced activity: glucose phosphate isomerase, to 69% of normal; glyceraldehyde-3-phosphate dehydro-
Table 1. Activity of Red Cell Enzymes Before and After Splenectomy

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>1974 (Before Splenectomy)</th>
<th>1976 (After Splenectomy)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IU/100 ml of RBC at 25°C</td>
<td>% of Normal</td>
</tr>
<tr>
<td>Hexokinase</td>
<td>18.3 (11 ± 1)</td>
<td>166</td>
</tr>
<tr>
<td>Glucose phosphate isomerase</td>
<td>512 (738 ± 64)</td>
<td>69</td>
</tr>
<tr>
<td>Phosphofructokinase</td>
<td>200 (246 ± 31)</td>
<td>81</td>
</tr>
<tr>
<td>Aldolase</td>
<td>31 (23 ± 3)</td>
<td>135</td>
</tr>
<tr>
<td>Triose phosphate isomerase</td>
<td>33815 (31,900 ± 3,500)</td>
<td>106</td>
</tr>
<tr>
<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
<td>1,790 (2,540 ± 237)</td>
<td>70</td>
</tr>
<tr>
<td>2-3-Diphosphoglycerate mutase</td>
<td>17 (30 ± 2.7)</td>
<td>57</td>
</tr>
<tr>
<td>3-Phosphoglycerate kinase</td>
<td>3,046 (3,300 ± 213)</td>
<td>92</td>
</tr>
<tr>
<td>Enolase</td>
<td>355 (343 ± 32)</td>
<td>103</td>
</tr>
<tr>
<td>Pyruvate kinase</td>
<td>110 (121 ± 10)</td>
<td>91</td>
</tr>
<tr>
<td>Glucose-6-phosphate dehydrogenase</td>
<td>210 (140 ± 9)</td>
<td>150</td>
</tr>
<tr>
<td>6-Phosphogluconate dehydrogenase</td>
<td>128 (81 ± 9.5)</td>
<td>158</td>
</tr>
<tr>
<td>Glutathione reductase</td>
<td>86 (78 ± 8)</td>
<td>110</td>
</tr>
<tr>
<td>Adenylate kinase</td>
<td>2,630 (3,915 ± 267)</td>
<td>67</td>
</tr>
<tr>
<td>Reduced glutathione</td>
<td>70 mg (60 ± 10)</td>
<td>117</td>
</tr>
</tbody>
</table>

Normal values for each method are indicated in parentheses under the values found for the patient; they are given ± 1 SD. The methods used are described in Refs. 2 and 4 for the assays performed in 1974 and in Ref. 14 for those performed in 1976.

Pyruvate kinase activity was normal (Table 1). By contrast with these lowered or normal enzyme activities, hexokinase, aldolase, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase showed increased activities, probably due to the high reticulocyte count found at that time. In 1976, new assays of erythrocyte enzyme activities showed the following: glucose phosphate isomerase, 45% of normal; phosphofructokinase, 64%; glyceraldehyde phosphate dehydrogenase, 73%; pyruvate kinase, 63%; adenylate kinase, 68% (Table 1). The activity of leukocyte GPI was normal.

Study of Deficient Pyruvate Kinase

The defect in PK activity could not be improved by cross-incubation of RBC in isologous plasma or by treatment of the hemolysate with SH reagents. The immunoneutralization experiment with crude hemolysate (Fig. 1) seemed to
Fig. 1. Immunoneutralization of erythrocyte PK in crude hemolysates (A) and in partially purified preparations (B). o-o, control; o-o, patient. Increasing quantities of anti-L-type-PK serum were added to hemolysates in A and to partially purified PK preparations in B, both diluted to the same enzymatic activity for the patient and the control. Incubation was for 1 hr at 37°C and about 4 hr at +4°C in a total volume of 85 μl of 100 mM Tris-Cl buffer, pH 8, containing 100 mM KCl, 10 mM MgCl₂, 0.1 mM 1,6-fructose diphosphate, 1 mM EDTA, 1 mM L-aminocaproic acid, 1 mM diisopropylfluorophosphate, 0.1 mM dithiothreitol, 2 mg/ml bovine albumin, and 500 mM sucrose. Then, the tubes were centrifuged for 10 min at 15,000 g, and the residual PK activity was assayed in the supernatant. From extrapolation to the X axis of the initial slope of the immunoneutralization curves it can be measured that, in the hemolysates, 1 ml of antiserum is able to neutralize 70 IU of normal PK and 45 IU of deficient PK; by contrast, in the partially purified preparation 1 ml of antiserum neutralizes 54 IU of both normal and deficient PKs. The slight decrease of the “apparent titer” of the antiserum as judged before and after partial purification of PK is a constant phenomenon, probably because of a slight inactivation of PK during the purification procedure.

This indicates that the concentration of the “PK-related antigen” was normal in the patient’s hemolysate and that, consequently, the ratio of enzyme activity to immunologic reactivity was lowered. By contrast, this ratio, measured by immunoneutralization or by electroimmunodiffusion was normal in the partially purified preparation. In the same way, PK-specific activity was similar in the partially purified preparation from the patient’s red cells (8.0 IU/mg of protein) and in that from control’s red cells (8.0 IU/mg of protein). In crude hemolysate deficient pyruvate kinase had a normal electrophoretic pattern.

Study of Deficient Glucose Phosphate Isomerase

The enzyme activity was not changed by cross incubation of RBC or by treatment of hemolysate with SH reagents. The electroimmunodiffusion experiment showed that the GPI-related antigen concentration was decreased parallel to the enzyme activity and that, consequently, the ratio of enzyme activity to immunologic reactivity was normal (Fig. 2). Residual GPI had a normal starch gel electrophoresis and electrofocusing pattern and was as stable to heat as a control enzyme. Heat stability and electrophoretic pattern of leukocyte GPI were also normal.

Study of Deficient Phosphofructokinase

The activity of deficient PFK, as well as that of both previously studied enzymes, could not be restored by cross-incubation of RBC, dialysis, or treatment with SH reagents of hemolysate. The experiments of immunoneutralization showed that the deficient enzyme was less neutralized by antimuscle PFK
Fig. 2. Electroimmunodiffusion of whole hemolysate glucose phosphate isomerase. The gels were 1% (w/v) agarose in a 25 mM barbital buffer, pH 8.2, containing 20 μl of antiserum per 20 ml of gel. The extracts (1:2, 1:4, 1:8, 1:16 and 1:32 hemolysates) were deposited in wells at the anodic side of the gel, glucose phosphate isomerase migrating towards the cathode. The migration ran for 150 min at 4°C, at a voltage of 10 V/cm. The immunoprecipitate peaks were specifically stained for glucose phosphate isomerase in 50 mM TrisCI buffer, pH 8, containing 0.2 mM NADP+, 1 mM fructose 6-phosphate, 10 mM MgCl₂, 0.8 IU/ml glucose 6-phosphate dehydrogenase, 0.2 mg/ml tetrazolium salt MTT, and 0.1 mg/ml phenazine methosulphate. After completion of staining, the reaction was stopped in 2.5% (v/v) acetic acid. On a graph the enzymatic activity applied to the gel (indicated for the 1:2 hemolysates) was then plotted versus the surface of the immunoprecipitate peaks. The slope of the straight line obtained represented the ratio of enzyme activity to immunologic reactivity, i.e., the molecular specific activity. In the experiment reported herein, it was clear that the deficient hemolysate contained both half of the enzymatic activity and half of the "antigen" concentration with respect to the hemolysate from the control.

Fig. 3. Immunologic studies of normal and deficient erythrocyte phosphofructokinase. In order to avoid all nonspecific inhibitory action of serum on phosphofructokinase activity, the antisera were diluted in a solution of serum from nonimmunized animals at the same concentration as that of the antiserum preparations. The antisera were added to crude hemolysate diluted in 50 mM Tris-Cl buffer, pH 8, containing 1 mM EDTA, 0.1 mM dithiothreitol, 1 mM diisopropylfluorophosphate, 2 mM L-aminocaproic acid and 1 mM ATP. The mixtures were incubated for 1 hr at 37°C and 16 hr at 4°C, then they were centrifugated for 5 min at 15,000 g and the residual enzyme activity was assayed in the supernatant. ○—○, control; ——, patient.

Fig. 4. Inhibition by ATP of erythrocyte PFK from normal controls (n = 7) and from the patient. ○—○, normal controls, mean value ±1 SD; ——, patient. Methods used were described into detail under Materials and Methods.
FIG. 5. Neutralization of antimuscle PFK serum of erythrocyte PFK from adult controls and from newborn infants. Methods similar to those described in Fig. 3. - - - , adult controls (n = 7), mean value ± 1 SD; Δ—Δ, unfractionated erythrocytes from a 21-wk-old premature infant; △—△, unfractionated erythrocytes from a 32-wk-old premature infant; ■—■, old erythrocytes from a full-term infant; six full-term infants were so studied, with results nearly identical.

FIG. 6. Inhibition by ATP of erythrocyte PFK from adult controls and from newborn infants. Symbols as in Fig. 5.

serum and more neutralized by antileukocyte PFK serum than normal enzyme (Fig. 3). In addition, the patient’s erythrocyte PFK was more markedly inhibited by ATP than a normal enzyme (Fig. 4).

**Study of Cord Blood Erythrocyte Phosphofructokinase**

The immunologic experiments with antimuscle PFK serum showed that PFK of unfractionated cord blood erythrocytes from two premature infants (21 wk and 32 wk of gestation), and PFK of “old” erythrocytes from six full-term infants was less neutralized than PFK of erythrocytes from adults (Fig. 5).

In addition, PFK from “fetal” erythrocytes was more markedly neutralized by ATP than PFK from “adult” erythrocytes (Fig. 6). In adults neutralization by antimuscle PFK serum and inhibition by ATP did not differ in either “old” or “young” erythrocyte fractions.

In full term infants, by contrast, PFK of young erythrocytes was clearly neutralized more by antimuscle PFK serum and less inhibited by ATP than the enzyme of old erythrocytes. As a matter of fact, PFK of young red cells from infants did not significantly differ from that of RBC from adults.

**DISCUSSION**

From the results reported here, it may be concluded that, in the patient studied, the observed decrease in enzyme activity of several enzymes was probably due to different direct mechanisms.

The pyruvate kinase defect involved enzyme activity, and not “PK-related antigen” concentration. Moreover, though the enzyme activity was not restored by dialysis or treatment with SH reagents as reported by Arnold et al., the inactive enzyme molecules seemed to be reactivated during partial purification; in the partially purified preparations, indeed, the patient’s PK had a normal “specific activity” and a normal ratio of enzyme activity to immunologic reactivity. A post-translational reversible inactivation of some PK molecules
in the patient’s erythrocytes, as reported by Arnold et al., seemed, therefore, the most likely hypothesis.

GPI deficiency in the patient’s red cells appeared to be a “true” molecular defect, with a parallel decrease in enzyme activity and in “GPI-related antigen.” A post-translational degradation of GPI was not probable, since the residual enzyme activity was as stable in the patient’s hemolysate, as in control hemolysates. In addition, residual enzyme showed normal electrofocusing and electrophoretic patterns. A fortuitous association between the blood disease and the heterozygous defect in GPI was also unlikely: the normal GPI activity in the patient’s leukocyte, indeed, ruled out the possibility of a silent gene; the normal stability of the leukocyte GPI excluded the likelihood of a very unstable GPI variant totally degraded in the red cells. Finally, reversion toward a fetal type of erythropoiesis could not be invoked since GPI has a very high activity in cord blood erythrocytes. Consequently, decreased synthesis of GPI by the abnormal erythroid precursors can be hypothesized.

Phosphofructokinase deficiency in the patient’s erythrocytes seemed to involve mainly the muscle-type subunit of the erythrocyte PFK, as indicated by the immunologic data. The greater inhibition by ATP of deficient PFK with respect to normal enzyme was consistent with this hypothesis, since muscle PFK is less inhibited by ATP than enzyme from other tissues. All these features were very similar to those described for congenital defects in muscle PFK. Whether or not this enzyme defect was involved in the mild hemolysis symptoms found before splenectomy can only be hypothesized. This possibility, however, should be considered, since the congenital defects in the muscle-type subunit of erythrocyte PFK have been associated with a moderate hemolytic syndrome.

In contrast with all other glycolytic enzymes, PFK from cord blood red cells has an activity significantly lower than that of red cells from adults; the defect is especially important in “old” cord blood erythrocytes, i.e., in fetal erythrocytes. Thus the question was raised of whether or not the defect in PFK activity found in the patient studied was similar to that characterizing fetal erythrocytes. The immunologic and kinetic results with unfractionated erythrocyte PFK from premature infants and with the “oldest” erythrocytes from full-term infants seemed to indicate that, indeed, fetal erythrocytes were relatively deficient in the muscle-type subunit of erythrocyte PFK. Consequently, the acquired PFK deficiency found in the patient could be due to a partial reversion of the genetic regulatory systems of the erythroid precursors towards a fetal type. The increase in the percentage of agglutinability of RBC by the anti-“i” serum could be interpreted in the same way.

Thus, it would appear that, in the same patient, several acquired defects in erythrocyte enzyme activity can be due to various direct mechanisms. We have previously shown that the defect in the activity of an enzyme (e.g., erythrocyte PK) can have different apparent mechanisms in different patients. In addition, the acquired PFK and GPI defects that have been reported by Arnold et al. are clearly of another type than those we have described above; in the observations reported by these authors, enzyme activity was restored by cross-incubation of red cells or by dialysis against buffers rich in SH reagents, while, in our obser-
vations, these treatments obviously had no influence, since the defects seemed to be due to a true decrease in enzyme molecule concentration.

It is unsatisfactory, however, to think that the whole range of biochemical abnormalities detected in some blood disorders cannot be ascribed to a common underlying cause. At present, we can only speculate upon the existence of this hypothetical “underlying cause.” Disorders in the mechanisms of gene regulation could account for both reduced synthesis of GPI and reduced synthesis of muscle-type PFK. The defect in PK activity, however, cannot be a direct consequence of such a disturbance in the control mechanisms. The metabolic abnormalities of cells which show disturbances in the gene control mechanisms could lead to modifications of the cell medium able to alter posttranslationally some enzyme molecules. From this viewpoint, the disturbance in the gene control mechanisms would be the “basic process” and could result in the decreased transcription of some genes, or in the derepression of other genes normally expressed in the fetus. The metabolic changes induced by these enzyme disorders could secondarily lead to modifications in the cell medium and, in this way, to postsynthetic alterations of other enzyme proteins.

In conclusion, the various hypotheses put forward for explaining the acquired erythrocyte enzyme activity defects in blood disorders do not exclude each other. Some defects may be due to post-translational events, others to modifications in the genetic regulatory systems, or to alterations in the genetic material. The relationship between these various abnormalities and the existence of a common underlying cause can only be hypothesized.

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Causal mechanisms of multiple acquired red cell enzyme defects in a patient with acquired dyserythropoiesis

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