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The Royal College of Pathologists

Molecular Variants in Disease

EDITED BY D. N. RAINE

Symposium organized by The Royal College of Pathologists and delivered in London in February 1974

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Erythrocyte enzyme deficiencies
Pyruvate kinase deficiency

E. C. GORDON-SMITH

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The identification, by Valentine and his colleagues in 1961, of a deficiency of a glycolytic enzyme in the red cells of some patients with congenital non-spherocytic haemolytic anaemia was the culmination of a long story of clinical and laboratory investigation. The chapters in this story illustrate well the steps required to identify a molecular disorder.

In 1954 Selwyn and Dacie published their observations on the effect of incubation of red cells at 37°C in autologous serum from patients with various congenital and acquired haemolytic anaemias. They studied the effect of addition of glucose to the blood on the amount of autohaemolysis and also the rate of utilization of glucose by the different red cells. They observed that red cells from two patients with congenital non-spherocytic haemolytic anaemia haemolyzed to the same, or even greater, extent when glucose was added (designated type II autohaemolysis, fig 1) and that these cells failed to utilize glucose normally. They concluded that in these patients there was a defect in glucose utilization by the red cells.

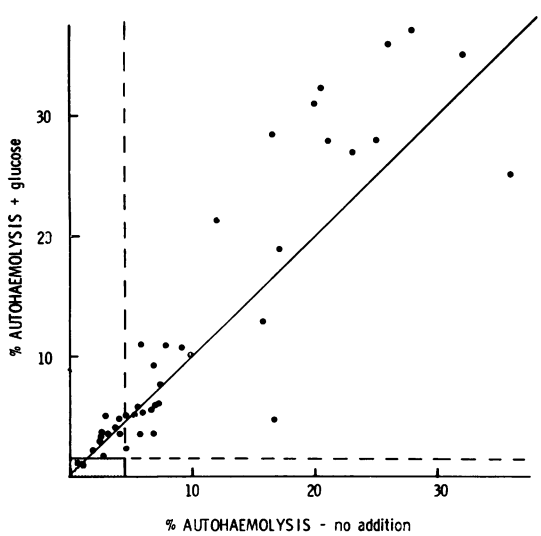

Fig 1 *The effect of the addition of glucose (2 mmol/l) to the autohaemolysis test in 43 patients with PK deficiency. The diagonal 45° line indicates no change when glucose is added. The shaded area includes the normal range.*

In 1960 de Gruchy and his coworkers (de Gruchy, Santamaria, Parsons, and Crawford) showed that the addition of adenosine triphosphate (ATP) to the blood in the autohaemolysis test reduced the lysis of these cells and later that these cells contained raised levels of 2,3, diphosphoglycerate (2,3DPG) (Robinson, Loder, and de Gruchy, 1961). They concluded that the defect in red cell metabolism lay in the late stages of the red cell glycolytic pathway. Later in the same year a systematic study (Valentine, Tanaka, and Miwa, 1961) of the glycolytic enzymes in these patients demonstrated that the red cells were deficient in pyruvate kinase (E.C. 2.7.1.40, ATP : pyruvate phosphotransferase).

The clinical and laboratory data reported here are derived from investigations on 43 pyruvate-kinase-(PK)-deficient patients from 35 families, seen at Hammersmith Hospital. Previous reports, have appeared on some of these patients (Grimes, Meisler, and Dacie, 1964).

Pyruvate Kinase in the Red Cell

Pyruvate kinase catalyses the transfer of phosphate from phosphenol pyruvate (PEP) to ADP in the final step of the glycolytic pathway. With this reaction there is a net gain of 2 moles of ATP generated from ADP for each mole of glucose metabolized. The conversion of PEP to pyruvate is strongly exergonic, so that equilibrium lies far to the side of pyruvate and the reaction is practically irreversible. Such irreversible reactions are commonly a site of control of a metabolic pathway, and the enzymes usually exhibit allosteric interactions. This is true of red cell PK, at least under defined conditions (Minakami and Yoshikawa, 1966).

Pyruvate kinase has many ligands apart from PEP and pyruvate. These are shown in table I. It is to be expected that molecular lesions of the red cell PK may affect any of these ligand-binding sites, thereby altering the function of the enzyme,

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Ligand	*Action*	$K_{\frac{1}{2}}$[1] *(approximate values) (mol/l)*	*Special Assay Conditions*	*Concentration of Ligand in Normal Red Cells*[2] *(mol/l)*	*Reference*
PEP	Substrate	$3{\cdot}0 \times 10^{-4}$	No F1,6DP	$1{\cdot}7 \times 10^{-5}$	Ibsen, Schiller, and Venn-Watson (1968)
		$0{\cdot}8 \times 10^{-4}$	$5{\cdot}0 \times 10^{-4}$M F1,6DP		
ADP Mg++)	Substrate	$3{\cdot}3 \times 10^{-4}$		$1{\cdot}8 \times 10^{-4}$	Ibsen *et al*
K^+	Activator	$4{\cdot}8 \times 10^{-3}$	6·4 mM Mg ++	$9{\cdot}0 \times 10^{-2}$	Ibsen *et al*
F1,6DP+	Activator	$1{\cdot}0 \times 10^{-7}$		$9{\cdot}0 \times 10^{-6}$	Blume *et al* (1971)
G6P	Activator	$2{\cdot}0 \times 10^{-4}$		$3{\cdot}8 \times 10^{-5}$	Black and Henderson (1972)
ATP	Inhibitor	$1{\cdot}0 \times 10^{-4}$	No organic phosphate pH 7·2	$1{\cdot}8 \times 10^{-3}$	Staal *et al* (1971)
	Partial activator	$5{\cdot}0 \times 10^{-4}$	F1,6DP 5×10^{-4}M		

Table I *Ligands of erythrocyte pyruvate kinase*

[1]$K_{\frac{1}{2}}$ Concentration of ligand at which half maximal activity is obtained *in vitro* under the conditions stated.
[2]Ligand concentrations from Minakami and Yoshikawa (1966).

or they may affect other parts of the molecule which have no effect on function. On the other hand, there may be a defect in synthesis of the enzyme so that a small quantity of normally functioning enzyme is produced.

There is now some evidence that at least two genetic variants of red cell PK exist. Figure 2 shows the activity of PK as assayed in 37 affected patients, 25 presumed heterozygotes and 24 normal controls. It will be seen that there is a considerable overlap between heterozygous patients who have no detectable clinical abnormality and the affected patients. Some of this overlap may be the result of contamination with white cells, which have their normal high levels of PK, though the assay is carried out on blood from which as many white cells as possible have been removed by dextran sedimentation.

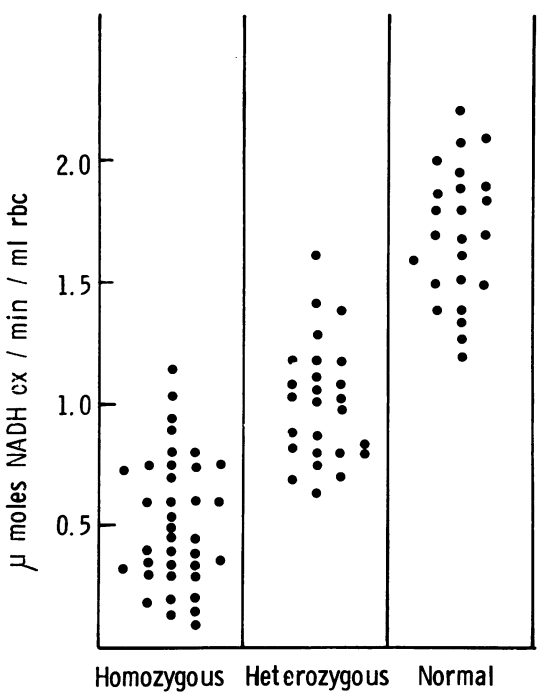

Fig 2 *Activity of PK in erythrocytes of deficient patients, heterozygotes, and controls assayed by the method of Valentine* et al *(1961). The Km for PEP for all propositi except two with the highest activities was between 0·8 × 10^{-4} mol/l and 2·1 × 10^{-4} mol/l measured on haemolysates. The two highest assay values had Km values of 30·6 × 10^{-4} mol/l and 11·6 × 10^{-4} mol/l respectively.*

An alternative explanation was suggested by the detection of a PK variant (called PK_2) in two unrelated families in which the activity in the assay system was normal but the affinity of the variant for substrate PEP was about 10 times less than normal (Paglia, Valentine, Baughan, Miller, Reed, and McIntyre, 1968). This means that at the physiological concentration of PEP the enzyme is functionally deficient as shown in figure 3. A second genetic variant (called type B) is suggested by the findings of Blume, Hoffbauer, Busch, Arnold, and Löhr (1971), who found that the enzyme from one family with PK deficiency required a much higher concentration of fructose 1,6 diphosphate (F1,6P) to activate enzyme than normal. The concentration required, 1×10^{-5} mol/l for half-maximal activation is higher than the concentration of F1,6P in the normal red cell. The common variety of PK deficiency (type A of Blume *et al*, 1971, or PK_1 of Paglia *et al*, 1968) appears to have normal kinetics but diminished activity and may represent a failure of synthesis, though this is not certain.

Biochemical Effects of PK Deficiency

The effects of PK deficiency on red cell metabolism *in vivo* are difficult to assess because of the large numbers of reticulocytes present. Reticulocytes have

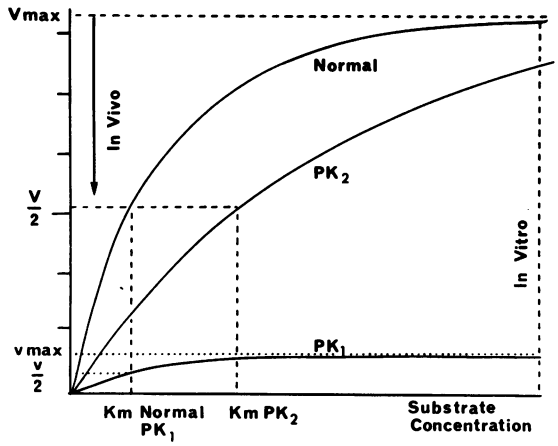

Fig 3 *Diagram to show the effect of abnormal binding characteristics (increased Km for PEP) on the* in-vivo *and* in-vitro *activity of PK variants. The* in-vivo *concentration of PEP is about* $3{\cdot}5 \times 10^{-5}$ *mol/l in PK-deficient cells whereas the usual assay concentration is* 15×10^{-3} *mol/l.*

alternative sources of energy to glycolysis and they may be able to utilize substrates other than glucose via the tricarboxylic acid cycle below the block of PK deficiency. The high reticulocyte count is considered to have metabolic advantages in PK deficiency, and Keitt (1966) has suggested that the *in-vitro* effect of glucose in increasing the autohaemolysis is the result of inhibition of oxidative phosphorylation by glucose (a Crabtree effect).

ORGANIC PHOSPHATE INTERMEDIATES

Deficiency of PK leads to an increase in phosphorylated intermediates which precede the enzyme block. Levels of PEP, 2,phosphoglycerate (2PG), and 2,3DPG are increased two or three times above normal. The increase in 2,3DPG has a marked effect on the oxygen dissociation curve (Benesch and Benesch, 1967; Chanutin and Curnish, 1967). The relationship between the $PO_2 50$ and packed cell volume in patients with PK deficiency is shown in figure 4. The physiological effects of such a shift to the right in the oxygen dissociation will be discussed by Professor Huehns later in this symposium.

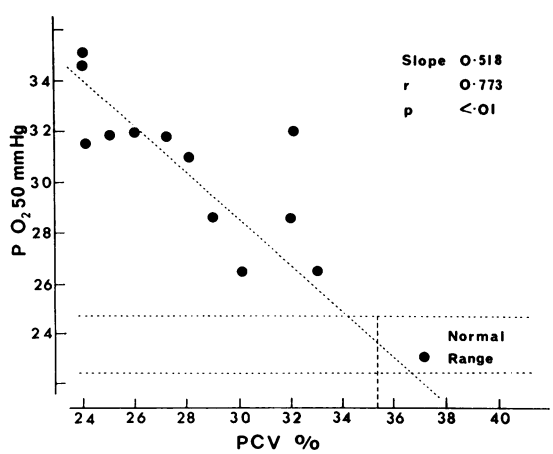

Fig 4 *Relationship between* $PO_2 50$ *and PCV in 13 patients with PK deficiency. The PCV in these patients was maintained in a steady state.*

Clinical Features of PK Deficiency

VARIABILITY IN EXPRESSION

One of the striking features of PK deficiency is the marked differences in the severity of the disease between different, unrelated patients. Table II shows the age of presentation in 43 patients with PK deficiency studied at Hammersmith Hospital. The majority were detected within a few hours of birth, sometimes with severe haemolytic disease requiring exchange transfusion. Other patients were not detected until adult life, often when a particular stress, such as pregnancy or intercurrent infection, brought their jaundice and anaemia to the attention of the doctor.

Age at Presentation	*No.*	
< 24 h	22	7 exchange transfusion 2 died with jaundice
Infancy	6	
Childhood	9	
Adult	6	2 during pregnancy

Table II *Pyruvate kinase deficiency*

When this variability is examined in relation to families, it is seen that similarities between sibs are more striking than differences (table III). Even in less severely affected families there is a close correlation between the metabolic effects of their deficiency. Figure 5a shows the remarkable similarity in osmotic fragility of fresh blood and blood incubated for 24h at 37°C from two sibs with PK deficiency. The decrease in osmotic fragility after incubation is a reflection of the decrease in total cation content during incubation. The relative potassium leak during this time is shown for one of the sibs in figure 5b.

The reasons for the concordance between sibs, but variation between families, seen in this series is not yet clear. In the majority of patients it has not been possible to demonstrate differences in the function of the PK enzymes but they may still

Patient	*Clinical Presentation (age)*	*Splenectomy (age)*	*Hb (g/100 ml)*	*Reticulocytes (%)*	*Comment*
ARM	Hereditary disease of the newborn, exchange transfusion thrice	3 y	6·5 - 8·5	70	Fair
SRM	Hereditary disease of the newborn	...	—	—	Died
SRM	Hereditary disease of the newborn	...	—	—	Died
PM	19 h	...	6·0	25	Fair
LM	Intrauterine and exchange transfusion twice	...	4·2	—	Poor
AQ	Infancy	1 y	9·0	26	Well
SQ	Infancy	2 y	6·3 - 8·2	—	Fair
MC	2½ y	3 y	8·0	80	Died 47 y
SC	1 y	2½ y	—	—	Died 12 y
MG	2 weeks	10 mth	10·1	32	Well
LG	1 week	2 mth	10·1	35	Well
JS	18 y	...	5·8	18	Well
BS	Infancy	3 mth	—	—	—
SF	5 y	...	9·2	6·5	Well
RF	8 y	...	9·0	7·2	Well

Table III *Pyruvate kinase deficiency in different sibships*

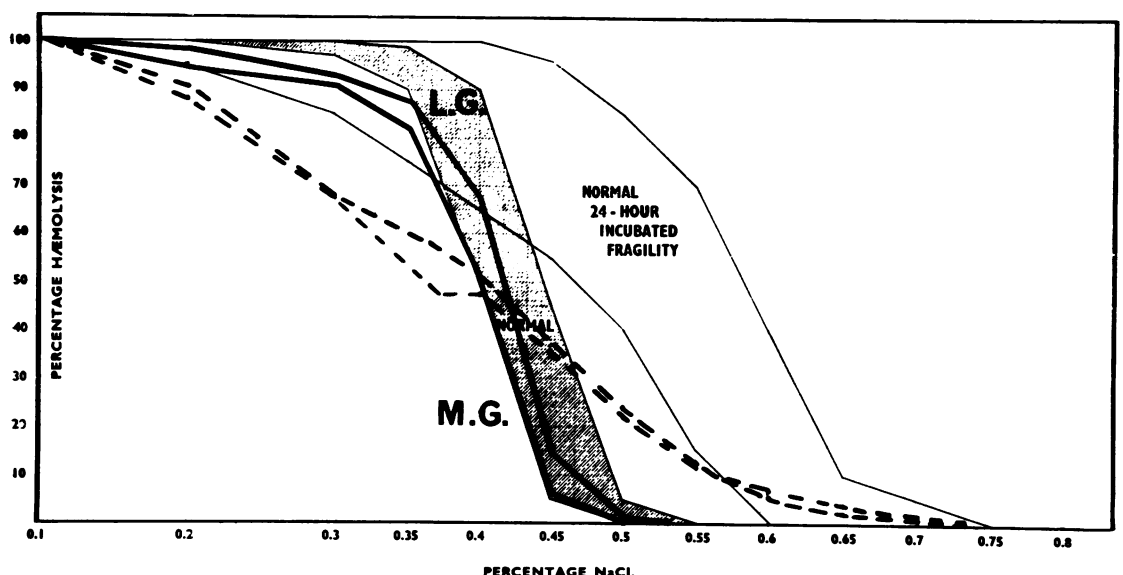

Fig 5a

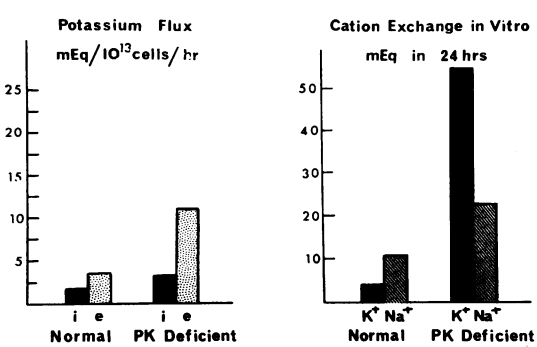

Fig 5b

Fig 5a *Osmotic fragility on fresh (solid lines) and incubated blood (24 hours, 37°C dotted lines) in a brother and sister with PK deficiency.*

Fig 5b *Potassium influx (i) and efflux (e) in fresh normal and PK-deficient cells in autologous serum and potassium loss and sodium gain after 24 hours' incubation.*

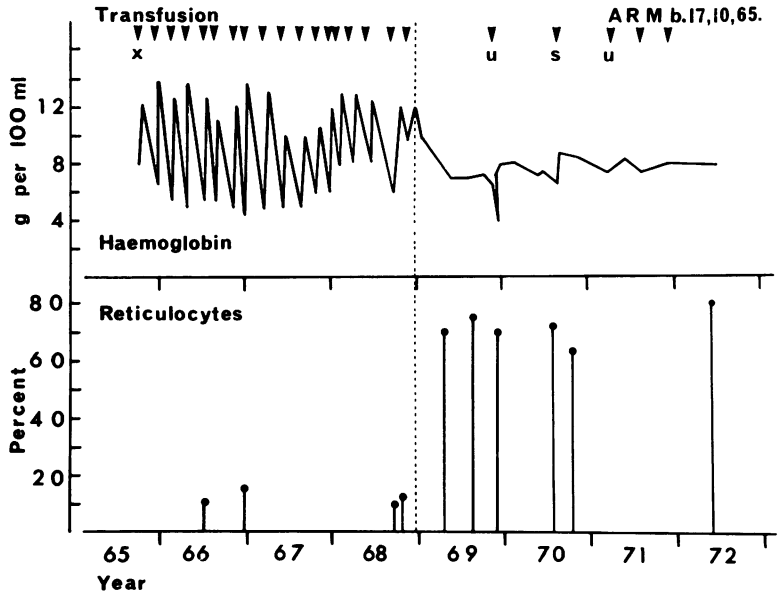

Fig 6 *Effect of splenectomy on transfusion requirements in patient A.R.M., the most severely affected PK-deficient patient in this series. U = urinary tract infection, S = staphylococcal septicaemia.*

exist. On the other hand, it may be that other polymorphisms play a part in determining the susceptibility to PK deficiency.

Management of PK Deficiency

The morbidity in patients deficient in PK arises from the anaemia and hyperbilirubinaemia. Patients with PK deficiency usually tolerate low haemoglobin concentrations very well and have normal exercise tolerance. One patient, AQ, despite a haemoglobin level of about 9·0 g/100 ml, regularly indulged in cross country running. This tolerance to exercise is probably related to the shift in the oxygen dissociation curve, produced by the raised 2,3DPG; but it should be noted that more severely affected patients usually have some degree of myocardial hypertrophy, and increased cardiac output plays an important part in compensation for the anaemia.

SPLENECTOMY IN PK DEFICIENCY

Early studies with ^{51}Cr suggested that splenectomy would be of little value in the management of congenital non-spherocytic haemolytic anaemias but this has not been borne out by clinical studies. Figure 6 shows the effect of splenectomy in the most severely affected child with PK deficiency in our series. After splenectomy, the haemoglobin stabilized between 6·5 and 8·5 g/100 ml, the lower values occurring particularly during infections. The very high reticulocyte counts found after splenectomy in this patient are usual in splenectomized patients (fig 7). There is evidence that PK-deficient reticulocytes are sequestered in the spleen so that mainly mature, metabolically inefficient red cells circulate (Bowman and Procopio, 1963). The timing of splenectomy is not always

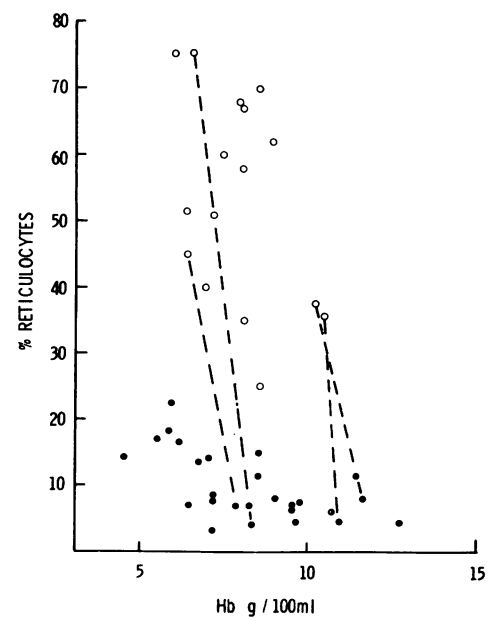

Fig 7 *Effect of splenectomy on reticulocyte counts in PK deficiency. Dotted lines join values from the same patient before (●) and after (○) splenectomy.*

straightforward because of the possible susceptibility of young splenectomized children to infections. The patient A.R.M. developed a staphylococcal septicaemia about one year after splenectomy which was carried out at the age of 3 years and 2 months. Children with PK deficiency who are splenectomized fall into the group of patients with only partially corrected haemolysis, and these seem to be particularly at risk from infections (Eraklis, Kerry, Diamond, and Gross, 1969). Prophylactic penicillin therefore seems a reasonable precaution.

The main complications of the haemolysis arise from hyperbilirubinaemia. Eight of the 43 patients in this series have required cholecystectomy for cholecystitis associated with pigment stones, and one other patient who refused surgery developed a biliary fistula to the skin and eventually died from complications of this disorder. So far none of the eight patients has developed stones in the biliary tract following removal of the gallbladder.

The total serum bilirubin may be reduced in patients with PK deficiency, as in other patients with haemolytic anaemia, by the administration of phenobarbitone but it is not yet known whether this reduces the incidence of pigment stones.

Conclusion

Pyruvate kinase deficiency still presents many unsolved problems in the relationships between molecular and clinical disorders. Even the pathogenesis of haemolysis in this disorder remains uncertain (Keitt, 1966).

I should like to thank Professor J. V. Dacie for the opportunity to study these patients, most of whom were referred to him for diagnosis and management.

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