5 J. A. GLOMSET, E. JANSEN, R. KENNEDY AND J. DOBBINS, *J. Lipid Res.*, 7 (1966) 638.
6 J. A. GLOMSET, *J. Lipid Res.*, 9 (1968) 155.
7 M. SUGANO AND O. W. PORTMAN, *Arch. Biochem. Biophys.*, 107 (1964) 341.
8 M. SUGANO AND O. W. PORTMAN, *Arch. Biochem. Biophys.*, 109 (1965) 302.
9 Y. AKANUMA AND J. A. GLOMSET, *J. Lipid Res.*, 9 (1968) 620.
10 G. R. WEBSTER AND M. J. COOPER, *J. Neurochem.*, 15 (1968) 795.
11 W. M. SPERRY, *J. Biol. Chem.*, 111 (1935) 467.
12 O. W. PORTMAN AND M. SUGANO, *Arch. Biochem. Biophys.*, 105 (1964) 532.
13 J. A. GLOMSET AND D. KAPLAN, *Biochim. Biophys. Acta*, 98 (1965) 41.
14 E. G. BLIGH AND W. J. DYER, *Can. J. Biochem. Physiol.*, 37 (1959) 911.
15 P. J. RIEKKINEN AND U. K. RINNIE, *Brain Res.*, 9 (1968) 136.
16 C. M. PLUM AND T. FOG, *Acta Psychiat. Neurol. Scand.*, 34 (1959) Suppl. 128, 11.
17 B. SWAHN, R. BRONNESTRAM, AND S. J. DENCKER, *Neurology*, 11 (1961) 437.
18 K. SOMEDA AND N. KAGEYAMA, *Klin. Wochschr.*, 43 (1965) 230.
19 D. R. ILLINGWORTH, *Ph. D. Thesis*, University of Liverpool (1970).
20 G. B. PHILLIPS AND J. T. DODGE, *J. Lipid Res.*, 8 (1967) 676.
21 A. RAZ, F. A. KUMMEROW AND T. NISHIDA, *Biochim. Biophys. Acta*, 176 (1969) 591.
22 W. W. TOURTELLOTE, *Neurology*, 9 (1959) 375.
23 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.

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Some properties of abnormal red blood cell pyruvate kinase

Erythrocyte pyruvate kinase (ATP:pyruvate phosphotransferase, EC 2.7.1.40) deficiency is a metabolic disorder in which a great variety in the parameters of enzymatic activity [V and K_m (phosphoenol pyruvate)] has been found. Three types could be distinguished based on these kinetic parameters. In most cases the parameters are measured on the crude hemolysate. MUNRO AND MILLER[1] concluded for a mutant enzyme that the ADP and PEP (phosphoenol pyruvate) sites show a greater cooperation than the normal enzyme. However they did not calculate the extent of cooperation, for instance, expressed as the Hill coefficient.

This paper deals with three unrelated pyruvate kinase-deficient patients from whom the enzymes are partially purified to stage 4, according to the method of STAAL *et al.*[2]. The enzymes were finally dialysed overnight against 0.01 M Tris–malate containing 50% (v/v) glycerol (pH 8.0). The enzymatic activity of pyruvate kinase was measured according to the method of BÜCHER AND PFLEIDERER[3].

Fig. 1 shows the $1/v$–$1/[PEP]$ plots at various ADP concns. of the normal, (highly purified) enzyme and the mutant enzymes. In contrast to the normal enzyme (Fig. 1A), the enzyme of patient J. v/d K. (Fig. 1B) gives straight lines. This was also found with the enzyme of an unrelated patient (N.S.) with pyruvate kinase deficiency (figure not shown). However, the enzyme of the third patient (B.H.) shows the same picture as the normal enzyme. The K_m values for PEP at $[ADP] = \infty$ are, within experimental error, identical. Moreover, in all $1/v$ *versus* $1/[ADP]$ plots of the data of these patients, straight lines are obtained (not shown) and the K_m (ADP) at $[PEP] = \infty$ are also normal. From these data it can be concluded that the enzymes of patients J. v/d K. and N.S., in contrast to normal enzyme and the enzyme of patient B.H. have lost their allosteric properties.

Further evidence for this conclusion is found in Fig. 2. This experiment shows

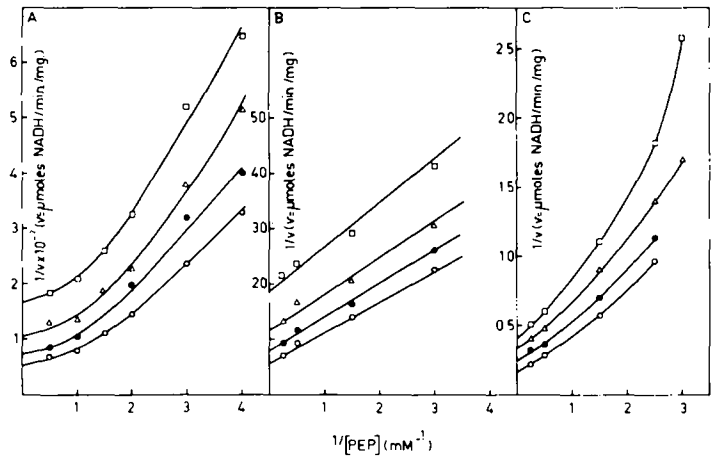

Fig. 1. The $1/v$ *versus* $1/[PEP]$ plot at various ADP concns.: ○—○, [ADP] = 0.33 mM; △—△, [ADP] = 0.66 mM; ●—●, [ADP] = 2 mM and □—□, [ADP] = ∞. A, normal enzyme; B, enzyme of patient J. v.d. K. and C, enzyme of patient B.H.

the v *versus* [PEP] plot in the presence and absence of Fru-1,6-P_2 for the normal and mutant enzymes. The mutant enzyme of patient J. v/d K. (Fig. 2B), like that of the patient N.S. (not shown), shows, instead of a sigmoidal curve, a hyperbolic one in the absence of Fru-1,6-P_2. However, the addition of Fru-1,6-P_2 still increases the activity. This is in contrast to the normal enzyme and that of the patient B.H. Addition of Fru-1,6-P_2 to these enzymes transforms the sigmoidal curve into a

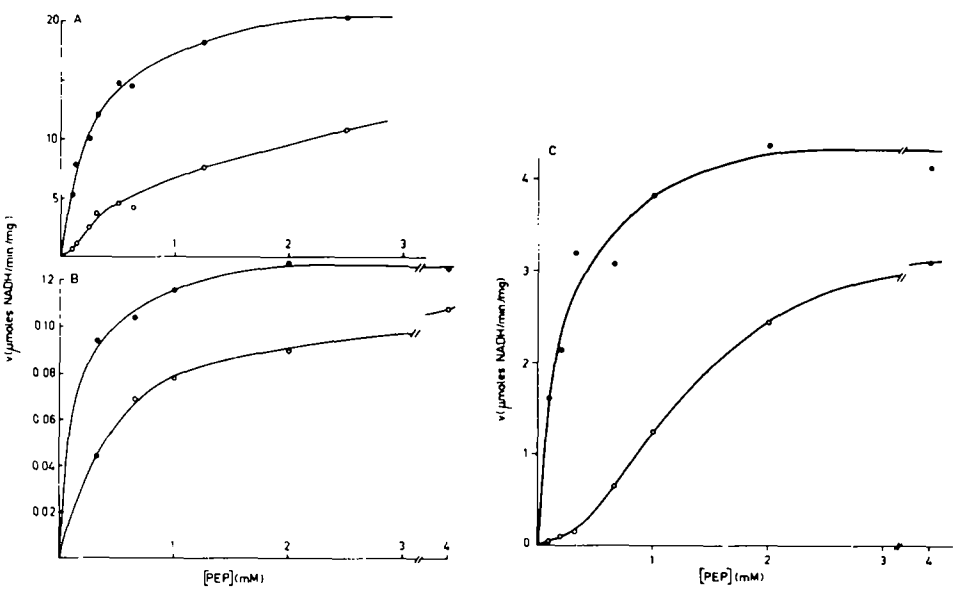

Fig. 2. The v *versus* [PEP] plot for the normal and the mutant enzymes. [ADP] = 2 mM, 0.2 M Tris–malate buffer, pH 7.6.
○—○, in the absence of Fru-1,6-P_2; ●—●, in the presence of Fru-1,6-P_2 (0.5 mM). A, normal highly purified enzyme; B, enzyme of patient J. v.d. K. and C, enzyme of patient B.H.

TABLE I

THE HILL COEFFICIENTS n FOR THE NORMAL AND MUTANT ENZYMES

The values are calculated from the data of Fig. 2.

Enzyme	n	
	— *Fru-1,6-P_2*	--- *Fru-1,6-P_2*
Normal	1.6	1.0
Patients J. v/d K. and N.S.	1.0	1.0
Patient B.H.	1.6	1.1

hyperbolic one. From Fig. 2 the Hill coefficients (n) were calculated. These data are summarized in Table I. The n values for the normal enzyme and the mutant enzyme of patient B.H. are identical and are normally influenced by Fru-1,6-P_2. However, the Hill coefficients of the mutant enzymes of patients J. v/d K. and N.S. are not influenced by Fru-1,6-P_2 and have a value of 1, meaning no cooperation.

With starch gel electrophoresis the highly purified enzyme shows one band[2]; however, the partially purified normal pyruvate kinase shows two bands (Fig. 3A). The electrophoretic behaviour of the mutant enzyme (patient B.H.) is identical to the normal enzyme, while the enzyme of patient J. v/d K. (Fig. 3B) shows a major and a very small band. Although, the positions of the bands are the same, the ratio between the intensities of the two bands is completely different from the ratio with the normal enzyme. It is also shown that the level just above the starting point is not the leucocyte enzyme, because the latter enzyme is found just below the starting point, as is also the case with the M type enzyme obtained from rabbit muscle (Boehringer & Sons).

The heat stability of the mutant enzymes of all three patients is considerably

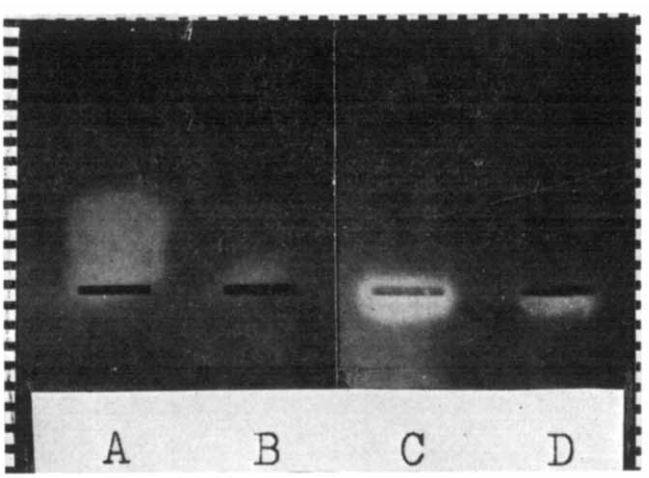

Fig. 3. Photograph of the starch gel electrophoresis of the normal and mutant enzyme. A, normal enzyme; B, mutant enzyme (J. v.d. K.); C, M type pyruvate kinase (Boehringen & Söhne); D_s leucocyte pyruvate kinase. Conditions, see ref. 2.

less than that of the normal enzyme. Heating for 1½ min at 60° causes a loss of activity of 40–50%, while the normal enzyme remains fully active. For the conditions of the heating procedure see ref. 2.

From the data it can be concluded that the mutant enzyme of patient B.H. is kinetically and electrophoretically identical with the normal enzyme. Probably the reason for the diminished enzymatic activity is due to increased instability of the enzyme *in vivo*. Evidence for this is found by measuring the pyruvate kinase activity in young and old erythrocytes. The young cells have 2.6-fold activity over the old cells. It is clear that the enzymes of the two other patients are completely different from the normal enzyme. The mutant enzyme has lost its allosteric properties and electrophoresis reveals a different pattern.

This mutant enzyme is also less stable than the normal enzyme. It is tempting to speculate that the loss of allosteric properties and the decreased stability in vitro are related and that the defect is responsible for the decreased activity of the enzyme *in vivo*.

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1 G. F. Munro and D. R. Miller, *Biochim. Biophys. Acta*, 206 (1970) 87.
2 G. E. J. Staal, J. F. Koster, L. Van Milligen-Boersma and C. Veeger, *Biochim. Biophys. Acta*, in the press.
3 T. Bücher and G. Pfleiderer, in S. P. Colowick and N. O. Kaplan, *Methods in Enzymology*, Vol. 1, Academic Press, New York, 1965, p. 435.

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